Blueprints of Life: Unlocking the Power of Modern Biotechnology

Edited by

Dr. Priyankar Pal

Swami Vivekananda University

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Contents

RECENT ADVANCES IN GUT MICROBIOTA-BRAIN INTERACTIONS: EMERGING INSIGHTS AND THERAPEUTIC IMPLICATIONS FOR NEURODEGENERATIVE AND NEUROPSYCHIATRIC DISORDERS

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ABSTRACT

Gut microbiota, also considered as the "second brain" play a pivotal role in regulating the physiological conditions as well as several neurochemical pathways through bidirectional communication in the gut-brain axis. The bidirectional pathway between microflora and the brain consists of vagus nerve, neuroendocrine, and immune system components. It indicates that a variety of neurodevelopmental, neurodegenerative, and neuropsychiatric disorders may be influenced by gut microorganisms, which may also affect behaviour, modify neurotransmission, and structure of brain development. The aim of this review article focused on the correlation between gut microflora and the brain and understand the significant role of gut microbiome in neurodegenerative disorder. The neuro-active metabolites such as short-chain fatty acids, neurotransmitters, and hormones derived from gut microbes can alter the enteric and central nervous system which causes various neurological diseases including Parkinson's disease, Alzheimer's disease, Huntington disease, multiple sclerosis, autism, and schizophrenia. It has been established that altering the gut microbiota has positive impacts on neural

networks, which delay the development of neurological diseases. Consequently, the adjustment of gut microbiota via tailored dietary approaches and oral bacteriotherapy could result in changes to gut microbiota and their byproducts. Therefore, this approach may offer a new therapeutic avenue for the medical treatment of neurodegenerative diseases.

Keywords: *Gut microbiota, Gut-brain axis, Neuro-active metabolites, Neurodegenerative disorder, Neuropsychiatric disorder.*

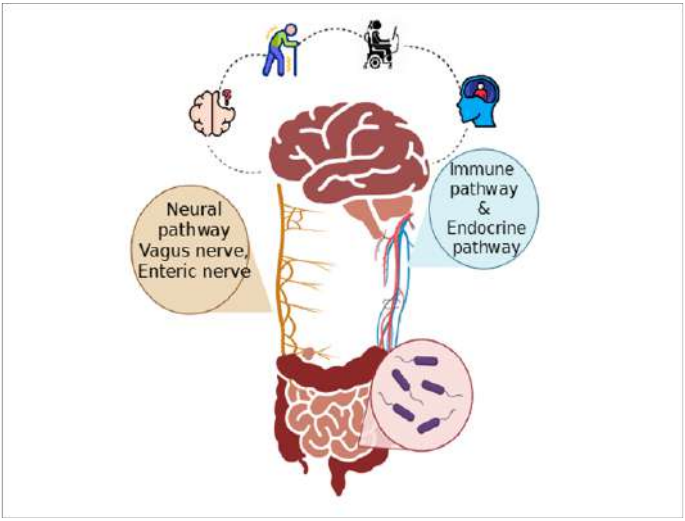

Figure 1.1:A Graphical representation of gut-brain axis(Image::BioRender)

1. Introduction:

The symbiotic bacteria that inhabit our gastrointestinal (GI) system, known as the gut microbiota, function as "metabolic machinery" (McGuinness et al., 2022).They affect multiple aspects of physiological condition through immunological, hormonal, and neurological pathways; in fact, certain individuals refer to the gut microbiota as a "virtual organ" (Clarke et al., 2014). Approximately 1014microorganisms exist in the gastrointestinal tract (GIT) reported by Human Microbiome Project (HMP) and Metagenomics of Human

Intestinal Tract (MetaHIT) (Singh et al., 2022).Firmicutes and Bacteroidetes are the two phyla and Verrucomicrobia, Proteobacteria, Actinobacteria and Fusobacteria speciesthat dominate the balanced proportions of the human GIT microflora(Martin et al., 2018). In past few years, gut microbes have drawn more attention among researchers, and several articles have been published on numerous diseases related to microflora such as irritable bowel syndrome, Crohn's disease, musculoskeletal diseases, neurodegenerative disorders, diabetes, obesity and colon cancer (Zhu et al., 2021). Correspond, studies shows that Actinobacteria, Firmicutes, Proteobacteria and Bacteroidetes are the most common microorganisms in the human the gastrointestinal tract, contributing for around 3%, 64%, 8%, and 23% of the overall microbial population, respectively (Zheng et al., 2020).Therefore, a number of studies are investigating the connections between gut microbiota and different diseases with the aim of developing novel diagnostic and treatment strategies (Lynch & Pedersen, 2016).

Chronic degradation of specific a group of neurons is an indicator of neurodegenerative diseases (Peilin et al., 2021). The main reason behind of the origin of neurodegenerative disease is environmental factors such as diet, lifestyle choice and xenobiotics exposure as well as genetic and aging is another key factor that influence the progression of neurodegenerative disorders (Dugger & Dickson, 2017). The emergence of accumulated proteins, such as amyloid-beta (Ab) and alpha-synuclein (a-syn), is a characteristic of the most common neurological disorders and significantly influences disease pathogenesis (Soto & Pritzkow, 2018). Surprisingly in the genesis of neurodegenerative diseases, the gut microbiota can communicate with these variables and function as an intermediary between the environment and the host. According to recent reports, gut microbes are thought to be crucial elements which communicate with both the extrinsic environmental factor and the central nervous system (CNS) (Jacob et al., 2021).Gut microorganisms use the sympathetic and parasympathetic nervous systems (SNS and PNS) as communication channels (Mohajeri et al., 2018). The diverse range of gut microbes influences both brain

functioning and behavioural patterns.The communication channel between gut microfloraand brain, known as gut-brain axis (Wang et al., 2021). Its communication held through vagus nerve, hypothalamic-pituitary-adrenal (HPA) axis, tryptophane metabolism, immune system, peripheral nervous system, endocrine signalling pathway (Gubert et al., 2022). Through these pathways metabolites derived from gut microbiota including short-chain fatty acids (SCFA), gamma aminobutyric acid (GABA), norepinephrine, serotonin, histamine, kynurenines, etc., control a number of cerebral physiological functions in the brain (Kennedy et al., 2017; Rooks & Garrett, 2016). When the intestinal microflora composition get imbalance (i.e. dysbiosis) the brain receives signalswhich further contribute to the pathological mechanisms of various neurological diseases, especially neurodegeneration, by manifesting as low-grade inflammation, increased oxidative stress, disrupted energy metabolism, and increased cellular degeneration (Mulak & Bonaz, 2015; Noble et al., 2017). However, it has also been documented that major changes in the composition of the gut microbiota occur with human aging, and the aging gut also exhibits a decrease of microbial diversity (Cheng et al., 2013; Friedland, 2015).Additionally, the GI tracts of older people with neurological diseases showed a significant change in the composition of their microbiota(Claesson et al., 2011). Therefore, researchers suggested that changes in the human gut microbiota could be one of the underlying causes of neurodegenerative diseases.

In this review suggest aim to investigate the convergent mechanism in between neurological disorders and gut microbes, and focusing on remarkable biomarkers for a novel therapeutic approach in order treat, diagnose and prevent the neurodegenerativediseases and also to compile the knowledge currently available on the several approaches for gut microbe identification and counselling.

1.1 The Gut-Brain Microbial Axis: A Bidirectional Communication Pathway Between the Brain and Intestinal Microflora

The bidirectional communication between gut microbes and the central nervous system is known as the microbiota-gut-brain

axis (Veiga-Fernandes & Pachnis, 2017).Through hormones, immunological signals, and neurons, the brain can exchange information with intestine (Ceppa et al., 2020).Once in the bloodstream, microorganisms synthesize neuromodulators such serotonin, short-chain fatty acids, host-derived vitamin B12, and choline (Nishiwaki et al., 2020).With the aid of G protein-coupled receptors, short-chain fatty acids like butyrate and acetate are crucial for altering brain behaviour (Mirzaei et al., 2021). These fatty acids play a major role in energy supply, production of regulatory T-cells (Treg) and activation of trophic factors, and simultaneously maintaining the unity of blood brain barrier and intestine barrier (Dalile et al., 2019; Vogt et al., 2017). It influences various mechanism including a) development and stimulation of the glial cells of brain to remove any unnecessary connections between synapses or neurons (Batarseh et al., 2016), b)upregulation of anti-inflammatory cytokines and Treg cell production (Cattaneo et al., 2017), c)reduction of neuroinflammation by preventing monocytes, macrophages, and dendritic cells from maturing and differentiating, which lowers their capacity to generate pro-inflammatory cytokines including TNF-α and IL-12 (Sampson et al., 2016), d)maintaining the blood-brain barrier's (BBB) integrity via maintaining the quantity of protein at the tight junction (Shi et al., 2018), e) Neurotrophic factors, neurotransmitters, and neuropeptides in the brain: homeostatic regulation (Foster and Neufeld, 2013), f)maintenance of comprehension and persistent memory function in the brain through histone acetylation-mediated gene expression regulation(Albenberg and Wu, 2014).Immune cells in the GI tract establish cytokines, which travel through peripheral arteries to the brain and stimulate the hypothalamic-pituitary-adrenal (HPA) system. Cortisol is released by the HPA axis and regulates a number of brain processes (Breit et al., 2018). This signalling pathway helps macrophages attach to the α-7 nicotinic cholinergic receptor, which inhibits TNF-α and other cytokines that promote inflammation (Costantini et al., 2012). Additionally, SCFA regulated the expression of 5-HT (5-hydroxytryptophan) and tryptophan hydrolase enzymes (TPH1 &TPH2) which is responsible for serotonin production (Lepage et al., 2013).

Quinolic acid and kynurenic acid is a major metabolite derived by kynurenine pathway where tryptophan is related Albenberg and Wu, 2014). Quinolic acid acts neurotoxically by attaching to the NMDA receptor, while kynurenic acid exerts neuroprotective effects by antagonistically binding to both the NMDA and the α-7 nicotinic cholinergic receptor (Li et al., 2014). Through the action of the enzyme tryptophanase, gut microbes also control these pathways and affect how tryptophan is converted to indole derivatives (Figure 2) (Goyal et al., 2020). These derivatives control the expression of inflammatory genes by acting on the aryl hydrocarbon receptor (Gulaj et al., 2010).

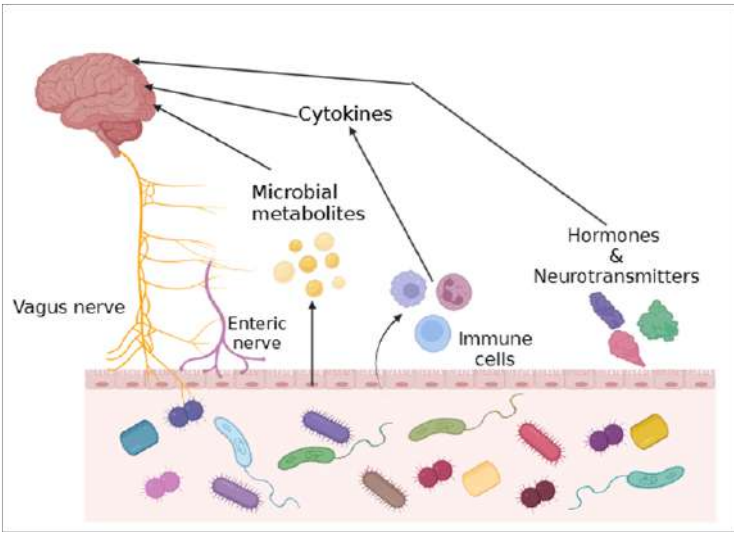

Fig 1.2: The pathways are involved in the Neurological disorders (Image :BioRender)

The direct stimulation of enterochromaffin cells to generate neuropeptides (such as neuropeptide Y, cholecystokinin, GLP-2, peptide YY, glucagon-like peptide-1, and substance P) or neurotransmitters (such as serotonin, GABA) by enteric bacteria and their byproducts (such as SCFA) can affect central nervous system function (Strandwitz et al., 2019). Hence, Myenteric neurons are directly activated by neurotransmitters

and neuropeptides generated from bacteria. These neurons then convey nerve signals to the brain via ascending fibres of the vagal nerve (Kaul et al., 2024).Eliminating the intestinal epithelium gives the gut bacteria and their byproducts the chance to enter and come into direct contact with VN, which activates the vagal afferent fibres via particular receptors or more straightforward means (Han et al., 2022). To trigger nodose ganglia (NG), Edwardsiella tarda can attach to the transient receptor potential ankyrin A1 (TRPA1) receptor, which is abundantly distributed on NG (Nodose Ganglia) (Strader & Woods, 2005).Through toll-like receptors (TLR) on their surface, enteroendocrine cells can identify bacterial compounds in the intestinal lumen, like LPS, or use other receptors to identify microbial metabolites, like SCFA. They can then send intestinal signals to vagal afferents via synaptic connections using glutamate as a neurotransmitter, or they can release more than 30 known peptides to stimulate vagal afferents (Ye et al., 2021). By activating enteroendocrine cells through the receptor transient receptor potential ankyrin A1 (TRPA1), Edwardsiella tarda can release the neurotransmitter 5-hydroxytryptamine (5-HT), which directly stimulates vagal sensory ganglia. When enteroendocrine cells get isovalerate stimulation, their olfactory receptor 558 can function as a sensory receptor for microbial compounds(Wu et al., 2021). The transmission of intestinal signals is then achieved by the production of 5-HT by enteroendocrine cells, which binds to the 5-HT receptors on vagal afferents (Bellono et al., 2017).

1.2 Role of gut microbiota in neurodegenerative and neuropsychiatric diseases:

Amyotrophic Lateral Sclerosis:

The progressive neurodegenerative disease, amyotrophic lateral sclerosis (ALS) is typified by the degeneration of motor neurons (Kaul et al., 2024).This eventually causes paralysis, muscle weakness, and breathing failure (Jain et al., 2023). A small number of ALS cases are inherited due to mutations in genes like SOD1 and C9orf72, but the majority are random (Kaul et al., 2024). Activated glial cells, impaired axonal transport, and improper protein folding all contribute to neurodegeneration in ALS (Jain et al., 2023).

In ALS, Bacteroidetes and Firmicutestwo phyla ratio shown decreased, reported by Zeng et al., 2020 & Zhai et al., 2019. Recent studies discovered that ALS patients had higher levels of Prevotella copri and lower levels of eight prominent butyrate-producing bacteria, such as Eubacterium rectale and Roseburia intestinalis (Nicholson et al., 2021). Also, it revealed that OTU10032 unclassified Acidaminococcaceae and Enterobacteriaceae were linked to an increased incidence of ALS (Zhang et al., 2022). Another study demonstrated alterations in the microbiota composition of ALS patients, with a decline in the relative abundances of Streptococcaceae and Ruminococcaceae in the patients, while the prominent families in healthy controls were Bacteroidaceae and Succinivibrionaceae (Ozaydin et al., 2024). Guo et al., 2024 revealed that microflora derived metabolites accumulation cause the ALS.This research may improve the reliability of putative biomarkers and provide mechanistic understanding of when ALS develops.According to a study by Davis and colleagues, cyanobacteria, or blue green algae, are connected to ALS and can cause the disease due to toxins in the algae blooms (Torbick et al., 2014). They suggested that the cyanotoxin b-N-methylamino-L-alanine (BMAA) would cause a similar pathology because microglial activation and protein aggregation in motor neurons are recognized to be the main pathology accompanying this disease. Degeneration of motor neurons was observed in BMAA-dosed vervets due to reactive astrogliosis, activated microglia, TDP-43 proteinopathy in anterior horn cells, and damage to myelinated axons in the lateral corticospinal tracts 9 (Hertzberg et al., 2022). Interestingly, vervets administered BMAA þ L-serine showed fewer neuropathological alterations, indicating that long-term nutritional exposure to BMAA results in pathological alterations of the ALS/MND type within the vervet and that coadministrationof L-serine lowers the degree of development and motor cortex damage (Kaul et al., 2024).

Parkinson Disease:

The primary neuropathological biomarker that emerged in Parkinson's disease was the accumulation of α-Synuclein in the stomach, nerve fiber, and ganglial α mucosa and

submucosa (Rai et al., 2017). Through the vagus nerve, the intestine's synuclein protein contributes to communication between the gut and the brain (Fitzgerald et al., 2019). Parkinson's disease is most consistently associated with changes in the gut microbiota. There has been a decrease in bacteria belonging to the genus Faecalibacterium and an increase in the populations of bacteria belonging to the genera Lactobacillus, Bifidobacterium, and Akkermansia (Romano et al., 2021). There were fewer Parkinson's disease patients with Lactobacillus, Bacteroides, Prevotella, and Bifidobacterium, among other helpful microorganisms. In addition, patients with Parkinson's disease had higher populations of harmful bacteria, including Enterobacteria, Streptococci, Staphylococci, Shigella, and Helicobacter (Vamanu & Rai, 2021). Additionally, the microorganisms in the gut affect the generation of dopamine, the buildup of α-synuclein, oxidative stress, local inflammation, intestinal permeability, and constipation (Doroszkiewicz et al., 2021).Through reducing pro-inflammatory nuclear factor-kB (NF-kB) signalling, the Pirin-like protein found in B. thetaiotaomicron's anti-inflammatory effect shields epithelial cells (Singh et al., 2022). Additionally, an increase in Lactobacillaceae and a decline in the population of Prevotellaceae in the gut microbiome are linked to lower levels of the gut hormone ghrelin, which controls the activity of nigrostriatal dopamine (Caputi & Giron, 2018). The bacteria Prevotella controls the intestinal layer's mucin production process. As a result, the Parkinson's disease patient is disturbed by the intestinal production of mucin.Moreover, Parkinson's disease patients' microbiomes are characterized by a decrease in butyrate-producing bacteria like Blautia, Coprococcus, and Roseburia and an increase in proinflammatory Proteobacteria, which may lead to inflammation-induced misfolding of alpha-syn (Singh et al., 2022).

Alzheimer disease:

Alzheimer's disease (AD) is a multifaceted, intricate illness that includes both neurodegeneration and persistent neuroinflammation (Goyal et al., 2020). The accumulation of amyloid beta (Aβ), hyperphosphorylated tau proteins, and

neurofibrillary tangles (NFTs) in brain tissue are pathogenic characteristics of AD (Zhu et al., 2020).Microbiota-derived compounds in patients' cerebral spinal CSF make gut microbiota a dynamic element in the aetiology of disease (Zhu et al., 2020).Both amyloid and lipopolysaccharides (LPS), which impair the gastrointestinal tract's permeability and the BBB's function, are produced when the gut microbial population experiences dysbiosis (Kowalski & Mulak, 2019). Amyloid molecules can be detected and eliminated by microglia's TLR4 and TLR2 receptors. The activation of nuclear factor kappa B and TNF α is caused by the production of a signal of myeloid differentiation main response 88 (MyD88) by microglial TLR2. Aβ is also stimulated by αMyD88 through the upregulation of β-secretase and -secretase (Kaul et al., 2024). The gut microflora distribution has been visualized in **Table 1.**

Schizophrenia:

Schizophrenia is a diverse neurodevelopmental condition that results from an accumulation of environmental risk factors throughout gestation and after delivery, as well as a complex and dynamic bidirectional interaction of genetic expression (Kelly et al., 2020).Remarkably, GI comorbidities are common in schizophrenia patients, and they also have a greater frequency of intestinal barrier failure and increased bacterial translocation (Socolata et al., 2021). Additionally, the chornic schizophrenia individuals had significantly increased level of the genera Lactobacillus and Prevotella and the orders Clostridiales, Lactobacillales, and Bacteroidales. In the phylum level, Proteobacteria and genus level Succinivibrio, Megasphaera, Mogibacterium,Corynebacterium, Collinsella, Clostridium, Klebsiella, and Methanobrevibacter, Anaerococcus, Ruminococcus and Eubacterium were shown an increased abundance whereas, Bifidobacterium spp., Escherichia coli, Lactobacillus spp., Haemophilus, Sutterella, Clostridium,Adlercreutzia, Anaerostipes, Ruminococcus and Faecalibacterium of abundance in microbial diversity were decreased in schizophrenia patients (Socolata et al., 2021).Notably, the degree of symptoms associated with schizophrenia was linked to Succinivibrio and

Corynebacterium, which could offer some new biomarkers for the diagnosis of schizophrenia (Li et al., 2020).

Table 1: Distribution of gut microbiota in neurological disorders.

Neurological disease	Gut microflora distribution		References
	Higher expression	Lower expression	
Parkinson's Disease	*Oscillospira, Ralstonia, Serratia, Enterobacteriaceae, Bifidobacterium, Akkermansia, Lactobacillus, Butyricicoccus, Clostridium, Escherichia coli, Helicobacter pylori*	*Shigella, Lachnospiraceae, Faecalibacterium, Coprococcus, Blautia, Prevotellaceae, Roseburia.*	Singh et al, 2022; Kaul et al., 2024
Alzheimer Disease	*E. coli, Bacteroidetes, Salmonella, Bacillus subtillis, Streptomyces coelicolor, Staphylococcus aureus, Bacteroidetes, Ruminococcacea, Blautia, Prevotella,*	*Eubacterium rectale, Firmicutes, Bifidobacterium, Firmicutes, Ruminococcacea, Lachnospirace, Faecalibacterium*	Wang et al., 2022; Heravi et al., 2023;
Amyotrophic Lateral Sclerosis	*Methanobrevibacter, Strptococcus salivarius, Citrobacter, Coprococcus, Lactobacillus, Ruminococcaceae, Sphingomonas, Erysipelotrichaceae, Atopobiaceae.*	*Eubacterium eligens, Bilophila, Eubacterium rectale, Oscillibacter, Roseburia, Firmicutes, Lachnospiraceae, Anaerostipes, Fecalibacterium, E. coli, Megamonas, Butyrivibrio fibrisolvens.*	Kaul et al., 2024; Singh et al, 2022

Schizophrenia	*Prevotella, Veillonela, Shigella, Escherichia coli, Megasphaera, Eggerthella, Lactobacillus, Alistipes, Proteobacteria.*	*Streptococcus, Haemophilus, Bacteroides, Roseburia, Coprococcus, Ruminococcus, Bifidobacterium*	McGuinness et al., 2022
Huntington's Disease	*Actinobacteria, Bacteroidetes, Proteobacteria*	*Firmicutes, Deferibacteres.*	*Zhu et al., 2021*
Multiple Sclerosis	*Patescibacteria, Methanobrevibacter, Akkermansia muciniphila, Bacillusfusiformis (Caproic acid producer)*	*Lachnospiraceae, Ruminociccaceae, Provotella, Fecalibacterium, Clostridium, Oscillibacter.*	Wang et al., 2022; Doroszkiewicz et al., 2021
Autism	*Clostridium, Lactobacillus, Bacteroidetes*	*Coprococcus, Provotella, Firmicutes*	Doroszkiewicz et al., 2021
Bipolar Disorder	*Desulfosporosinus, Desulfotomaculum, Desulfovibrio, Methanobrevibacter, Bifidobacterium, Oscillibacter, Enterococcus, Strptococcus, Flavonifractor*	*Faecalibacterium, Ruminococcus*	McGuinness et al., 2022

2. Implication of Microbiota- gut axis in neurological diseases:

Role of Gut Microbes as Potential Biomarkers:

The relationship between neurodegenerative diseases and the gut microbiota may lead to new avenues for early detection and therapy. According to recent studies, changes in the composition of gut bacteria and metabolite profiles could

serve as markers for neurodegenerative diseases (Jain et al., 2023). This study concludes that many bacteria (at phyla level or genus level) share common abundance in different neurological disorders. It might be higher possibility to applied them as per diagnosis and to build an early prevention method. In order to genus level Prevotella has been decreased in PD, AD, ALS, MS, HD, autism, bipolar and schizophrenia. It is implied as a biomarker for all different neurological diseases. Additionally, the increased level of Megasphaera, Eggerthella, Lactobacillus, Escherichia, Shigella, Veillonella were note as a biomarker for neuropsychiatric disorders whereas decreased level of Roseburia, Coprococcus were also reported (McGuinness et al., 2022).Incase of PD and AD, the abundance of Akkermansia, Bifidobacterium, Ruminococcaeae, Verrucomicrobia, Allistipes, Lachnospiraceae bacterial communities sever as a biomarker for diagnosis purpose (Heravi et al., 2023). Therefore, the relation between gut microflora and brain possesses several pivotal roles in diagnosis, early treatment, and prevention of neurological diseases.

3. Emerging and Future Therapeutic Approaches:

Complex disorders associated with neurodegenerative illnesses typically encompass motor, systemic,and cognitive dysfunctions. Their pathogeneses are thought to be influenced by both genetic and environmental factors, with gut microbiota potentially playing a role. Although there is little evidence, the manipulation of intestinal microbiota by FMT may influence the possible symptoms of diseases through metabolic, endocrine, immune, and neural pathways mediated by intestinal microorganisms. As a result, it may be a viable treatment opportunity for a number of neurodegenerative and neuropsychiatric conditions. Recent studies reveal that biopeptides play a pivotal role to treat the neurodegenerative disease by improving the gut dysbiosis. The impair arrangement of gut microflora has been influenced by the biopeptides.Low molecular weight peptides often display superior structural stability and increased bioavailability in the stomach. Gly-Pro-Hyp and Pro-Hyp, for instance, were able to penetrate the intestinal cell monolayer and remained

stable in simulated gastrointestinal fluid for two hours. These characteristics, such as intestinal permeability and stability against further enzymatic hydrolysis, are crucial for ensuring the functional performance of peptides. As for example, walnut, sesame cake and soyabean peptides suggest a beneficial approach to treat the AD, PD in human (Wu et al., 2021).These peptides regulate gut homeostasis in the initial phases of CNS ailments, which can help cure the unfavourable signs associated with neurodegenerative diseases. Other than that, probiotics and prebiotics intake, fecal microbiota transplantation are also involved to prevent and treat the gut-microbiota's distribution in neurological diseases.

Conclusion:

Since, the ratio of pollution and highly refined food intake was increased day by day, neurological diseases have become emerged as the most common adolescent life-threatening illnesses. A precise coordination of gut microflora is required for optimal health, according to a multitude of research, and any disturbance of this equilibrium may result in neuropsychiatric disorders. In this review, describe the correlation between gut microbes and neurodegenerative and neuropsychiatric disease, and their various pathway involved in gut-brain axis, which modulation led to various neurological diseases. Also, this study summarizes the biomarkers present in both neurological disorders provide a suitable treatment approach as per futuristic possibility.As previously reported, peptides have the ability to control gut microbiota, which may play a useful part in daily dietary intake to fend against neurodegenerative illnesses. Additionally, it offers insight into whether changes in a single gut microbe impact host immunity.

CONFLICT OF INTEREST: There is no conflict of interest related to the study.

AUTHOR CONTRIBUTIONS: Acquisition and interpretation of data is done by Priti Nandi. Conception, design and revising of the article are done by Dr. Bidisha Ghosh.

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NON-CODING RNAs, AS BIOMARKER AND AS A THERAPEUTIC MODALITY FOR COLORECTAL CANCER

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ABSTRACT

Non-coding RNAs (ncRNAs) have been a matter of interest since time of their discovery in late 1950s. There are various types of ncRNAs available like the micro RNAs, long non-coding RNAs (lncRNAs), small nucleolar RNAs (snRNAs) and Piwi Interacting RNAs (piRNAs). They itself do not code for any proteins but plays a vital role in progression of many fatal diseases by their dysregulation. Similar case is also for colorectal cancer, a life-threatening disease that comes as a bane with mal-lifestyle practices. Various ncRNAs are often abundantly found in different colorectal cancer patient samples like the miR-17-92, miR25, H19, Xist, SNORD44, circRNA-000014, whose hyper production promotes colorectal cancer cell growth, metastasis and cancer cell survival. These biomolecules act as the biomarker for the disease as they are very particular with their implication in colorectal cancer. So makes an advance opportunity for faster diagnosis of the patients.Another novel approach to suppress colorectal cancer is via ncRNA mediated gene silencing. The target mRNAs like the miR25, MALAT-1, SNORD44 have oncogenic activity and are related to the advent of colorectal cancer in the patients which can be silenced by application of complementary therapeutic ncRNAs thereby progression

of the disease can be effectively controlled. Recent studies even focus on role of Probiotics as potential anti-CRC agents as these are strains of gut microbes whose active metabolites has been proven to supress CRC progression with greater efficiency than many of the modern medications.

The review focuses on joint approach towards diagnosis and cure of colorectal cancer, an emerging world problem with the ncRNAs as diagnosis biomarker mediated by RNA-seq read mapping, real-time RT-PCR and eventual suppression of the dysregulated ncRNA via ncRNA mediated gene silencing and intake of potential Probiotics.

Keywords:*Colorectal Cancer, Gene Silencing, Non-Coding RNAs, RNA Biomarkers,Probiotics*

Introduction

Non-coding RNAs are the type of RNAs that do not code for proteins, yet play a significant role in regulating gene expression and other cellular functions. First Non-coding RNA was discovered by George Palade and Paul Zmecnik in mid 1950s.Their dysregulation has been implicated in various diseases, including cancer.These are often identified to be disease biomarkers for several carcinomas and often being used as diagnosis monitor to predict the progression of the disease in the patients.There are multiple variants of non-coding RNAs available such as the Micro RNAs, Long Non-coding RNAs, Small Nucleolar RNAs and Piwi Interacting RNAs(John S Mattick, Igor V Makunin.,2006). These have an impressive role to play as potential cancer therapeutics and Carcinoma Biomarkers(Yokugawa Y et al.,2015). Recent implications of circular non-coding RNAs in cancer therapy have been a revolutionary work as it kills the major drawback that was arising with the use of therapeutic linear non-coding RNAs as the former are covalently closed in nature and are resistant to cellular nuclease resulting in increase of their half- life.Dysregulation of non-coding RNA refers to the phenomenon of abnormal expression, processing or functioning of ncRNAs which leads to severe and chronic diseases and even loss of cell cycle, arrest of cellular check points leading to tumorous outgrowths and ultimately cancer. Over and under expression of ncRNAs causes mutations

leading to loss of vital functionality of the cell or even gain of new functionality leading to cellular abnormalities.This may even cause epigenetic modifications like changes in DNA methylation and Histone modification.Over the timencRNAs have been under strict observation as their abnormalities can be a triggering point for any kind of diseases , and abnormal behavioural patterns of ncRNAs serves as the first ever alarming symptom for cancer as this starts happening at a very early period before the progression of the disease to its fatal nature, thus considering ncRNAs as biomarkers for life threatening diseases like cancers helps easy and early diagnosis of the patient so that proper treatment could be given to prevent further progression of the disease. Recent studies with the virtue of RNA-seq read mapping revealed that upregulation of four long non-coding RNAs namely CRCAL-1, CRCAL-2, CRCAL-3, CRCAL-4 have been seen in case of colorectal cancer and so the above colorectal cancer associated long non-coding RNAs can be used as perfect biomarkers for the disease(Atsushi Yamada et al.,2018).

Using ncRNAs as an effective molecular tool for gene silencing have been used since date of its discovery.This process effectively subdues the targeted gene by the mechanism of complementary hybridization, this means the ncRNAs are selected such that they are complementary to the target mRNA sequences to silence the effect of the corresponding gene, this binding of the ncRNA to the target mRNA is due to presence of complementary nitrogenous bases and referred as RNA hybridization.This acts as a novel anti-angiogenic targeted therapy.(A Mulkeen et al.,2004).

Recent research has revealed the fact that probiotics have strong impact in suppression of CRC even with better efficiency than any other modern medications. These are actually strains of human gut microbiome whose active metabolites cause cytotoxicity to CRC cell lines (Hold GL et al.,2016).

Application of thementioned processes paved a life-saving path towards faster diagnosis and treatment of the colorectal cancer patients. Chronic nature of the disease that can make its way into the body due to certain mal practices like smoking,

unhealthy diet, obesity, alcohol consumption etc that comes together with the modern lifestyle. Curing of the disease becomes a vitality to suppress the adverse effect of the bad lifestyle choices and save millions of lives annually.

ncRNAs as biomarkers for colorectal cancer

Biomarkers are the molecular tools to make a birds view over the occurrence and progression of a disease. Studies revealed that there is pivotal relation with over expression of particular ncRNAs that comes with the advent of a particular disease as a result monitoring the abnormalities of these molecules could make out a gradual relation to the occurrence of the disease and can be used as a detection tool. Previously colonoscopy was the only reliable method for the detection of colorectal cancers but eventually this is avoided due to its high cost and longer time consumption. Hence need of biomarker discoveries for better and faster diagnosis of the disease with greater proximity and yet cost cutting. Proteins are being used these years as biomarkers for colorectal cancers.

M2PK, MMP9 and RBP4, TFF3 are the important protein biomarkers that have been found profusely in the patient stool and blood serum samples respectively(Alexandre Loktionov et al.,2019). Another such biomolecule that has high potential to be a biomarker for colorectal cancer are the ncRNAs. There have been extensive research showing that over expression of certain ncRNAs are associated with the advent of the colorectal cancer. The case of their over expression is suspected to be the hypoxia condition that is inducing the cell to become cancerous (Hsiao-Mei Chao et al.,2022).Some of the pseudogene that are produced by some ncRNA that epigenetically silences tumour suppressors one such example is a pseudogene derived from lncRNA DUXAP10 that promotes colorectal cancer growth by suppression of p21 and pTEN(Yifan lian et al.,2017). Apart from siRNA, miRNA and LncRNA biomarkers there are also piRNAs.The piRNAs are produced from pre-piRNAs by a dicer independent process, such piRNA whose up regulation is seen in case of CRC are piR-1245 and piR-823(Mandana AmeliMojarad et al.,2022). Some of the vital ncRNAs that have shown over expression in the colorectal cancer patients have been listed below in Table

1 along with their sample sources and types of those ncRNAs are mentioned.

Table 1: List of vital ncRNA, their biomarkers with the source

Slno.	Biomarker	Type of ncRNA	Source sample	Reference
1	CRCAL-1,2,3,4	lncRNA	Colorectal neoplasms	Atsushi Yamada et al.,2018.
2	CRNDE	lncRNA	CH16 of CRC	Jie Ding, Juan Li et al.,2017
3	CASC9	lncRNA	HT-29 and SW680 cell line	Hui-Zi Li et al.,2021
4	BLACAT1	lncRNA	Blood serum	Alexandre Loktionov et al.,2021
5	MiR-92a	miRNA	CRC cells	Xiaoya Luo et al.,2011
6	piR-1245	Piwi-interacting RNA	CRC cells	Mandana AmeliMojaradet al.,2022
7	Circ-HIPK3	circRNA	Urine	Mandana AmeliMojaradet al.,2021

Detection of these ncRNAs in high amount in the mentioned sample of the suspected colorectal cancer patient are to be done via RNA-seq read mapping and their dysregulation can be further validated using real-time RT-PCR(Atsushi Yamada et al.,2018). This process of patient diagnosis has been found cost effective and less time consuming which paved out crucial opportunity for the patient to proceed with the treatment.

mRNA silencing approach to suppress Colorectal Cancer

In the contemporary period, gene silencing has evolved to be a novel tool for suppression of deadly diseases like the colorectal cancer. Any type of therapeutic application needs a biological target at molecular level which is to be controlled. Non-coding mRNA mediated gene silencing is a process in which neutral non-reactive ncRNAs are produced that are complementary to the target such that when they are placed into the same environment with the target which is specifically an mRNA, binds to it by complementary base pairing and as a result the effect of the very specific gene whose transcribed product is the targeted mRNA, is suppressed. These ncRNAs are produced by the action of an enzyme called dicer from double stranded RNA and get loaded over their target by the virtue of RISC complex, that means RISC helps in location and proximation of the target mRNA(Gregory J Hannon.,2002).

Studies successfully demonstrated the therapeutic anti-colorectal cancer activity of the ncRNAs as in case of a lncRNA derived pseudogene DUXAP10 that was initially used as diagnosis biomarker from HCT116 and SW480 cell lines, but later studies revealed its biological role in colorectal Cancer cell proliferation and metastasis as it is associated with knocking down of p21 and pTEN tumour suppressors. Knocking down this lncRNA DUXAP10 using DUXAP10 siRNA led to higher apoptotic levels in the CRC cells incomparison to the control cell line which was not transfected with the mentioned siRNA(Yifan lian et al.,2017). Another lncRNA upregulation was noticed in case of CRC that is lncRNA CASC9. On silencing this lncRNA CASC9 in HT-29 and SW680 cell lines using si-CASC-1 or si-CASC-2, CRC cell proliferation and growth was hampered. This was because on silencing CASC9 depletion in cyclin D1 and CDK2 was noticed that are associated to G1 cell cycle arrest(Hui-Zi Liu et al.,2021). Another Long non-Coding RNA whose deregulation causes CRC is CRNDE that is located on human chromosome 16.Knockdown of this lncRNA using si-CRNDE 1 and si-CRNDE 2 increased apoptotic levels in CRC cells. It was also experimentally proven that down regulation of lncRNA linc-00152 by miR-376c-3p miRNA restricts viability

of CRC cells(Jie Ding , Juan Li et al.,2017).Circular ncRNA are one of their kind, discovered in1990s.The are synthesized without intervention of dicer enzymes, by following ways : Exon reverse splicing into loops(exonic circRNA,ecircRNA), intron-preserving transcript reverse splicing (exon-intron circRNA, elcircRNA), and intron reverse complementary pairing (circular intronic RNA, ciRNS). Two of the circRNA namely hsa_circ_0066631 and hsa_circ_0082096 are seen to be up regulated in CRC stem cells and targets and downregulates CRC stemness miRs such as miR-382, miR-548c-3p and miR-579(Xiangyu Jian et al.,2020). NUSAP1(aberrant nucleolar and spindle associated protein 1) is a highly conserved protein in humans and recently its upregulation has been specifically noticed in case of CRC cell lines such as CACO2, LoVo, LS274T and SW480. On silencing the mRNA responsible for NUSAP1 production by NUSAP1 siRNA, adequate lowering in cell growth and proliferation of CRC cells is noticed (Guoda Han et al.,2019). A lncRNA PANDAR that was previously suspected only as a biomarker for CRC, have shown a peculiar phenomenon that when silenced jointly by PANDAR siRNA 1,2 and 3 increased apoptotic rates in CRC cells even at relatively low doses of Curcumin, a drug use to treat CRC. This serves as a joint therapy in combating CRC.

Combined Therapy for Combating CRC: ncRNAs and Probiotics

The review has enlisted the role of ncRNA in CRC therapy both as a diagnosis biomarker and as a molecular tool to silence dysregulated villain molecules and thus are proven to be a boon for controlling CRC cell proliferation and metastasis. This have been proven to be much cost cutting yet latest and more the most effective tool to replace orthodox methods of cancer diagnosis and treatment such as colonoscopy and chemotherapy. Studies even revealed that probiotics plays a significant role to supress CRC cell proliferation as these are always naturally directed into the gut. Probiotics are practically strain of gut microbes that comes along with the food we intake and exerts a positive impact to our health. The positive role of probiotics has been confirmed as they demonstrated that active or inactive strain

of probiotic like Lactobacillus acidophilus and Lactobacillus casei have high apoptotic capacity over 5-fluorouracil (5-FU) of CRC cell line LS513 (Baldwin et al.,2010). Not only the live or inactive bacteria but also the cell supernatant of probiotic strains L.casei and L.rhamnosus can successfully supress CRC progression. CFS of above the mentioned strains were proven to significantly reduce HCT-116 cell invasion. Inhibitory activities were not found when HCT-116 cells were exposed to commensal bacteria Bacteroidesthetaiotaomicron CFS. The active molecules were detected in the 50-100kDa and >100kDa fractions of Lactobacillus strains' CFS. The research established that L. casei and L. rhamnosus GG secretory metabolites have anti-invasive effects on HCT-116 cells (Escamilla J et al.,2012). Further research identified that L. johnsonii BCRC17010 was an effective probiotic strain with high adhesion capacity and proapoptotic process induction and lactate dehydrogenase release in HT-29 cells and efficiently suppressed the growth of HT-29 cells (Chen et al.,2017). Methanolic extract of secreted metabolites of Pichia kudriavzevii AS-12 (MEPK) was found to be cytotoxic to Caco-2 and HT-29 cells and the cytotoxicity in HT-29 cells was found to be equivalent to that of 5-FU. BAD, caspase-3, caspase-8, caspase-9, and Fas-R expression increased, whereas the expression of Bcl-2 was inhibited in MEPK-treated cancer cells. The expression level of proapoptotic genes (caspase-3, caspase-9, Fas-R in HT-29 cells, and Fas-R in Caco-2 cells) was greater in MEPK treated cells compared to 5-FU treated cells (positive control). According to the findings, the MEPK can be regarded as an effective anti-colorectal cancer agent (Saber et al.,2017).

Conclusion

Colorectal Cancer is the third largest cancer in the world leading to millions of deaths every year. Classical treatment of colorectal cancer like other cancers are very unpredictable and tedious with colonoscopy, chemotherapy and other modern medication. Advent of RNA therapy to combat CRC paved a way towards faster diagnosis and better suppression of CRC in the patients. This can be also conducted on severe Chemo-resistant patients creating another chance for their

survival. ncRNA biomarkers have been identified to be the most valid and efficient tool for cancer diagnosis. Targeting the ncRNA biomarker have increased the efficiency of the ncRNA mediated gene silencing therapy in CRC cells. Apart from these probiotics have been also proven to be a magic supplement in controlling cancer cell proliferation. These foods are often easily available and are not majorly subjected to cause any allergic reactions in the patients. The active compounds of probiotics even showed better anti-cancer effect that many modern medicines. This review linked up all the possible ways to combat CRC sustainably in a less cost-effective way expecting to promote awareness and to make cancer treatment within the means of common folk. Diagnosis using ncRNA biomarkers and cancer control using ncRNA mediated gene silencing and Probiotics have potential to be the most effective combined therapy to CRC saving millions of lives globally.

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COMBINATIONAL THERAPY OF NANOPARTICLES WITH ANTIBIOTICS& ANTIMICROBIAL PEPTIDES TO INHIBIT BIOFILM FORMATION

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Abstract

Biofilms are intricate communities of bacteria grouped together in a matrix that works against the effects of antibiotics and against the immune system of its host. Some of the most severe diseases occur due to the formation of resistant microbial biofilms. Most of the conventional antibiotics have become redundant due to the emergence of antimicrobial resistance that has rendered their inability to be used as a sole drug to be used as a therapeutic module. Recent advancement in the benevolent approach of nanomedicines has led to the minimization of overuse of antibiotics. One possible approach in treating these chronic diseases that come as an excellent platform for repurposing conventional drugs is to combine established antibacterials like antibiotics with nanoparticles. This technique has been successful in the removal of notorious biofilm associated with a number of pathogenic diseases. The latest developments in the use of nanoparticles used in conjugation with antibiotics and in certain cases with antimicrobial peptides for the possible eradication of microbial biofilms. This review thus focuses on the recent advancements in these synergistic combinational drug

therapies that can be used as a broadspectrum antimicrobial and as an effective strategy to meet antimicrobial resistance.

Keywords: *Antibiotics, antimicrobial peptides, biofilms, drug-resistance, nanoparticles.*

1.INTRODUCTION

Over the last few decades, rising bacterial infections, particularly those caused by antibiotic resistant bacteria, have emerged as a serious threat to global public health. Within the last century, these antibiotic-resistant bacteria have begun manifesting both intrinsic resistance to antimicrobial agents and acquired resistance from other strains through mutation and gene transfer. A number of bacterial strains termed "superbugs" have emerged due to these mutations and selection for years of antibiotic therapy, becoming complex challenges to manage. As older antibiotics used to treat these bacteria become less effective, infections caused by these resistant strains are becoming life threatening. In order to solve this issue, the use of nanoparticles that can be used as delivery agents such as liposomes, polymersomes, or solid lipid NPs as well as effective antimicrobials on their own such as metal and metal oxide NPs, has increased in hopes of improving existing antibiotics. Most of the modern antibiotics target bacteria using a single mechanism. On the other hand, metal and metal oxide NPs which possess intrinsic antibacterial activity are suggested to treat bacterial infections by employing several mechanisms simultaneously. While recent evidence suggests that the generation of reactive oxygen species (ROS) is a common contributor to cell killing downstream of drug-target interactions, antibiotics, particularly bactericidal drugs, typically cause cell death by inhibiting DNA replication (quinolones), RNA synthesis (rifamycins), cell wall synthesis (β-lactams), or protein synthesis (macrolides). However, through ion release, physical interactions, and ROS formation, metal and metal oxide nanoparticles can concurrently target intracellular targets as well as cell walls. This is why, the bacteria find it considerably more difficult in resisting nanoparticle antibacterial treatment (11).

When traditional antibiotic therapy is used, it is particularly challenging because many internal bacteria are dormant or latent. These bacteria are capable of undergoing periods of quiescence where they are alive, but non-culturable, which makes them able to survive for extended periods of time. Therefore, how susceptible they are to antibacterial drugs is affected due to drug targets being downregulated and cellular permeability changing drastically. Microorganisms in damaged tissues are protected by multiple biological structures that surround the infection foci. Under pathological conditions, these adhere to the tissues and show their protective qualities by secreting glycocalyx, which causes enhanced protection and increased resistance to antibacterial drugs(7).

The increase in antibiotic resistance is just as alarming as the infections caused by implanted devices. Biofilm forming microbes usually cause infections related to devices. It is estimated that 80% of hospital-acquired infections involve biofilms. A phenomenon called quorum sensing happens when the population of microbes on the surface of the host is high because there is sufficient cell-to-cell communication to trigger the expression of genes that lead to biofilm formation (11). Antibiotic resistance of biofilms in medical implants and catheters is one example of how the failure of traditional antibiotic therapy has resulted in worsening conditions worldwide. Because biofilms dynamically alter theirExtracellular Polymeric Substance(EPS), they are resistant to a variety of antibiotics (6).Perhaps these chronic infections can be treated with nanoparticles-infused anti-biofilm to combat the microbial biofilm(1).

Novel nanotechnology-based antimicrobials have recently been employed to target biofilm formations, antibiotic resistant species and planktonic bacteria. Antibacterial agents, along with these novel techniques, have become increasingly effective (1). Hydrogels, liposomes and even polymeric particles are micro- and nanocarriers that are being researched for the treatment of bacterial infections due to their ability to encapsulate and distribute therapeutic substances over an extended period of time. Polymeric biodegradable

nanoparticles(NPs), based on the biocompatible poly (lactic–co- glycolic acid) PLGA, are being researched in our lab as drug delivery vehicles. The great advantage of these NPs is that they can be produced with a variety of methods, most of which are readily scalable, making it possible to encapsulate many different compounds with a wide range of chemical and physical properties (3). The chronic nature of intracellular infections, even with advanced antibiotics in the market, are often not treated completely. Amid these challenges, emerging therapeutic approaches like loading antibiotics into liposomes, nanoparticles, and colloidal carriers have been utilized to improve contamination of infected cells. Hence, we will discuss and elaborate the issue concerning the limitations and pharmacological advantages of these particulate carriers for the targeted antibiotic therapy of intracellular bacterial infections (7).

Antimicrobial peptides, also known as cationic host defense peptides, are a very diverse class of tiny proteins with a range of amino acid counts. Numerous synthetic AMPs have been created in labs, but in addition to those that are naturally present in plants and animals, a vast array of AMPs are also made by bacteria and yeast. In addition to their anticancer and anti-inflammatory qualities and their capacity to function as immune modulators, AMPs have been shown to engage in a range of biological activities, such as antibacterial, antiviral, antifungal, and anti-mitogenic agents. AMPs are therefore a viable substitute for a large range of widely used medications. The majority of the research that is currently accessible also shows that AMPs have therapeutic effects in both in vitro and in vivo settings(16).

2. BIOFILM

Biofilms are collections of microorganisms (including bacteria, protozoa, algae, and fungi) that stick to surfaces and are encased in a very moist matrix of extracellular polymeric substances (EPS). It is estimated that around ninety percent of microorganisms in the world are structured in biofilms that are found in surface water, lakes, underwater bodies, and land- based ecosystems. They are also found in ecological niches that undergo extreme pH, chemicals like biocides and

antibiotics, as well as hydrodynamic stress, osmotic pressure, temperature, and pressure. Different forms of biofilms such as marine or subaerial biofilms are critically important for a variety of small-scale ecosystems(4). Bacteria that can develop biofilms have a higher tolerance against antibiotics and other antimicrobial treatments. Bacterial biofilms are thought to present the biggest problem, it is said to be from the increased degree of aminoglycoside resistance to macrophage and antimicrobial agents. Research has shown that the polysaccharide matrix within the biofilm, which anchor the whole group, is the main source of this type of bacteria. When used, the biofilm alters osmosis for the better. Furthermore, within the biofilm, oxygen levels and rate of nutrient supply differ, and so do the bacteria's metabolism and growth rate. Cell division consequently proceeds at substantially slower rates, resulting in persister cells that are extremely resistant to a range of antimicrobial treatments (2).

2.1. Biofilm Formation

• **Planktonic bacteria attachment:** -In aqueous circumstances, planktonic bacteria have the ability to adhere to surfaces. This includes transport mechanisms like motility and diffusion, environmental influences including organic conditioning films, and bacterial surface features like curli and fimbriae. Weak, non-specific interactions characterize initial attachment, which is reversible. Biofilms form when adhesion is stronger and more permanent.

• **EPS Production:-** Cyclic di-GMP, or c-di-GMP, is a signalling molecule found in bacteria that mostly controls the production of biofilms. A sequence of reactions occurs inside the bacterial cell when the bacteria detect changes in their surroundings, such as temperature, pH, or the availability of nutrients. In the end, this results in c-di-GMP production. High c-di-GMP levels encourage the production of biofilms. It facilitates bacterial adhesion to surfaces and promotes the synthesis of extracellular polymeric substances (EPS), a matrix that protects the bacteria by encasing them in a biofilm.

• **MicrocolonyFormation:-**The critical stage in the development of biofilms is microcolony formation. Bacteria

multiply and create tiny, three-dimensional clusters known as microcolonies after first adhering to a surface. By promoting cell-to-cell contact and the synthesis of the extracellular matrix, which offers structural support and protection, these microcolonies form the basis for the mature biofilm structure.

• **Biofilm formation: -** Microcolony proliferation during biofilm maturation results in the formation of a larger, three-dimensional structure because of the creation of EPS. Antimicrobial resistance and biofilm protection are improved by this EPS. In contrast to planktonic bacteria, the mature biofilm exhibits unique and varied gene expression.

• **Dispersion: -** Dispersion is the last stage of biofilm formation. The life cycle of the bacterial biofilm is completed when some unattached bacteria attach to a new surface and create a new biofilm after the biofilm has matured(5).

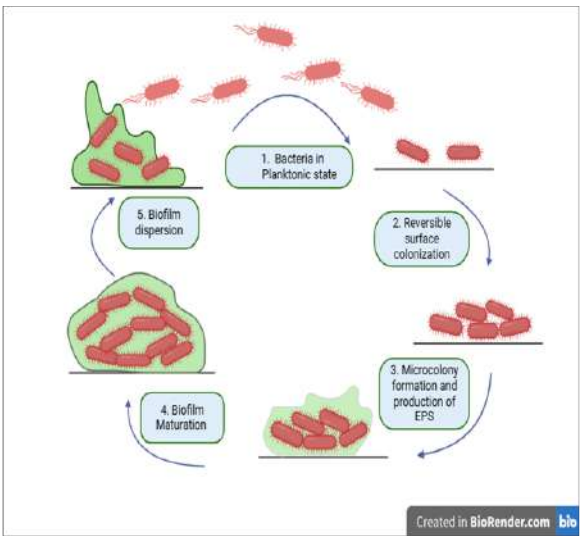

Fig. 1: LIFE CYCLE OF BIOFILM FORMATION

3. NANOTECHNOLOGY

Nanomedicine and other areas of nanotechnology have provided new possibilities in the diagnosis and therapy of a wide variety of diseases. Because of their size and surface

properties, nanoparticles can cross biological barriers (5). In addition, there is a great deal of research in various NPs for its potential antibacterial activity. For instance, metallic oxide NPs like CuO-NPs and ZnO NPs have been shown to be low in cytotoxicity while being highly effective as drug delivery systems. Numerous studies have shown that shape, surface charge, and many other factors can significantly affect the anti-microbial and anti-biofilm efficiency of a nanomaterial. It is worth mentioning that ZnO-NPs are also better in terms of solubility, bioavailability, and biocompatibility(2).

Some nanoparticulate **drug delivery carrier system's** function will be covered in detail in the sections that follow.

3.1. Solid lipid Nanoparticles

As nanoparticulate drug delivery devices, solid lipid nanoparticles were developed. Triglycerides, glycerides, fatty acids, steroids and waxes were the constituents of these nanoparticles.

The advantages of these nanocarriers are

- Control drug release
- Targeted delivery
- Improved drug stability
- Biocompatibility and
- Simple scale-up process (1).

According to the recent studies, the applications of this nanoparticlesas a carrier of the compound with anti-biofilm activity are discussed here –

- Researchers assessed the in vitro anti-biofilm properties of SLNs loaded with rifampin against Staphylococcus epidermidis, which produces biofilms. They demonstrated that these nanoparticles could decrease biofilm biomass in a manner that was dependent on both time and concentration (1).
- In vitro anti-biofilm activity against Staphylococcus aureus biofilm was evaluated after the formulation of

clarithromycin-loaded solid lipid nanocarriers (CLA-SLNs). When compared to free drug, the results showed that CLA-SLNs had superior anti-biofilm activity in biofilm eradication at lower drug concentrations (1).

- Rifampin and cis-2-decenoic acid were recently added to SLN formulations, and anti-biofilm properties were evaluated against staphylococcal biofilm at both the creation and eradication stages. The formulation can be used as an effective nanoparticulate system with potential antibiofilm activities, as evidenced by the superior anti-biofilm activities that were observed (1).

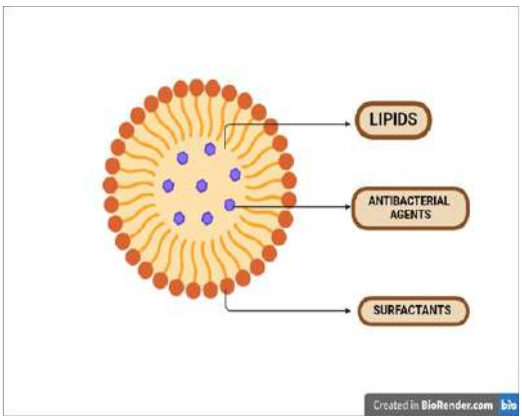

Fig. 02:SOLID- LIPID NANOPARTICLE

3.2. Polymeric Nanoparticle

Drug delivery systems widely incorporate the use of polymers. The polymeric carrier systems are primarily comprised of hydrophilic and hydrophobic segments. The hydrophobic segment of the molecule predominantly acts on a polymeric core which holds the drugs in place. While Elimination of the core is efficiently done by the hydrophilic segments and it is also responsible for the degradation of the core. These systems also present an opportunity to infiltrate hard to reach areas such as biofilm and super dense tissue (1).

Hydrogels

Hydrogels are cross-linked, hydrophilic copolymers or homo polymers which are highly biocompatible and form polymers of low or high degree of composition. These types of delivery systems are made up of natural or synthetic polymers in a three-dimensional network. These polymers can apply in the infusion of antibiotics owing to their unique net structure. For the controlled and sustained release of some antibiotics, they can take in and retain large volumes of water while exhibiting mass swelling characteristics (1).

Despite these benefits, the delivery of antibiotics via hydrogels has shown limited success; as a result, a few studies have concentrated on the delivery of antimicrobial hydrogels based on polymers -

a. In one investigation, the hydrogel/silicone composite made of cross-linked poly (2-hydroxyethyl methacrylate) embedded in a poly (dimethylsiloxane) silicone elastomer was sustained after ciprofloxacin was loaded into it. The antibiotic's release profile was noted. The danger of bacterial colonisation and subsequent biofilm formation on the device material was decreased by coating medical devices with hydrogel containing antibiotics (1).

b. Anderson et al. investigated polymeric hydrogels composed of crosslinked poly (2-hydroxyethyl methacrylate) (pHEMA), surface modified with octadecyl isocyanate, and loaded with norfloxacin. The results of this study showed that not only zero-order releasing profile of drugs be achieved from these hydrogels, also the antimicrobial activity of norfloxacin was improved to eradicate both planktonic and biofilm forms of S. epidermidis from medical implant surfaces (1).

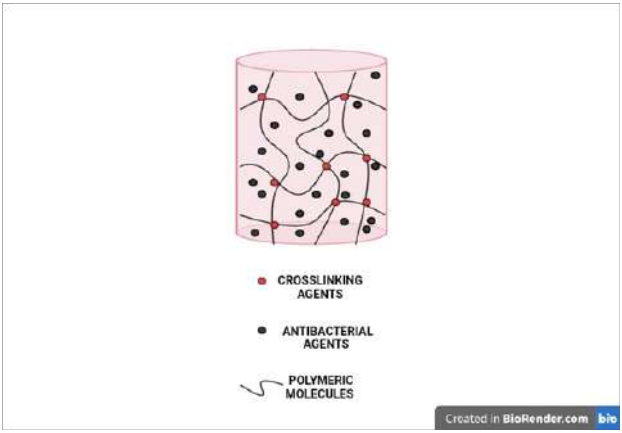

Fig. 03: HYDROGEL MATRIX

An overview of the various lipid-based carriers used to deliver antibacterial drugs to biofilm structures is given in this section of the article. These carrier systems were depicted, and Table 1 included some significant data(1).

Table 01: The lipid-based drug delivery systems to biofilm.

Type of carrier	**Encapsulated agent(s)**	**Active against**	**Advantages**
1. SLN	Clarithromycin	*Staphylococcus aureus*	• High potential for eliminating biofilms • cellular biocompatibility and characteristics of reduced acute toxicity
2.SLN andNLC	Levofloxacin and DNase type I	Pseudomonas aeruginosa	• Reduced biofilm formation

3. Liposomes	Vancomycin	Staphylococcus aureus	• Fusogenic liposome-encapsulated vancomycin exhibits improved biofilm penetration.
4.Hydrogels	Norfloxacin	S. epidermidis	Targeted drug delivery • Sustainedrelease of the antibiotic • Improved tissue compatibility • The potential for sustainedtherapeutic effects • Controlled drug release

SLN :Solid LipidNanoparticle

NLC : Nanostructured Lipid Carrier

4. SilverNanoparticle:

The silver cationic nanoparticles pose a promise in battling bacterial infection linked with burns and wounds. Silver nanoparticles have shown promise for kitchen as an antibacterial adjuvant for treatment of several infectious diseases due to the synergy that they have with other antibiotics such as ampicillin and vancomycin. The results obtained by the joint efforts against some Gram positive and negative bacteria including Streptococcus pneumonia, P. aeruginosa, S. aureus, and Shigella flexneri, demonstrate Ag-NP's anti biofilm and antimicrobial activity. (1).

Ag-NPs have been shown in numerous surveys to be useful in reducing infections linked to biofilms. The synergistic effect of Ag-NPs and curcumin NPs (Cur-NPs) against S. aureus and P. aeruginosa, which are Gram-positive and Gram-negative bacteria, was examined by Loo C-Y et al. A phenolic plant extract called curcumin has anti-biofilm qualities by

weakening the quorum sensing systems of bacteria. When Ag-NPs and 100 µg/ml Cur-NPs are combined, 50% of bacterial biofilms may be disrupted(https://doi.org/10.1016/j.jddst.2020.101880).

But from several studies Ag-NPs has been showing several disadvantagesthat are listed below:

- Cytotoxicity that might restrict usage in therapeutics
- Silver nanoparticles cause the central nervous system to become inflamed by releasing pro-inflammatory cytokines and producing reactive oxygen species (ROS) and nitric oxide.
- Additionally, the cytotoxic effects of silver nanoparticles on the circulatory, respiratory, osteoclast andosteoblast systems may limit their usage in biomedical applications(2).

But according to recent research by Panacek et al., following repeated exposure, Gram-negative bacteria such Escherichia coli 013, Pseudomonas aeruginosa CCM 3955 and E. coli CCM 3954 become resistant to silver nanoparticles (11).

5. Zinc Oxide Nanoparticle:

A broad range of NPs are being evaluated for their antibacterial properties. For example, metallic oxides NPs like copper oxide (CuO-NPs) and zinc oxide (ZnONPs) are unique because they have low cytotoxicity and enhanced drug delivery properties. It has been shown that a lot of parameters, for instance shape and surface charge, can affect a nanomaterial's antibacterial and anti-biofilm properties (2).

ZnO-NPs in particular possess a wide range of benefits including soluble, bioavailable, and biocompatible. Thus, ZnO-NPs can reside within numerous biological systems, mimic the function of biomolecules, and maintain the equilibrium of cells. Because ZnO-NPs are less expensive and is of lower toxicity than other nanomaterials, they are more likely to be utilized for the treatment of diabetes, inflammation, cancer, and infection. ZnO-NPs are differed from other nanosized materials by their catalytic effectiveness

combined with superior adsorption capacity and chemical stability (2).

Evaluation of the impact of ZnO-NPs and antibiotics together on the formation of S. aureus biofilms. The test tube approach was used to examine the synergistic effect of ZnONPs and tested antibiotics on biofilm inhibition against S. aureus ATCC 6538. The findings indicate that ZnO-NPs by themselves could reduce the production of biofilm by 34–37%. However, a 65–85% inhibition of biofilm formation was seen when ZnO-NPs and different antibiotics were combined (2).

Characterization of ZnO-NPs-induced suppression of S. aureus biofilm formation under a microscope: ZnO-NPs were further studied, with emphasis on their effect on S. aureus biofilm, through SEM and light microscopy. S. aureus biofilm was grown on polystyrene discs with 0.78 mM ZnO-NPs (64µg/mL), gentamicin (2 µg/mL), and control, and was compared to the untreated cells. The structure of S. aureus biofilm was illustrated using a photograph captured on the microscope at different magnifications. Untreated cells displayed in a dense fashion at higher magnification (>5000x) showed evidence of a thick biofilm. After the treatment of either gentamicin or ZnO-NPs there was a significant decrease in biofilm formation which resulted in minimal biofilm mass on the polystyrene discs. Microscopic examination of the infected surfaces revealed the presence of a scattered single layered biofilm of bacteria of 1–2 cell thickness with clumped cells in multilayered structures. Higher magnification revealed the cruder form of untreated S. aureus (>10000x) which showed monolayer thick dense clumps. Whereas after treatment, both organisms appeared to have turned into single cell structures resulting in a smooth uniform cell surface. The combination treatment of gentamicin and ZnO-NPs managed to halt bacterial biofilm development leading to the significant disruption of biofilm production and formation resulting in a minimal biofilm mass.

6.Gold Nanoparticle

The range of surface plasmon resonance (SPR) exhibited by Ag, Au, Pt, Zn, and Cu green synthesized metal nanoparticles

is unique, and versatile, with Au-NPs demonstrating a distinct anti-biofilm activity against clinically relevant multi-resistant enterococcal strains. Augmented antibacterial, antimalarial, anti-viral (anti-HIV), anti-tumour, and anti-biofilm effects have been accredited to Au-NPs. Au-NPs, synthesized with the use of MPPE, were tested by Vijayakumar S. et al. for the anti-biofilm activity against Gram-positive Enterococcus faecalis, a multiple antibiotic resistant (MARS) bacterium (1).

It was also reported by Giri K et al. that the surface charge of Au-NPs is responsible for the antibacterial activity against S. aureus and P. aeruginosa, the major pathogens in ventilator associated pneumonia (VAP) and also important in the destruction of biofilms. The growing importance of Au-NPs as anti-biofilm agents can be attributed to the ease of surface functionalization (1).

Table 02 lists the antibacterial characteristics, potential antibacterial mechanisms, and key variables influencing the antibacterial activity of other metal and metal oxide nanoparticles, including gold nanoparticles, iron oxide nanoparticles, and zinc oxide nanoparticles. These NPs have also been thought to be effective antimicrobials (11).

Table 02 : Nanoparticles and nanomaterials used for antibacterial applications.

Nanoparticle	**Mechanism of Action**	**Antibacterial properties**	**Active against**
1.Ag-NPs	o ROS production o silver ion release o membrane integrity disruption	Antibacterial activity against Gram-negative bacteria is superior to that against Gram-positive bacteria.	o S. aureus o p.aeruginosa

2.ZnO-NPs	o ROS generation o Zinc ion release	Good antibacterial activity against both Gram-positive and Gram-negative bacteria	o S. aureus o p. aeruginosa
3. GoldNPs	o ROS generation o disruption of membrane integrity o suppression of rRNA binding to the ribosomes	High stability, nontoxicity, simple surface modification, photothermal therapy, and effective antibacterial qualities	o Enterococcus faecalis

7. Antimicrobial Peptides:

Small molecular weight proteins have a wide range of antimicrobial properties against bacteria, viruses, and fungiknown as antimicrobial peptides (AMPs).The molecule can be soluble in aqueous conditions and penetrate lipid-rich membranes because these evolutionarily conserved peptides are often positively charged and possess both hydrophobic and hydrophilic sides (12).

There is great potential for using AMPs as novel antibiofilm agents, and this is a field of study that is being investigated more and more. The microbial, animal, and plant kingdoms all include AMPs, which are important elements of innate immunity.Most AMPs are amphipathic, cationic, small molecules (12–50 amino acids) with a mechanism of action that disrupts bacterial membranes.AMPs have a broad spectrum of activity, a lower propensity to induce resistance, and a high ability to target metabolically inactive cells because of the low specificity of their molecular target.Numerous investigations have shown that AMPs can disrupt different phases of biofilm formation by stopping bacterial cells from adhering to surfaces in the first place, focusing on planktonic cells before they

integrate into the biofilm structure, or eliminating established biofilms by separating and/or eliminating bacteria that are embedded in the biofilm (13).

An international summary of the most promising AMP-based combinations against biofilms is given in this article, along with potential explanations for the synergistic effect. The following combinatorial strategies are given special attention: (i) traditional antibiotics; (ii) compounds that can break down the extracellular matrix of biofilms or prevent their synthesis; (iii) compounds that inhibit QS and/or other signalling pathways involved in biofilm formation; and (iv) additional AMPs and/or peptide-based molecules. Examples of combinations where AMPs are used to combat bacterial biofilms in combination with other substances (13). By disrupting their essential biological functions, including DNA and protein synthesis, AMPs can efficiently eradicate MDR bacteria. Compared to antibiotics, their modes of action are less likely to cause bacteria to develop drug resistance. Additionally, it has been observed that AMPs can destroy bacterial biofilms once they have formed and prevent the production of the signals (ppGpp) needed for biofilm development and spread. To increase the antibacterial arsenal, a variety of synthetic AMPs with strong antimicrobial efficacy against MDR bacteria and biofilms have been created based on an understanding of the structure-activity connections of AMPs (15).

7.1.2 Chitosan

N-acetyl-D-glucosamine and D glucosamine are two monosaccharides connected by glucosidic β-1-4 bonds to form chitosan, a linear high molecular weight molecule that is produced by the alkaline deacetylation of chitin. In addition to its bactericidal effect, its biodegradability via hydrophilic enzymes is one of its most significant biological characteristics.

Chitosan has antibacterial properties because of:

- Presence of amino group in its composition
- disrupt the bacterial membrane's regular processes and cause intracellular components to seep out

• as well as the breakdown of nutrient delivery to the cell.

Chitosan's antibacterial action is only effective at pH values below 6, hence it needs to be artificially altered in order to be studied in physiologically neutral environments.

• Derivatives of chitosan have demonstrated notable bactericidal action when combined with other substances. For instance, certain bacteria belonging to the ESKAPE group can be effectively combatted by chitosan-based biocomposites containing multi-walled carbon nanotubes (MWCNT). Additionally, when chitosan and chlorhexidine are used in endodontic sealants, the antibacterial action of chitosan against Enterococcus faecalis is greatly enhanced.

• Furthermore, two efficient combinatorial approaches against resistant bacteria have been investigated; one showed that nisin, teapolyphenols (TP), and chitosan work best together to inhibit both Gram-positive and Gram-negative bacteria, while the other showed how chitosan hydrolysates work in concert with divalent metal ion-EDTA compounds. Lastly, it was shown that, when combined with the zinc-EDTA chelate, active molecular chitosan (AMC) exhibited superior inhibitory efficacy against Penicillium italicum (14).

7.1.3 Alginate

Mannuronic and guluronic acids combine to form alginate, a naturally occurring polymer. It is non-toxic to human cells and possesses exceptional biocompatibility and biodegradability qualities. By treating them with aqueous alkaline solutions, brown algae (Phaeophyceae) are the primary source of this biopolymer. The alginate salt precipitate is obtained by filtering the extract and combining it with calcium chloride. The water-soluble sodium alginate (Na-Alg or SA) is then produced by purifying the alginate salt with diluted hydrochloric acid (HCL), which transforms it into alginic acid.

• Zinc oxide-cellulose sodium alginate nanocomposite fibres (ZnO-SACNF), which have demonstrated strong antibacterial activity against E. coli, are an example of how

sodium alginate's (SA) antimicrobial qualities have recently been investigated in combination with other substances. At the same time, S. aureus and E. coli were instantly eliminated by copper alginate hydrogels in the form of microspheres.

- Moreover, the best antibacterial efficacy against Listeria monocytogenes was seen in sodium alginate film containing 5% hydroxyapatite nanoparticles (HA NPs).

- The green production of sodium alginate (SA)-silver nanoparticles (AgNPs), in which SA served as a stabilizer for AgNPs, was reported in another study. Because of their increased membrane permeability and breakdown of the bacterial wall, these NPs caused S. aureus and E. coli to undergo cell death.

- Zinc alginate films with and without 1% graphene oxide (GO) have also been created using sodium alginate, and they exhibit strong antibacterial action against S. aureus. Unfortunately, adding 1% w/w of GO had an impact on a number of mechanical properties. Similarly, films made of crosslinked alginate, hydroxypropyl methylcellulose, and ε-polylysine with minimal plasticizer content demonstrated a 99.9% reduction in bacteria in strains of S. aureus and E. coli. Because ε-PL competes for alginate chains, making the films more fragile, it should be used sparingly. Last but not least, a brand-new antimicrobial nanocomposite was created using corona-treated polypropylene (CPP) and copper oxide nanoparticles and alginate. This nanocomposite shown outstanding antibacterial activity against Candida albicans, S. aureus, and E. coli (14).

8. Conclusion and future directives:

The inability of the antibiotic targeting approach to effectively clear infected cells may be due to the development phase of the bacteria, and the inability of non-dividing bacteria to effectively respond to the traditional antibiotics. When bacteria attach themselves into the glycocalyx matrix, their resistance to antibacterial treatment increases, as earlier studies have shown. New strategies for the use of antibacterial medicines when the target bacteria are stubbornly inaccessible or in inactive states are essential. In addition, chronic carrier or

persistent infection models of Salmonella spp. can be of great assistance. The advance in the antibacterial treatment will require a mix of both genetic and biochemical skills. Perhaps a more reasonable option for enhancing the antibacterial treatment is the combination of different forms of carriers(7). One example is Linhaliq, a liposomal formulation of ciprofloxacin aimed at P. aeruginosa infections in cystic fibrosis patients, which is currently pending regulatory approval (3).

The way devices get infected with biofilm is becoming a more serious problem, caused somewhat by the overuse of antibiotics. This will lead to the future design of more efficient systems being based of the comprehension of how the nanoparticles and nanostructured surfaces interact with the bacteria and which technologies will be used to them for material science and biology (3). Although antibiotics have proven to be an effective weapon against pathogenic bacteria, it is crucial to develop new therapies for pathogens that have devised means to evade their mechanisms. New materials like polymers, nanoparticles, and AMPs provide distinct benefits compared to traditional antibiotics in the fight against different infectious diseases. It was noted, however, that certain therapies can have undesirable toxic effects on human cell lines. Only a small number of combinatorial treatments have made it to the clinic. Challenges regarding the clinical use of combination strategies include cytotoxicity effects, production costs, bioavailability, and efficacy. To address these challenges, a number of strategies have been developed, including chemical modifications, innovative delivery systems, and advanced synthesis technologies like electrospinning and three-dimensional printing(14).

This review article discusses some research studies were all the nanotechnology came into practice for drug delivery systems, especially for biofilms. It is expected that it will be used for the treatment of microbial biofilms as well. The NPs' ability to deliver the conventional antibiotics to the infection sites and increase the anti-biofilm properties makes them promising for biofilm elimination (1).

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MECHANISTIC APPROACH OF ESOPHAGEAL CANCER DUE TO MICROBIAL GROWTH

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ABSTRACT

One of the main subtypes of esophageal cancer (EC), esophageal adenocarcinoma (EAC) is a developing worldwide health issue. The incidence of EAC has been continuously increasing in spite of gains in survival rates. Although genetic factors to EAC have been extensively studied, little is known about the involvement of the human microbiome in its pathophysiology. Recent research indicates a link between changes in the microbial ecology and the advancement of EAC, with particular bacterial genera acting as possible early detection indicators. Gastroesophageal reflux disease (GERD) is a significant risk factor for EAC because it causes long-term inflammation and molecular alterations that result in Barrett's esophagus, a condition that precedes EAC. Long-term exposure to acid and bile causes Barrett's esophagus, which in turn causes cellular change via pathways involving DNA damage, oxidative stress, and resistance to apoptosis. Important genetic changes, including modifications to COX-2, p53, and cell cycle regulators, are linked to the transition from Barrett's esophagus to EAC.Depending on the stage of the disease, endoscopic resection, chemotherapy, radiation therapy, targeted therapy, and immunotherapy are among

the current treatment options for EAC. Although surgical procedures and proton pump inhibitors (PPIs) are used to treat GERD symptoms, their ability to stop the spread of malignancy is still up for discussion. Furthermore, innovative approaches to managing EAC risk include dietary changes, biomarkers for early identification, and developing treatment techniques including microbiota modification. Clarifying the molecular processes behind GERD-induced carcinogenesis, improving biomarker-based diagnostic methods, and creating treatments that target the microbiota should be the main goals of future research. Improving early detection, prevention, and individualized treatment plans requires an understanding of the complex interactions of genetic, microbial, and environmental variables in the course of EAC.

Keywords: *Barrett's Esophagus,Bile Reflux, Carcinogenesis, Dysplasia, Gastroesophageal Reflux Disease (GERD), Human Microbiome, Metaplasia, Oxidative Stress*

Introduction

Esophageal adenocarcinoma (EAC) is one of the main subtypes of esophageal cancer. While 5-year relative survival rates were low throughout the previous 20 years, the incidence rate of EAC gradually climbed. More research has been done on the mechanism of EAC in connection to genetic variables than in relation to the human microbiome. Studies on the connection between EAC and the human microbiota are now in their infancy (Dan et al.,2022).The seventh most frequent malignancy, esophageal cancer (EC), is expected to cause 604,000 new cases globally in 2020. Additionally, it ranks as the sixth most common cause of cancer-related mortality, with an anticipated 544,000 deaths in 2020(Sung et al.,2021). Except in Korea and Japan, the age-standardized 5-year net survival of EC patients ranged from 10 to 30 percent between 2010 and 2014. Between 2000 and 2014, the age-standardized 5-year net survival trends in several nations rose by 6–10% (Allemani et al.,2018).Although esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) are the two main subtypes of EC, there are notable differences between the two in terms of risk factors, regional patterns, and temporal trends (Abnet et al.,2018; Uhlenhopp et al.,2020).

One of the biggest concerns of this century is addressing the EC's worldwide load.The genetic composition of humans is almost the same. The human microbiome's metagenome exhibits more variety than the human genome(Lloyd-Price et al.,2016).The human microbiota is a very unique, intricate, and ever-changing community found in every healthy person (Consortium,2012;Gilbert et al.,2018).The human body's largest microbial habitat, the digestive tract is home to the greatest variety and quantity of microorganisms(Gupta et al.,2017;Adlerberth & Wold ,2009).There is a correlation between EC symptoms and the course of the illness. Patients may present with progressive dysphagia, persistent back or retrosternal pain, and noticeable cachexia in the latter stages, but in the early stages, they may be asymptomatic or complain of dysphagia. In early-stage disease, endoscopic treatment is frequently utilized to remove malignant material. For locally advanced disease, chemotherapy and radiation therapy are typically administered either prior to or following surgical resection. It has been determined that obesity and gastroesophageal reflux disease (GERD) are significant risk factors for EAC. Alcohol use and tobacco use may promote the development of EAC (Coleman et al.,2018;)For metastatic disease, treatment options include chemotherapy, radiation therapy, immunotherapy, targeted therapy, or a combination of these treatments to control symptoms and slow the progression of the disease (Rubenstein & Shaheen, 2015;Li et al.,2023; Dan et al.,2022). The three primary community types that make up the esophageal microbiota have been shown to differ significantly from one another. Streptococcus in type 2 and Prevotella in type 3 are the two most common genera among them. In addition to Streptococcus and Prevotella, type 1 is an intermediate between types 1 and 2, containing higher concentrations of Haemophilus and Rothia (Deshpande et al.,2018: Kaakoush et al.,2015).According to recent research, esophageal carcinogenesis is associated with changed human microbial communities and decreased microbial diversity, particularly among Enterobacteriaceae, Campylobacters, acid-producing bacteria, and periodontal pathogens (Aggregatibacter actinomycetemcomitans and Tannerella forsythia). Tannerella forsythia, Helicobacter pylori,

Prevotella, and Aggregatibacter actinomycetemcomitans can all be used as biomarkers for EAC screening and individualized precision diagnosis. The classification of the oral microbiota, the expression of biomarkers linked to HPV, and the DNA status of Fusobacterium nucleatum can all be significant prognostic indicators of EAC. Notably, new clinical treatments pertaining to the human microbiota, including as proper oral hygiene, a diet high in fiber and low in fat, exercise, PPI, mucosal protective drugs, and probiotics, may also be used to treat EC. These treatments may be very beneficial to patients (Chen et al.,2017).

Gastroesophageal reflux disease (GERD)

Esophageal cancer and gastroesophageal reflux disease (GERD) are strongly linked. Given that the target organs for GERD lack the esophageal's inherent protective mechanisms, the carcinogenic qualities of the gastroduodenal contents may also cause cancer in other organs .With an increasing incidence, gastroesophageal reflux disease (GERD) is a highly common condition. The illness is unquestionably connected to the pathophysiology of Barrett's esophageal adenocarcinoma. Given that the target organ for GERD lacks the esophageal inheritance protective mechanisms, the carcinogenic qualities of the gastroduodenal contents may also cause cancer in other organs (Herbella et al.,2015).

GERD-induced carcinogenesis mechanism:

The most researched cancer associated with GERD is esophageal adenocarcinoma, which started in Barrett's esophagus. Nevertheless, little is known about its cellular causes and molecular mechanisms (Fang et al.,2013).

Esophageal inflammation brought on by GERD causes oxidative stress, which damages DNA. bile and acid both have an effect on carcinogenic pathways. Acid causes apoptosis, reduces proliferation, and damages DNA. Bile salts produce resistance to apoptosis,damages DNA, and has a pH-dependent effect on proliferation (Fang et al.,2013;Denlinger & Thompson,2012).

Barrett's esophagus

Barrett's esophagus is intimately associated with long-term exposure to acid columnar epithelium. Theoretically, squamous epithelium could metaplasia and develop into intestinalized columnar epithelium via two separate route methods. In the first pathway, mature squamous cells undergo direct transdifferentiation into intestinal cells. On the other hand, esophageal intestinal mucosal cells might be the consequence of aberrant stem cell differentiation in the esophageal mucosa (Dvorak et al.,2011).

Male sex, advanced age, cigarette smoking, obesity with an intra-abdominal distribution of body fat, hiatal hernia, chronic GERD, and white race are risk factors for Barrett esophageal cancer. Approximately 0.25% of patients without dysplasia and 6% of patients with high-grade dysplasia are at risk for esophageal cancer each year. According to high-quality research, persons with Barrett esophagus who receive medication or surgical treatment for their GERD do not significantly differ in their risk of developing cancer. Patients with high-grade dysplasia who receive endoscopic eradication therapy with radiofrequency ablation had a much lower chance of developing cancer(Spechler, 2013).

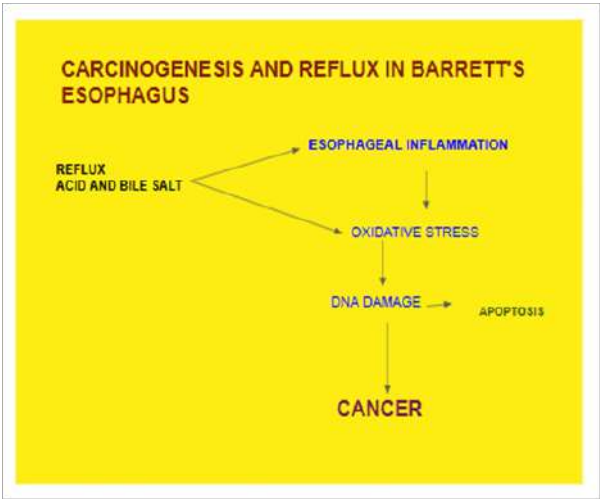
How GERD may induce Barrett's metaplasia

GERD that persists over time might lead to Barrett's metaplasia. By triggering transcription factors or generating developmental signaling pathways that establish an intestinal phenotype, elements of the refluxed gastric juice (such as acid or bile salts) and/or the ensuring esophageal inflammation (reflux esophagitis) may result in esophageal metaplasia (Wang & Souza, 2011).

Progression of Barrett's esophagus to esophageal cancer:

The risk of developing esophageal cancer is 30-150 times higher for patients with Barrett esophagus than for those without Barrett. Patients with Barrett esophagus have a 0.5% to 1.0% annual chance of cancer advancement. The molecular mechanisms underlying the metaplastic cells malignant transformation are still being understood.The idea that gastric

and bile reflux have a causal role has been validated by in vitro studies. The histologic alteration of the distal esophageal mucosa that lead to cancer when repeatedly exposed to bile salts in an acidic environment over a protracted period of time are replicated by immortalized non transformed esophageal Barrett cells cultivated in cell culture (Das et al.,2011;Denlinger & Thompson ,2012).When cells were treated for five minutes every day to glycochenodeoxycholic acid at pH of 4 . The expression of the colon-specific antigen Das-1 revealed phenotypic alteration as early as two weeks. Following 30 weeks of daily treatment, the histologic alteration showed that the cells had rounded and started to form units that resemble glands. More aggressive growth patterns were observed over 65 weeks later, and in a naked animal model, the cells developed tumorigenic properties. Numerous genes were found to become activated through analysis of the molecular processing that takes place concurrently with the morphologic alterations. Between weeks 22 and 62, the most noticeable alteration was 10-to 20-fold rise in COX-2 expression. In addition, there was a 2-fold rise in p53 depression and a 3--fold increase in TC22 expression. Furthermore, as cells evolved into a more aggressive phenotype, many factors involved in cell cycle regulation were elevated. After 20 weeks of daily bile acid exposure, ATM (ataxia telangiectasia , mutated) and ATR (ATM and Rad3-related), crucial cell cycle checkpoint regulator that stop cells from leaving values at 20 weeks showed that the following genes were elevated in comparison to untreated control cells: RB1 (2.18) , E2F1 (5.55), CCNE1 (6.56), CDC25A (8.24), BRCA1 (3.82), TP53 (2.2) and CHEK2 (2.13) (Fang et al.,2011;Denlinger & Thompson ,2012). It is still unknown if these gene products are initially responsible for the fast cell cycle progression or if the tumor's growth is the secondary cause of their upregulation (Denlinger & Thompson ,2012).



Targets that have been previously identified in other more prevalent types of carcinomas are the most clinically important molecular targets in esophageal cancer, and the significance of these proteins in esophageal cancer was later demonstrated. Clinical safety profiles provided by prior clinical studies of targeted drugs in the treatment of various cancers have made it easier for esophageal cancer to be treated with medication including VEGF, Her2/neu, and EFGR inhibitors. The development of drugs specifically suited for the treatment of Barrett's esophagus and esophageal adenocarcinomas may result from ongoing research into the particular pathophysiology involved in their development (Denlinger & Thompson, 2012).

Locating the Biomarkers for Barrett's Development into Adenocarcinoma

The clinical results of patients with Barrett esophagus may be significantly impacted by the early detection of those who are most at risk of developing adenocarcinoma or who have the illness in its early stages. As a deeper comprehension of the process behind the development of disease is created, potential biomarkers have been examined.As Barrett esophagus develops into cancer, inactivation of p16 is often observed,

which led to its investigation as a potential biomarker. The utility of p16 as a biomarker is limited because it is frequently suppressed in nonneoplastic tissue. Methylation of p16, HPP1, RUNX3, CDH13, TAC1, NELL1, AKAP12, AND SST were the only promoter methylation markers that were more common in patients who developed cancer than in those who did not, according to a retrospective double-blind study of biomarkers for esophageal adenocarcinoma. The biomarkers' respective sensitivity and specificity were 0.443 and 0.629 while maintaining a specificity of 0.9 or 0.8 (Denlinger & Thompson, 2012; Jin et al.,2009).Independent research is required to validate these findings, and the findings are not entirely reliable. They do, however, give us optimism that we will be able to create tests that would allow us to group patients based on their progression risks(Denlinger & Thompson, 2012).

Prospective therapies for GERD and Barrett's esophagus-related esophageal cancer

When the lining of the esophagus changes from its normal squamous epithelium to columnar epithelium, it is known as Barrett's esophagus. More current definitions of Barrett's esophagus stipulate that the columnar epithelium must be of the intestinal type, which means it must contain goblet cells, even if earlier definitions permitted any type of columnar epithelium (Sampliner,1998).In order to exclude intestinalized epithelium lengths that involved less than 3 cm of the esophagus, several researchers have defined Barrett esophagus according to a length criterion. Any length of intestinal metaplasia in the tubular esophagus is considered Barrett esophagus because even brief segments of intestinalized metaplasia raise the risk for esophageal cancer (Rudolph et al.,2000).Reflux symptoms are closely linked to Barrett's esophagus. Although less than 1% of people in the general population have Barrett esophagus, endoscopic investigations show that 5% to 15% of people who experience reflux symptoms for a long time will have some degree of Barrett esophagus (Menges et al.,2001; Csendes et al.,2000). Researchers have tried to stratify the risk for progression to esophageal cancer among patients with Barrett's esophagus,

as the illness may affect over 3 million people in the United States. Individuals with those risk factors may benefit from more rigorous endoscopic surveillance if trustworthy risk variables could be found. The degree of Barrett esophageal dysplasia, or precancerous cellular atypia, is currently the strongest predictive predictor. Adenocarcinoma at subsequent monitoring endoscopies is unlikely to occur in people without dysplasia, while it is more than 25% likely to occur in those with high-grade dysplasia (Reid et al.,2000). Moreover, there is a significant sample error in patients with high-grade dysplasia because of the random site of Barrett esophageal endoscopic biopsies. Up to half of the resection specimens from patients with high-grade dysplasia who have their esophagus removed show adenocarcinoma that has not been identified before. Because of this, a number of authority have recommended esophageal resection for patients with Barrett's esophagus and high-grade dysplasia. Others have proposed that people with Barrett esophagus and high-grade dysplasia undergo an intensive endoscopic surveillance protocol, with esophagectomy reserved for those who develop adenocarcinoma. They base this recommendation on the low incidence of incurable metastatic disease among those who develop cancer and are closely monitored with frequent surveillance endoscopies. To better define the best course of treatment for people with Barrett's esophagus who develop high-grade dysplasia, more longitudinal studies will be required (Schnell et al.,2001).Whether any medication, surgery, or endoscopic procedure may successfully restore Barrett's esophagus to its normal squamous epithelium is one area of growing therapeutic interest. When proton pump inhibitors were developed, doctors hypothesized that patients with Barrett's esophagus would benefit from severe acid suppression. Unfortunately, it is now known that proton pump inhibitor therapy does not completely prevent Barrett esophageal cancer from progressing to adenocarcinoma or significantly reduce the quantity of metaplastic tissue that is present. Therefore, the reduction of reflux symptoms is now the main reason for using proton pump inhibitors to treat patients with Barrett esophagus. There is no evidence to support the use of high doses of acid suppressants to treat

Barrett's esophagus patients in the hopes of lowering their risk of adenocarcinoma.It's unknown how surgical antireflux procedures relate to the management of Barrett's esophagus. 59 individuals with Barrett esophagus and no dysplasia participated in a randomized trial comparing medical and surgical antireflux therapy. Ortiz et al. found that the surgical group experienced a statistically significant reduction in the progression to any type of dysplasia, although the cancer rate decreased insignificantly. Among the 108 participants with Barrett esophagus who were monitored for an average of nearly 10 years, a randomized trial comparing medical and surgical antireflux therapy for reflux disease conducted in various US Veterans Affairs hospitals showed no statistically significant difference in the development of cancer between those treated medically and those treated surgically (4% vs. 3%). The study's sample size may have been insufficient to show such a difference, assuming one existed, according to the authors. Furthermore, a number of researchers have reported that adenocarcinoma can develop in the context of Barrett's esophagus in patients who had surgical antireflux operations months or years before the onset of cancer.Therefore, it is uncertain if surgical antireflux methods reduce the incidence of cancer, and patients who receive surgical treatment are still susceptible to adenocarcinoma.Barrett esophageal cancer risk reduction has been investigated using endoscopic ablation of Barrett esophageal regions. A number of endoscopic treatment methods, such as lasers, argon plasma coagulation, multipolar electrocoagulation, and photodynamic therapy, have been used to remove Barrett esophageal segments. It has been demonstrated that any of these methods can eliminate the metaplastic epithelium. After healing, the resulting epithelium is typically squamous when one or more of these techniques are used in conjunction with substantial acid suppression. However, islands of Barrett mucosa may remain in the treated area if the reversion to squamous epithelium is not complete. Additionally, what appears to be normal squamous epithelium may be covered with Barrett mucosa following endoscopic ablative therapy. Adenocarcinoma has been observed to grow beneath squamous mucosa in regions where there appears to be a return to squamous epithelium.

Consequently, it is still unknown how likely it is that regions of the Barrett esophagus treated with ablative therapy may develop cancer(Macey et al.,2001; Shand et al.,2001 and Van Laethem et al.,2001).These therapy approaches are now regarded as experimental, and it is unclear how useful they will be in the long run. These treatments might be most helpful for those with dysplasia who are at a higher risk for cancer, while those with Barrett esophagus and no dysplasia have a low chance of developing adenocarcinoma (Shaheen, & Ransohoff,2002).

Conclusion

Esophageal adenocarcinoma (EAC) is becoming a more significant global health concern, especially because of its correlation with Barrett's esophagus and gastroesophageal reflux disease (GERD). Even while our knowledge of genetic and environmental risk factors has advanced, research on the human microbiome's function in EAC development is still in its infancy. Potential pathways for early identification and targeted therapy are suggested by the association between esophageal carcinogenesis and altered microbial populations.

The development of Barrett's esophagus into EAC is significantly influenced by GERD, mainly due to persistent inflammation and molecular alterations brought on by acid and bile reflux. Male sex, advanced age, obesity, smoking, and persistent GERD are risk factors for Barrett's esophagus and its development into cancer. The efficiency of current treatments, like as proton pump inhibitors and surgical procedures, in halting the advancement of cancer is still up for debate, despite their goal of managing GERD symptoms.New therapeutic approaches that hold promise for improved risk classification and individualized therapy include endoscopic procedures and the use of biomarkers. To completely comprehend the molecular processes involved in the shift from Barrett's esophagus to EAC and to enhance treatment procedures, more longitudinal research is necessary. To lessen the burden of this cancer in the interim, it is still essential to lead a healthy lifestyle, manage GERD well, and regularly check on high-risk individuals.

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THE IMPACT OF SOAPS AND COSMETIC PRODUCTS ON SKIN MICROBIOTA AND IMMUNE MODULATION

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ABSTRACT

The human skin microbiota is a complex ecosystem of microorganisms that plays a major role in the maintenance of skin health and immune modulation [1], [2]. Our modern hygiene and nutrient, such as soaps and cosmetic products, are applied very frequently and directly disrupts this skin wall of equilibrium. Accordingly, consideration in the present review is given to the multilayered skin microbiome impacts of these products in terms of dysbiotic mechanisms, immune modulation, and systemic health implications. This paper integrates prominent studies with a comprehensive survey of the literature, presenting a subtly calibrated and complex relationship between microbial diversity, chemical agents, and immune pathways. In fact, this investigation indicates the need to re-evaluate current practice: microbiome-compatible formulations and personalized therapeutic approaches may mitigate deleterious effects and bolster the skin barrier against insult. The cross-field initiative must also do its due diligence to preserve that wellness as aligned with scientific verity regarding skin and microbiome research.

Key words: *Skin microbiota, Immune function, Dysbiosis, Skincare products, Microbial diversity, Skin barrier, Microbiome-*

friendly formulations

1. Introduction

The human skin is recognized as one of the most intricate and multifunctional organs metabolism-wise, acting as one of the most critical windows that links the internal body and the outside world, as well as being a changing environment for multitudes of communities of microbes collectively termed as skin microbiota (Grice & Segre, 2011; Kong & Segre, 2012). This microbiota, consisting of bacteria, fungi, archaea and viruses, is most crucial in preserving the structure of the epidermis, inhibiting pathogen encroachment as well as through immune regulation (Byrd et al., 2018).

Table 1: Composition of The Skin Microbiota

Skin Type	Actinobacteria	Firmicutes	Proteobacteria	Bacteroidetes	Fungi	Other
Dry Skin	**~50%**	**~25%**	**~15%**	**~5%**	**~3%**	**~2%**
Moist Skin	**~25%**	**~30%**	**~35%**	**~5%**	**~3%**	**~2%**
Sebaceous Skin	**~60%**	**~15%**	**~15%**	**~3%**	**~5%**	**~2%**

The physical aspects of the skin microbiome, which include genetic factors, hormonal changes, age and even lifestyle, such as the environment and skin care practices, affect the functional features of the skin (Proksch et al., 2008).

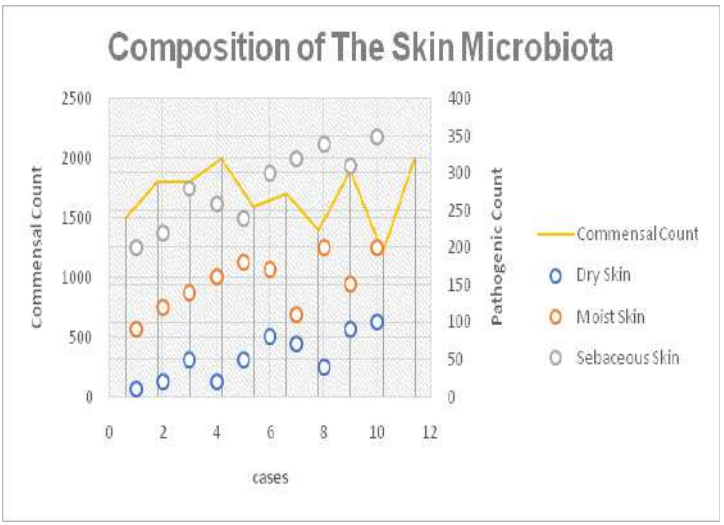
In the past decade, there was development of more and more confident concern associated to the potentially damaging effect of traditional soaps and cosmetics, as the influence of skin microbiota on dermatological, as well as, on systemic health has been found to be significant. Such products, even those meant to cleanse, protect and beautify the integument have added substances which alter the existing microbial status.

The disbalance of the microbiome caused by diminished diversity and proliferation of opportunistic pathogens has been associated with inflammatory skin conditions as well as generalized dysfunction of the immune system. These associations are important to follow in course of developing

novel strategies for the improvement of the skin and general health supporting the microbiome.

2. The Skin Microbiota: Composition and Functional Contributions

The four major bacterial phyla that characterize the skin microbiota are Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes (Sanford & Gallo, 2013). Many of these notable genera of the four phyla live in defined niches created within the varied micro-environments existing on the skin. Other essential members include the fungal populations, primarily by the genus Malassezia, as well as archaea. Bacteriophages and other viral components make the microbiota more complex (Belkaid & Segre, 2014).



The functional contributions of the constituents of the skin's microbiome are numerous and include the following:

A critical function, such as immune modulation is to be discussed. There, commensal microorganisms interact with the host's PRRs. Such forms of interaction have implications for both innate and adaptive immune responses, promoting tolerance and regulating inflammatory pathways. Thus, it ensures the correct balance in which the immune system does

not overreact to innocuous stimuli but responds to harmful pathogens appropriately (Cogen et al., 2008; Prescott & Björkstén, 2007).

One more critical function that the skin microbiome performs is its ability to reinforce the epidermal barrier. SCFAs and ceramides, two types of microbial metabolites, maintain this barrier by reducing the water loss across the epidermis. Furthermore, these metabolites strengthen the major defenses of the skin, allowing it to better endure injuries and withstand environmental stress. The structural integrity of the epidermis has to be maintained through the existence of these metabolites (Egert & Simmering, 2016).

The skin microbiome also protects the body against pathogens by inhibiting their growth and activity. In this regard, commensal microbes suppress pathogenic microorganisms by competing for nutrients with these pathogens and releasing bactericidal peptides, like defensins, which may directly kill harmful bacteria. This results in a skin ecosystem that remains balanced and less susceptible to possible infections, as it continues to act as a barrier (Grice & Segre, 2011).

Table 2: Common Skin Microbiota Phyla and Their Functional Roles

Phylum	Genera	Functional Contributions
Actinobacteria	Cutibacterium, Corynebacterium	Skin homeostasis, pathogen suppression, production of antimicrobial peptides
Firmicutes	Staphylococcus	Immune modulation, antimicrobial peptide production
Proteobacteria	Pseudomonas, Acinetobacter	Pathogen competition, inflammatory response
Bacteroidetes	Bacteroides	Short-chain fatty acid production, skin barrier reinforcement

Changes in these functions caused by factors inside or outside the body, can throw off the balance of microbes. This imbalance plays a role in skin problems that cause inflammation (like

eczema and psoriasis), makes it easier to get infections, and can affect the whole body leading to autoimmune and metabolic issues.

3. How Soap and Cosmetic Products Affect Skin Microbes

Soaps and cosmetics, while key for staying clean and caring for skin often have ingredients like surfactants, preservatives, and scents that mess up the balance of microbes on the skin (Zeeuwen et al. 2013). The main ways they do this include:

Overuse of harsh cleansers and soaps severely diminishes microbial diversity of the skin. These chemicals remove natural oils and helpful microbes, such as Cutibacterium acnes and Staphylococcus epidermidis, that are vital in keeping the skin healthy. Leyden et al. (1975) and Sanford and Gallo (2013) emphasized how such loss desynchronizes the natural ecosystem of the skin and renders it more susceptible to environmental insults as well as infections.

And once the protective microbes are eradicated, pathogenic microorganisms often occupy their niche. Without commensal microbes, pathogens that cause harm, like Staphylococcus aureus and Candida albicans, can more easily colonize the skin. The result is hypergrowth resulting in increased risk of infection and inflammatory response, further compromising the health and balance of the skin (Findley & Grice, 2014). As these on the periphery fall away or suffer from overgrowth, the chance for infectious and inflammatory responses increases, further weakening skin health and balance (Findley and Grice, 2014).

Numerous cleansers and soaps also disrupt the pH of the skin by being too alkaline. The skin's natural acidity is responsible for the balance of microbes and for maintaining the integrity of the skin barrier. Proksch et al. (2008) showed that a disruption of the skin's pH using alkaline soaps damages both the microbial community and the barrier function of the skin, reducing its capacity to defend against external insults.

Table 3: Effects of Triclosan on Skin Microbial Populations

•Microbial Genus	Control Group	After Triclosan Exposure
Staphylococcus epidermidis	20%	15%
Cutibacterium acnes	30%	22%
Candida albicans	5%	20%
Staphylococcus aureus	1%	10%

4. Case Study: Triclosan's Role in Dysbiosis

Parlet et al. Triclosan's Impact on Gut Bacteria Balance - Triclosan, a common antimicrobial ingredient in soaps is impactful in decreasing the diversity of microbes and facilitates the proliferation of micropes with a resistant gene. 2019). Prolonged use can potentially damage skin strength and encourage pathogenic contamination. certain antimicrobials, e.g., chlorhexidine, are not commonly used but have the potential to disrupt the balance of microbiota, and therefore should be carefully applied.

5. How Microbiota Dysbiosis Affects Immune Function

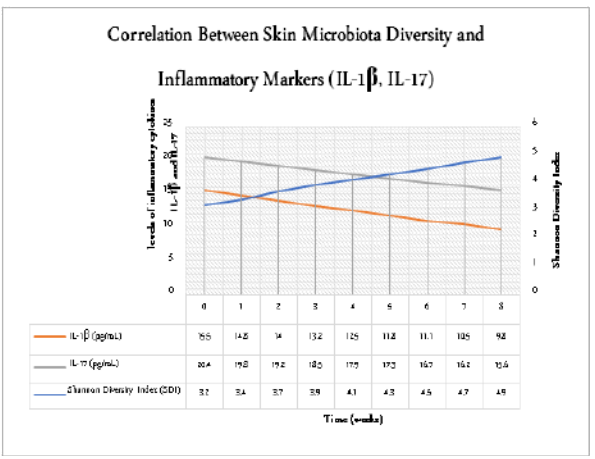
The skin microbiota and immune system work together to keep both local and overall balance. When this balance is upset, it leads to:

The immune system and the skin microbiota work in concert to achieve both systemic and local homeostasis. But, when this homeostasis is disrupted, it can have a variety of undesirable effects. Chronic inflammation is one of the byproducts. Overgrowth of pathogenic microbial communities can lead to overproduction of pro-inflammatory cytokines, including IL-1β and IL-17, which contribute to skin inflammatory diseases like psoriasis and atopic dermatitis. This overwhelming of the immune mechanisms emphasizes the need for an optimal balance required for maintaining skin homeostasis (Prescott & Björkstén, 2007; Zhu et al., 2018).

Dysbiosis of the skin microbiota also disrupts natural immunity of the skin. This derangement diminishes the level of antimicrobial peptides that are one of the key components of the skin immune defense system. When proper amounts of these peptides are not present, the skin becomes exposed to infections, as its ability to fight off harmful microbes becomes compromised (Sanford & Gallo, 2013).

Moreover, skin microbiota composition changes may have extra-cutaneous effects that are related to the development of systemic immunological diseases. These alterations have been linked to autoimmune diseases like lupus and rheumatoid arthritis and to metabolic problems. This association demonstrates that the skin microbiota is involved not only in local immunity but also in broader physiological processes that shape general health (Belkaid & Segre, 2014).

New studies point out a skin-gut connection where skin imbalance affects gut microbiota and overall immune responses. Neuroinflammatory pathways also show the two-way link between skin health and mental well-being.



6. Highlighted Studies

Dysbiosis is exacerbated by immune dysregulation and disturbances in the function of the skin barrier itself, which have been shown to be relevant in congenital ichthyosis

[116–118]. Studies by Ho et al. (2024) demonstrated that the respective effects are mediated through the JAK/STAT signaling pathway. In addition to compromising the skin's physical barrier function, the abnormal epidermal barrier may also create a permissive environment for microbial dysbiosis, resulting in increased immune dysregulation that worsens disease severity.

They are also involved in the destruction of the Tonus of skin microbiome. Studies have shown that pH-disruptive cleansers can take a toll on microbial diversity.Proksch et al. According to (2008), alkaline soaps affect the skin's natural pH and thus eliminate its potential to maintain a balanced population of healthy microbes. The skin becomes susceptible to external irritants and the general defence of the skin weakens.

Moreover, cosmetic and personal care products that often contain preservatives like parabens and sulfates have been recognized to alter the human skin microbiota. Gilbert et al. (2018) described the manner in which such chemical agents lead to suppression of commensal populations with the risk of opportunistic microbial overgrowth. This kind of imbalance can also have negative implications for overall skin health and even poses risks for infections or inflammatory responses which highlights the need for the formulation of microbiota-compatible formulations.

7. Strategies for Preserving Microbial Homeostasis

Several approaches can mitigate the adverse effects of soaps and cosmetics on the skin microbiota:

At that point, there is a need for pH balanced formulations to stabilize the skin because healthy microbial stability depends on maintaining natural pH balance of the skin. A product with a pH of around 5.5 is formulated to follow the skin's natural environment, increasing the chances of a balanced skin microbiota. This method does preserve the microbial diversity and the natural defenses of the skin (Egert & Simmering, 2016).

Table 4: Comparison of pH Levels in Various Soap Formulations

Product Type	pH Level	Impact on Skin Microbiota
Alkaline Soap	9.0	Disrupts microbial balance, increases pathogen growth
Neutral pH Soap	7.0	Moderate impact, some disturbance in microbial equilibrium
Acidic pH Soap	5.5	Supports natural skin pH, minimal impact on microbiota

A second innovative approach for improving skin health is probiotic and prebiotic skincare products. These products deliver live beneficial bacteria or nutrients to encourage these microbes to grow. Probiotic and prebiotic products improve the skin's protective barrier against intruding pathogens and its overall homeostasis by encouraging a more diverse and resilient microbial community (Belkaid & Segre, 2014). Plant-based natural antimicrobials are a more subtle way of preserving the health of skin microbiota than synthetic chemicals. And some ingredients, including tea tree oil and colloidal oatmeal, have been shown to kill the bad microbes without causing imbalances in the good ones. Unlike synthetic agents like triclosan, these natural options support the balance of the delicate skin microbial ecosystem (Parlet et al., 2019).Moderation in product use is just as important for a healthy skin microbiome. Overwashing, or using too many germ-killing products, can upset the microbial balance — risking certain skin problems. Using mutagenic cleansers and anti-microbial agents lessens the chances of imbalance of the microbiota, allowing the skin to use its natural barrier functions. This minimalist approach to skincare helps maintain a healthy microbiome long-term.

8. Future Research and Innovations

Moving microbiome-friendly skincare forward needs teamwork from different fields:

The effect of routine "cosmetic" habits on the skin microbiome

needs to be better understood, further research with a long-time assessment for the "skin health" is required.Grice and Segre (2011) considered that the daily routines and product usage should be studied over long-time periods to further analyze their influence on the microbial diversity. This research may help more adequately maintain a microbiota balance and avoid skin conditions.

Personalized skin-care products based on a person's unique skin microbiota offer a tremendous opportunity to enhance outcomes. Research by Byrd et al. (2018) noted the potential of custom formulations tailored to each person's microbial profile.

Therapeutic approaches that deliver beneficial bacteria and their metabolites to the skin are showing potential for restoring microbial homeostasis. Gilbert et al. (2018) investigated the probiotics and microbial by-products as therapeutic agents; they showed their ability to repair the dysbiosis and restore the skin condition. It is likely these treatments will form an integral part of managing microbiota-associated diseases.

Coordinating worldwide rules and standards for skin care product formulation that integrate skin microbiota is a key step in this direction. Standards will have to be created that encourage microbiota-friendly ingredients and formulations, leading to safer, more effective products. Not only would such standards drive up product quality, but they would also create consumer trust in microbiome-oriented skin care solutions.

9. Conclusion

This is, of course, a simplification of the complex relationship between skin microbes and immune function, and the efficacy of soaps and cosmetics on skin microbes and shows that we have to be aware of the microbiome when it comes to our personal care. These products are vital to our cleanliness and skin care, but they can also wreak havoc. So, we have to be mindful when we make and use them." Advances in microbiome science, plus bespoke skincare regimens, may provide new solutions for achieving skin health and general well-being.

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OPTIMIZING CAR T-CELL THERAPY: THE ROLE OF EZH2 INHIBITORS IN B CELL LYMPHOMAS

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ABSTRACT

CAR T-cell therapy has emerged as a potentially revolutionary treatment modality for refractory B-cell lymphomas, particularly in cases of DLBCL and FL. Despite huge success, more challenges are also seen in generating durable responses to the treatment approach. This article reviews the hope of EZH2 inhibitors, which can strengthen CAR T-cell efficacy. EZH2, a histone methyltransferase, is pivotal in germinal center B-cell lymphomas; gain-of-function mutations contribute to oncogenesis. Recent research indicates that EZH2 inhibition reprograms tumor cells to be more immunogenic but also modulates the tumor microenvironment by affecting the activity of regulatory T cells (Treg). Combining CAR T-cell therapy with EZH2 inhibitors is a promising avenue to overcome current therapeutic limitations.

Keywords: *CAR T-cell, EZH2, tumor microenvironment, oncogenesis, immunogenic*

Introduction

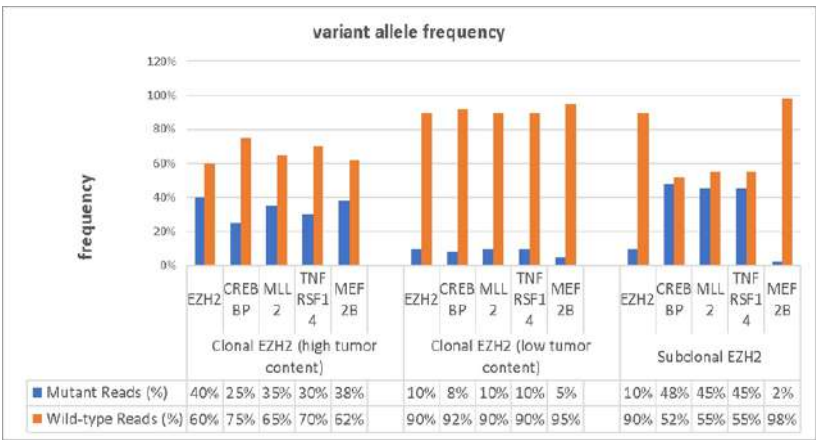
CAR(Chimeric Antigen Receptor)T-cell therapy has dramatically changed the therapeutic scenario of B celllymphomas, especially the refractory and relapsed type. CD19-targeting CAR T-cell therapies, such as CTL019, have demonstrated an impressive response rate, including durable

remissions in subsets of patients.T cells are engineered genetically, producing CARs which precisely detect and eradicate malignant cells carrying a specified antigen (Ali et al., 2016).A CAR integrates antigen-binding areas, typically a single-chain variable fragment (scFv) sourced from the variable motifs of antibodies (June & Sadelain, 2018). Unlike the traditional TCR T-cells, they don't require MHC presentation on tumor targets, which confers an anticancer advantage as the MHC-related antigen recognition is downregulated in tumor immunoevasion (Garrido et al., 2016).However, the efficacy of CAR T-cell therapy remains variable, and resistance or relapse is a huge challenge (Neelapu et al., 2017; Locke et al., 2018).About 10 to 20 percent of people who get anti-CD19 CAR T cell treatment doesn't go into remission.Loss or suppression of CD19 and/or CD22 on malignant B cells shows antigen manipulation which lets antigen exit, causing resistance to CAR T cell therapy (Shah and Fry, 2019). EZH2 is the enzymatic component found in polycomb repressive complex 2 (PRC2), which catalyses the trimethylation of histone H3 lysine 27 (H3K27me3) to repress gene expression. EZH2 functions as a principal mediator of cell cycle growth, autophagy, and apoptosis, facilitates repair of DNA damage, reduces senescence in cells, and is integral to cell lineage identification and associated signalling networks. This signifies its involvement with diseases, which includes cancer as well (Duan et al., 2020). Numerous investigations demonstrated how EZH2 can function by itself, without the intervention of PRC2 and its histone methyltransferase properties (Kim et al., 2018). It is highly involved in silencing anti-tumor genes.Somatic gain-of-function mutations in EZH2—notably Y641, A677, and A692—are common in FL and GCB DLBCL, driving lymphoma pathogenesis (Morin et al., 2010;). Targeting these mutations and regulating its function with EZH2 inhibitors like tazemetostat and GSK126 could offer a novel therapeutic approach.

EZH2 Mutations and Their Role in B Cell Lymphomas

This mutation is observed in 25% of FL cases and in 7–22% of GCB-DLBCL. EZH2 mutations increase H3K27 trimethylation, which leads to a repression of genes for tumor

suppressors, thus promoting the onset of oncogenesis (Morin et al., 2010; Bin Zhang et al., 2012; Dawson et al., 2012). EZH2 is also important in maintaining GC structure through the repression of genes in cell cycle checkpoints and plasma cell differentiation (Chénard et al., 2018). Mutant and wild-type EZH2 contribute to survival in lymphomas, and hence, patients of both categories will gain from suppression of EZH2 (Stein et al., 2018).



Frequencies of the EZH2 Mutations. The variant allele frequency of mutant and wild-type reads across different genes (EZH2, CREBBP, MLL2, TNFRSF14, MEF2B) in samples of tumors with subclonal, low, and high tumor concentration.

EZH2 Inhibitors for Enhancing CAR T-cell Therapy

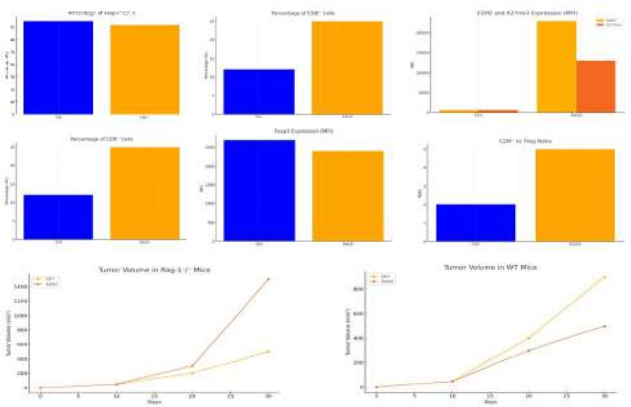
Tumor Cell Intrinsic Effects

EZH2 inhibition repositions lymphoma cells to have upregulated genes encoding for antigen presentation and T-cell engaging. Such cells become more immunogenic, rendering them more vulnerable to cytotoxicity mediated by CAR T-cells (Smith et al., 2024). Cells exhibit a substantial rise in pro-apoptotic expression of genes when treated with Tazemetostat, including genes such as BCL2L11 and BMF, and thus increasing susceptibility to immune-based attack (Morschhauser et al., 2020).

Tumor Microenvironment Modulation

Tregs are essential for immunological homeostasis but suppress anti-tumor immunity in malignancy.The suppression of Treg function by the inhibition of EZH2 has been shown to convert TI-Tregs into pro-inflammatory cells, enhancing the activity of TCR effector cells. The remodelling of the tumor microenvironment supports the recruitment and activation of CAR T cells, amplifying their therapeutic efficacy (Overacre-Delgoffe et al., 2018).

Studies has been conducted to support recent findings, saying that inhibition of EZH2 promotes the success rate of CAR T-cell therapy on B cell lymphomas. This reprograms lymphoma cells towards an over-expression of the T cell engaging gene. They accomplish this while also enhancing activity and diminishing the sign of fatigue. It increases and improves immunogenicity. Both functions synergized to allow improvement in control for tumor, shown in a few preclinical models (Isshiki et al., 2024).Intravital vision research have shown thatEZH2 inhibition increases CAR T-cell recruitment and interaction within the tumor microenvironment, improving lymphoma cell killing (Isshiki et al., 2024)



EZH2 Blockage Stops the Growth of Tumors through a T Cell-Dependent Process. *Progression graphs of MC38 tumors in Rag-1 mice infused with EZH2 inhibitors and control*

Effect of EZH2 Inhibitors in disrupting Tumor Progression.

Recently, Yan et al. (2024) found that in addition to the improvement of CAR T-cell cytotoxicity, EZH2 inhibition also suppressed the mobility of immune checkpoint molecules such as PD-L1 on the outer layer of tumor cells, implying that the synergistic use of EZH2 inhibitors along with CAR T-cell therapy and checkpoint inhibitors might produce a broader immunotherapeutic impact (Yan et al., 2024).In another preclinical study, Li et al. (2023)found that the combination of EZH2 inhibitor and CAR T-cell therapy provided better results in models with a high tumor burden. In these studies, the inhibitors of EZH2 decreased the expression of genes linked toimmune clearance in tumors, including CTLA-4 and TIM-3 pathways.

Role of Tazemetostat and Emerging Drugs

Tazemetostat is an oral inhibitor of EZH2 that has demonstrated efficacy in both preclinical and clinical and preclinical trials.It suppresses both mutant and wild-type EZH2 in follicular lymphoma. In models of B cell lymphoma, it has been demonstrated to improve CAR T-cell infiltration and activity. CYP3A breaks down tazemetostat in the liver into two main neutral metabolic products, M5 (EPZ6930) and M3 (EPZ006931) (Straining and Eighmy, 2022).Tumors subjected to tazemetostat treatment exhibited increased susceptibility to immune-mediated destruction due to elevated MHC-II molecules and enhanced antigen processing pathways(Morschhauser et al., 2020; Isshiki et al., 2024).The advised intake of tazemetostat is 800 mg taken orally twice a day, either with or without food, till progression of the disease (Hoy, 2020).Other EZH2 inhibitors, such as CPI-1205 and SHR2554, have been studied in clinical investigation for the purpose of their ability to synergize with CAR T-cell therapies. Preclinical data indicate that these agents can augment CAR T-cell expansion and persistence, which allows for long-term control of lymphoma growth (Li et al., 2023).

Clinical Advances and Challenges

EZH2 inhibitors such as tazemetostat have demonstrated efficacy in relapsed/refractory FL and DLBCL, with proven

clinically long-term responses in a proportion of patients (Morschhauser et al., 2020). The integration of EZH2 inhibitors with CAR T-cell treatment may circumvent resistance mechanisms such as antigen escape and immunosuppressive microenvironments(Chénard et al., 2018).Recent clinical studiesnamely NCT05934838, and NCT05994235 that assess EZH2 inhibition in combination with CAR T cell therapy in lymphoma patients (B cell); preliminary information coming from these will show a form of synergy promising better outcomes compared to those drugs used in treatments of resistant lymphomas (Isshiki et al., 2024).Given these advancements, uncertainties persist regarding the optimal integration of EZH2 inhibitors into CAR T-cell treatment.In order to ascertain whether synergistic interactions with other immunomodulatory drugs are feasible, it is necessary to comprehend the distinct effects of EZH2 inhibition in mutant versus wild-type tumors (Stein et al., 2018). Another challenge is the potential toxicities of combined therapy, which must be carefully evaluated in clinical trials (Yan et al., 2024).

Conclusion

In general, immuno-oncology signifies a transformative advancement in the fight against cancer, providing greater hope and opportunities for the victims and their families. Despite the transformative impact of CAR T-cells on the treatment of blood-related malignancies, numerous obstacles remain in effectively extending these drugs to solid tumors. Enhancing CAR engineering is presently the primary method to advance CAR T-cell treatment.In this context, EZH2 inhibitors constitute a viable approach for expanding the efficacy of CAR T-cell treatment in B cell lymphomas.These medicines may bypass significant obstacles to sustained responses by reprogramming tumor cells and altering the tumor microenvironment. Future research should focus on optimizing combination strategies, exploring biomarker-driven patient selection, and elucidating the long-term impacts of EZH2 inhibition on CAR T-cell functionality.

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MICROBIAL DIVERSITY IN HEAVY METALS CONTAMINATED SOIL & ITS CORRELATION WITH HEAVY METALS REMEDIATION

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ABSTRACT

Heavy metal pollution of soil has emerged as an environmental issue linked with various human-induced activities such as industrial activities and poor disposal practices. Soil microorganisms are involved in vital nutrient cycling processes, organic matter degradation, and health promotion to plants. The breakdown of organic matter, the cycling of nutrients, and the enhancement of plant health are all facilitated by soil microbes, particularly bacteria.Microbial communities are adversely affected by heavy metal stress because it decreases their metabolic efficiency and diversity.However, certain bacterial species possess several defence systems, like efflux metal ions, sequestration, and enzymatic reduction, for their survival and thus lessening toxicity of heavy metal poisoning. The mechanisms of microbial resistance are highlighted in this review, along with the causes and effects of heavy metal pollution on soil microbial diversity and their role in eliminating heavy metals from polluted soils.

Keywords: *Heavy metals, Soil microorganisms, Microbial diversity, Toxicity, Heavy metal pollution.*

Introduction

The estimation of soil microbe population density is crucial for understanding the microbiological processes taking place in the soil. The development of new, sophisticated techniques altered the ideas of microorganism biomass and population density. The highest concentration of bacterial cells is found in biotopes such as forest litter, sod, and upper humus layers, where the bacterial population is 5–10 times denser than the bacterial population found in mineral horizons[1]. Microfauna (nematodes and protozoa), mesofauna, macrofauna, and microscopic bacteria and fungi are all present in soils, making them most diverse microbial ecosystems on the globe[2]. Anthropogenic activities in industrial areas have the ability to polluted the soil in this ecosystem,resulting in substantial heavy metal pollution and being the main reason for the increase in pollutants [3-5].Industries release an extensiveamount of heavy metals,which can disrupt balance in ecosystem and harm humans, animals, and plants by altering the growth rate and soil environment[6]. Various statistical methods can be used to studythe buildup of heavy metal contaminants in the soil.The majority of contaminants are often found in the topsoil layer.The adsorption capabilities of soil particles primarily determine the amounts of contaminants in soil.A number of factors influence the solubility of heavy metal ions in soil, including moisture content, conductivity, and pH [7]. Among all the contaminants, exposure of heavy metals into soil took place through many industrial activities such as,the transportation of raw material and scrapes, metal forging, the production of alkaline storage batteries and synthetic organic compounds [8-9].

High levels of heavy metals in soil can disbalance the microbial diversity of soil.Soil microorganisms are essential componentfor soil ecosystems, as these microorganisms influence soil ecosystems through their involvement in biogeochemical cycles [10].As among all microorganisms, bacteria are the most abundant,they significantly regulate the soil environment, preserving soil structure and promoting nutrient uptake and utilisation by plants[11].Soil bacterial communities are usually highly sensitive to heavy metal stress,

which causes bacterial protein denaturation and disruption of cell membranes [12] [13].According to earlier research, heavy metal contamination disrupts soil structure and bacterial diversity[14] [15].There is a strong negative connection between cadmium exposure and bacterial diversity; although Proteobacteria,Verrucomicrobia, and Nitrospirae are sensitive to cadmium, Actinobacteria were tolerant [13].The bacterial which are tolerant to heavy metals are capable of altering the mobility and availability of heavy metals through the release of chelating agents and acidification [16]. In this review paper we are going to discuss about heavy metal contamination in soil and the impact of heavy metals pollution on microbial diversity in soil and relation between microbial diversity and heavy metals remediation.

Heavy metal pollution in soil

Heavy metal pollution is a main concern for ecosystem. Heavy metals are xenobiotic, stable, and non-biodegradable, once ingested they persist in body for longer period of time [17]. Heavy metal pollution refers to the excessive accumulation of heavy metals in soil due to human activities. These heavy metals include mercury (Hg), cadmium (Cd), lead (Pb), chromium (Cr), arsenic (As), and other metals that are biologically hazardous. Additionally, they comprise various heavy metals with specific biological toxicity, such vanadium (V), nickel (Ni), copper (Cu), zinc (Zn), stannum (Sn), and so on [18].

The soil naturally contains lead. The majority of environmental lead concentrations are caused by human activity [18]. An unnatural lead cycle has been generated due to the use of lead in petrol. Automobile engines burn lead, which produces lead salts such oxides, bromines, and chlorines. Furthermore, Pb is widely employed as a crucial industrial raw material, which causes huge quantities of solid waste containing the element to accumulate, such as in mining slag, making it a serious environmental hazard. Solid waste is frequently heaped and improperly handled, which makes it easy for heavy metal ions to leach out through rain, snow, and weathering and seriously pollute the soil. In fact, soil lead pollution has emerged as a major global environmental issue [19].Lead poisoning causes

a number of negative consequences, including: Anaemia and disruption of haemoglobin biosynthesis; elevated blood pressure; kidney damage; miscarriages and subtle abortions; nervous system disruption; and brain damage etc [18].

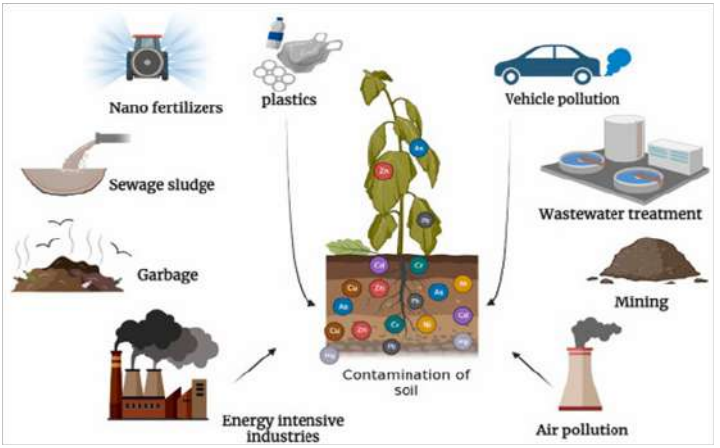

Fig 1: sources of heavy metal [20]

Microbial diversity in soil & its role

One of the most prevalent and varied biological groups in nature is the soil microbial community [21]. 1g of soil contains a variety of microorganisms, including viruses, fungus, bacteria (including actinomycetes), algae, protozoa, and archaea.Soil bacteria, the most common microbial community in soil, have great impact on biodiversity, plant health, soil texture, and the nutrient cycle [22].

As shown in FIG2, soil bacteria have the following main effects on plant growth and soil structure:

1. Bacteria contribute to the degradation of organic matter in the soil by releasing enzymes that transform organic materials into nutrients that plants can utilise. For instance, Bacillus subtilis and Bacillus licheniformiscan release several proteases and polysaccharide hydrolases. A wet cellulose bacterium called Cellulomonas uda belongs to the Cellulomonas genus

and is capable of decomposing complicated macromolecular organic molecules into simpler ones. Another common use of bacteria is to decompose organic pollutants in soil. For instance, the species Flavobacterium is capable of decomposing phenolic chemicals and bothPseudomonas and Bacillus species are capable of decomposing polycyclic aromatic hydrocarbons (PAHs). Enhancement of soil quality, purification of the soil environment, and balancing of the soil ecosystem are all facilitated by the breakdown processes of the soil bacteria [22].

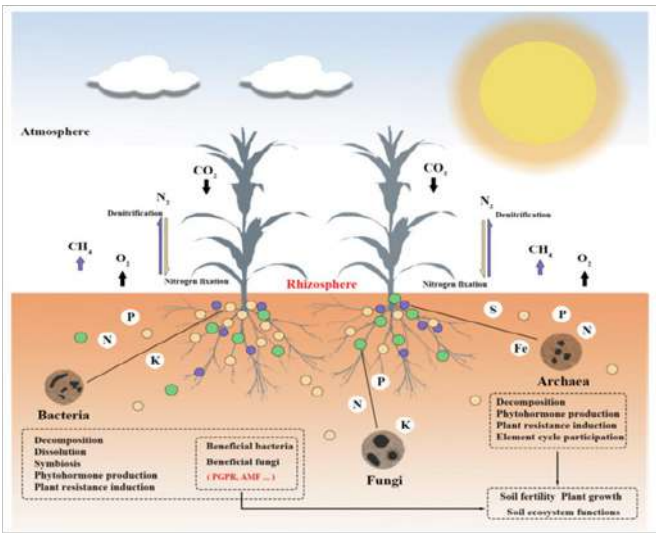

Fig2: Schematic representation of mechanisms of action of important microbial communities of soil. [22]

2. Soil bacteria facilitate the cycling of nutrients, including nitrogen, phosphorus, and potassium [22]. For example, feldspar can be broken down by Bacillus mucilaginosus Krass, which can also change insoluble potassium components into soluble nutrients [23] [24]. Some soil bacteria, such as those belonging to the genera Erwinia, Pseudomonas, Agrobacterium, and Bacillus, possess the capacity to transform insoluble phosphate into soluble phosphorus that plants can use, thus helps in promoting plant development[22].

3. Beneficial bacteria and plants can form symbiotic partnerships by providing nutrients that promote plant growth and development. For instance, phosphorus-solubilizing microbes form symbioses with plant roots, while Rhizobiumforms symbioses with legume plants [22]. Nitrogen fixation [25-29]and phosphorus solubilizing actions [30-35]are the process by which they transform nitrogen and phosphorus respectively that plants cannot directly absorb into forms that they can use for growth.

4. Plant illnesses can be prevented and pathogens can be suppressed by soil microorganisms. For example, to inhibit germs, Streptomycesand Bacillus subtilis can create antibiotics. Bacillus can induce the production of antioxidants, antimicrobial peptides, and other compounds that help plants fight against infections and halt their spreadin plants [22].

5. Plant hormones like cytokinins, gibberellins, and indole acetic acid (IAA) can be produced by some soil bacteria. By affecting physiological processes including flowering, fruit development, and plant growth, they encourage the growth of plants. Gibberellins can be produced by Micromonospora and Streptomyces[36]and IAA can be produced by PGPR-like Bacillus [37] [38].

6. Soil microorganisms can improve soil texture and structure. To develop polysaccharide adhesives that adhere to soil particles, help in the creation of soil aggregates, enhance soil structure, promote soil aeration and water retention, and boost soil fertility, bacteria such as Pseudomonas aeruginosa, Bacillus subtilis, and Chromobacterium violaceum can collaborate [39].

In conclusion, soil bacteria support plant growth, influence soil structure, and take part in ecological processes as a group. Plant and soil ecosystems are significantly impacted by their relationships with other microorganisms and plants. Together, these processes keep ecosystems stable and in balance. [22]

Mechanisms of heavymetal resistance bacteria that helps them to tolerate heavy metal toxicity

As we discussed how some bacteria are non-tolerant to heavy metal toxicity and how adversely heavy metal pollution effect those bacteria, here we are going to discuss about how somebacteria are able to tolerate heavy metal toxicity. Bacteria have adapted to withstand high amounts of heavy metals in areas near active volcanoes, hydrothermal vents, and mineral deposit sites. As a result of this adaptation, sensitive cellular components mechanisms to defend against heavy metal ions. A number of factors, including the forms of metal ion transport into the cell, the location of metal resistance genes (chromosome, plasmid, or transposon), and the function of metal ions in cellular metabolism, may affect a bacterium's ability to tolerate metals[50].

The five primary methods of bacterial resistance to heavy metals are listed by Bruins et al. and Choudhury et Srivastava. It need to be emphasized that a single bacterium may have many defence mechanisms:

- extracellular barrier;
- active transport of metal ions (efflux);
- extracellular sequestration;
- intracellular sequestration;
- reduction of metal ions; [50,51]

Extracellular barrier to stop metals from entering the cell: Metal ions may be kept out of the cell by the cell wall, plasma membrane, or capsule. Ionizable groups of the cell wall or capsule (carboxyl, amino, phosphate, and hydroxyl groups) allow bacteria from various taxonomical groups to adsorb metal ions [52] [53]. Since metal ions can also be bound by dead bacterial cells, this adsorption is a passive process.It has been shown that the adsorption capacity of bacterial cells destroyed by heat treatment is equal to or greater than that of living cells.Pseudomonas putida, Brevibacterium sp., and Bacillus sp.non-viable cells showed a high degree of passive adsorption of heavy metal ions.According to several writers,

the accumulation of metal ions by living cells occurs in two stages: first, the cell wall adsorbs them quickly and non-specifically, and then the metal ions are slowly transported into the cytoplasm.Bacterial capsules have the ability to adsorb heavy metal ions, primarily through the carboxyl groups of polysaccharides. Metal ions have been found to accumulate in the extracellular biopolymers of Enterobacter chloaceae, Klebsiella aerogenes, and Acinetobacter sp[55].

Active transport of metal ions (efflux): Efflux, or active transport, is the most prevalent type of bacterial heavy metal resistance system. To export metal ions from cells, bacteria take advantage of these systems. The genetic determinants of efflux systems can be found on plasmidsand on chromosomes. Certain metal ions can enter the cell through the mechanisms that allow critical elements to be absorbed; for instance, the sulphate transport system is used to move chromate inside the celland the magnesium transport systems allow ions of cadmium, zinc, cobalt, nickel, and manganese to enter the cells of Ralstonia metallidurans (Alcaligenes eutrophus)[55].

Extracellular sequestration: The accumulation of metal ions by cellular constituents in the periplasm or outer membrane, or the complexation of metal ions as insoluble compounds, is known as extracellular sequestration [55].

In order to bind copper ions, copper-resistant Pseudomonas syringae strains produced copper-inducible proteins CopA, CopB (periplasmic proteins), and CopC (outer membrane protein) [54]. As a result of metal buildup, bacterial colonies appear blue.The similar blue bacterial colonies could be observed during growth of copper-tolerant Pseudomonas pickettii US321 strain on agar medium supplemented with copper, copper ions were accumulated in the periplasm or outer membrane [55].

Intracellular sequestration: When different substances in the cell cytoplasm bind with metal ions, this is known as intracellular sequestration. Two kinds of eukaryotic metal-binding peptides, phytochelatins and metallothioneins, are abundant in cystein residues and use sulfhydryl groups to bind metal ions [56]. Plants and fungi contain peptides of

low molecular weight, called phytochelatins. They are made from glutathione and have 5 to 11 amino acid residues [57]. Among prokaryotes, the cyanobacterium Synechococcus sp. PCC 7942 has been shown to be capable of synthesising metallothionein. Compared to the corresponding eukaryotic peptide, this peptide has less cysteine residues. Cadmium and zinc ions cause Synechococcus sp. to synthesise metallothionein, which is encoded by the genes smtA and smtB [58]. Cadmium-tolerant P. putida strain have the capability of intracellularsequestration of copper, cadmium and zinc ions through the help of cysteine-rich lowmolecular-weight proteins [59].

Reduction of heavy metal ions by bacteria: Bacteria may reduce a broad range of heavy metal ions. Numerous ecological niches yielded bacteria that reduced chromate, molybdate, and vanadate [60]. Certain bacteria are able to generate energy by using metals and metalloids as electron donors or acceptors. Oxidised metals may act as terminal acceptors of electrons when bacteria are respiring anaerobically. Additionally, enzymatic reduction of metal ions would produce a less toxic form of mercury and chromium. Bacillus cereus,Klebsiella pneumoniae,P. stutzerican reduce Hg2+ and Hg0 from the soil.Desulfomicrobium norvegicum, Microbacterium sp., Ochrobacterium intermedium, Brevibacterium sp and Pseudomonas spp. Can reduce the toxicity of Cr6+ and Cr and reduce its availability from soil [55].

Conclusion

Heavy metal contamination has been regarded as one of the major threats to soil ecosystems as it changes microbial diversity and diminishes the quality of the soil. Microorganisms play a critical role in soil health through nutrient cycling, decomposition of organic matter, and ecosystem stabilization. Thus, the harmful impacts of heavy metals on microbial communities, which include reduced diversity and metabolic inefficiency, stress the need for effective mitigation strategies. However, certain bacteria have strong resistance mechanisms with metal ion efflux, sequestration, and enzymatic reduction. This has made it possible for the survival of these bacteria and their contribution to detoxification in heavy metals.

Utilization of these microbial capabilities opens avenues for bioremediation of contaminated soils, thereby contributing to environmentally friendly management practices. Further studies are required to fine-tune applications of microbes in remediation.

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INVESTIGATING THE RELATIONSHIP BETWEEN GUT MICROBIOME AND IBS

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ABSTRACT

Irritable Bowel Syndrome (IBS) is a multifactorial gastrointestinal disorder affecting a significant portion of the global population, characterized by symptoms such as abdominal pain, bloating, and irregular bowel movements. Although there is still much to learn about the pathophysiology of IBS, mounting data has linked the gut microbiota to the onset and progression of the condition. With a focus on microbial diversity, particular bacterial populations, and their impact on gut health and symptoms, this study aims to investigate the connection between gut microbiota makeup and IBS. Stool samples from people with IBS and healthy controls were thoroughly analyzed, and we profiled the microbial communities using next-generation sequencing methods.

Our findings show clear alterations in the relative abundance of specific bacterial species and decreased microbial diversity in the microbiome of IBS patients as compared to controls. In particular, IBS patients showed an overgrowth of pathobionts such Enterococcus and Escherichia coli together with a decrease of beneficial Firmicutes and Bacteroidetes. The degree of IBS symptoms, including bloating and stomach pain, was also connected with these alterations. We also looked at how changes in gut motility, visceral hypersensitivity, and low-grade inflammation—three important characteristics of

IBS—may be influenced by microbial dysbiosis.

The results of this study demonstrate the potential use of the gut microbiota as a therapeutic target and diagnostic biomarker for IBS. We can develop new microbiome-based therapies, such as probiotics, prebiotics, and fecal microbiota transplantation, to restore gut health and enhance patient outcomes by comprehending how certain microbial imbalances impact the course of disease. In addition to providing opportunities for individualized therapy based on microbiome profiles, this research emphasizes the significance of microbiome-gut-brain connections in the management of IBS.

Keywords: *Microbiome, gut microbes, IBS, microbial association*

INTRODUCTION:

Irritable Bowel Syndrome (IBS) is a common, chronic gastrointestinal disorder characterized by a group of symptoms that include abdominal pain, bloating, and alterations in bowel habits such as diarrhea, constipation, or a combination of both[1]. IBS is considered a functional disorder, meaning it does not involve any structural abnormalities or visible damage to the digestive system, but instead, the symptoms arise from disturbances in the normal function of the gut. The exact cause of IBS is not well understood, but it is believed to result from a complex interplay of factors, including abnormal gut motility, heightened visceral sensitivity (increased sensitivity to pain in the intestines), dysregulation of the gut-brain axis, alterations in the gut microbiome, psychological stress, and in some cases, post-infectious changes.[16]

IBS can be classified into several subtypes based on the predominant bowel symptom: IBS-D (diarrheha-predominant), IBS-C (constipation-predominant), IBS-M (mixed type), andIBS-U (unsubtyped), which refers to patients whose symptoms do not clearly fit into one of the other categories. The condition affects both men and women, though it is more prevalent in women, and it can be diagnosed using the Rome IV criteria, which emphasize the presence of abdominal pain at least once per week, along with associated changes in bowel movements, over a period of at least three

months.[2]

The diagnosis of IBS is primarily clinical, often requiring the exclusion of other potential causes of gastrointestinal symptoms such as inflammatory bowel disease (IBD), celiac disease, or colorectal cancer[3]. No specific biomarker exists for IBS, which makes the diagnosis challenging and largely reliant on symptom patterns and clinical evaluation.[17] Additionally, IBS symptoms can be highly variable, with some individuals experiencing symptom flares and others going through periods of symptom relief.

Treatment for IBS is multifaceted and aims to manage symptoms rather than cure the condition. It includes dietary modifications such as the Low FODMAP diet, pharmacological interventions like antispasmodics, laxatives, antidiarrheal agents, and antidepressants, as well as psychological therapies such as cognitive behavioral therapy (CBT) and gut-directed hypnotherapy. Probiotics and prebiotics are also often used to help restore balance to the gut microbiome, which is thought to play a crucial role in IBS pathophysiology. While IBS does not lead to serious, life-threatening conditions such as cancer, its impact on daily life and well-being can be profound, with many individuals experiencing significant emotional distress, work limitations, and social isolation due to the unpredictability of symptoms.

GUT MICROBIOME:

The distal small bowel and colon are home to the majority of the 100 trillion bacteria that make up the human gastrointestinal tract. Despite the fact that bacteria have received a lot of attention, it's crucial to keep in mind that viruses, fungi, archaea, and eukaryotes also contribute to the communities that live in the GI tract's microenvironment. More than 2,000 distinct species of bacteria from 12 phyla have been identified by studies; 93.5% of these species belong to the four dominating phyla: Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria. According to recent studies, environmental variables including nutrition, medications, and lifestyle have a bigger impact on the gut flora than genetics. Furthermore, it's possible that the gut

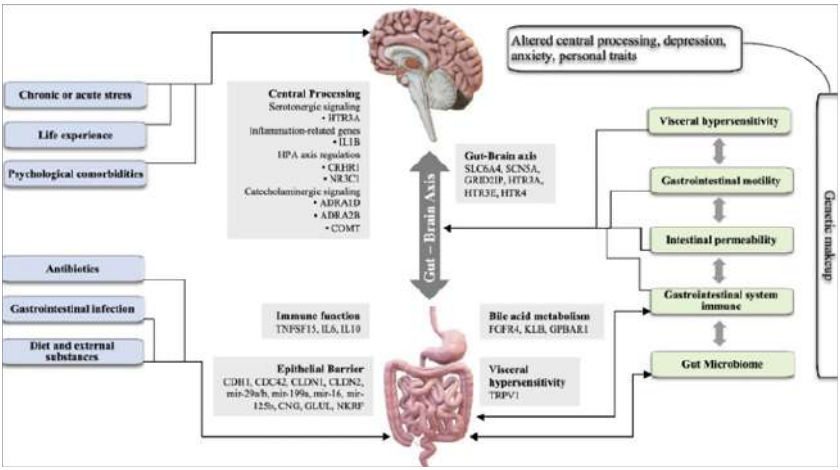
microbiota can predict metabolic variables and clinical phenotypes more accurately than genetics.[4]

Normal gut growth and health depend on bacteria. For instance, as compared to animals grown in a typical laboratory setting, germ-free mice exhibit reduced cycling and propagation of the migrating motor complex, delayed intestinal transit and stomach emptying, and decreased expression of the enteric nervous system genes GABA and VAP-33. By supplying vital amino acids, vitamins, and short-chain fatty acids, as well as encouraging the healthy growth and operation of the gut immune system, bacteria help support the host's health.

Mircobial association with IBS:

The integrated actions and communication between the microbiota and the autonomous nervous system are central players in the perpetuation of IBS symptoms. This signaling pathway is called the Gut-Brain Axis (GBA). The GBA is a bidirectional neurohumoral communication system that integrates brain and GI functions, such as gut motility, appetite and weightand here the microbiota plays a critical role.[5] Changes in gastrointestinal or central nervous system physiology may result in an altered habitat, which again may cause changes in the composition of the microbiota.

Dysbiosis, a disruption of the physiologic symbiotic interaction (eubiosis) between the human host and the microbiota, is thought to be a fundamental cause in the onset and maintenance of IBS in most patients. Evidence now available indicates that IBS is actually a problem of the microbiota and the GBA, since the dysbiosis seen in IBS and the immunological reaction that follows may cause and maintain the gastrointestinal symptoms of IBS.[6] It is unknown if gut alterations are caused by brain abnormalities or whether vagal and sympathetic pathways in the brain impact how the stomach functions.



Microbiome in healthy individual vs individual affected by IBS

In an initial investigation including 80 IBS patients and 65 matched controls who did not have IBS, Jeffery and associates discovered that Ruminococcus gnavus and Lachnospiraceae were abundant, but Coprococcus catus and Barnesiella intestinihominis were less prevalent [7]. IBS patients had lower abundance ofBifidobacterium, Lactobacillus, and Faecalibacterium prausnitzii, according to a meta-analysis of 13 publications [8]. A different meta-analysis that included 16 publications and 777 IBS patients discovered that, at the phylum level, there were more Firmicutes and less Bacteroidetes (with a higher Firmicutes:Bacteroidetes ratio). At lower taxonomic levels, they also found several alterations, such as a rise in Clostridiales and Clostridium and a fall in Bacteroidia and Bacteroidales [9]. Similarly, in the gut microbiome analysis of IBS patients compared to healthy controls, researchers found lower levels of Lactobacillus and Bifidobacterium and highly abundantE. coli and Enterobacter in subjects with IBS, but no difference in fecal Bacteroides or Enterococcus [10].

The majority of research have failed to identify substantial differences across IBS subtypes, despite evidence that the

microbiome of IBS patients and controls vary [9,11,12,13]. It should be noted that a lack of standardized techniques may contribute to the difficulty to identify meaningful variations in microbiome phenotypes in individuals with IBS. The current standard for analyzing gut microbial assemblages is whole shotgun metagenomics; however, this method depends on bioinformatic methods to interpret the data, which have drawbacks of their own [14,15]. When examining these functional characteristics, it's also critical to use metagenomics, metatranscriptomics, and metabolomics since taxonomic composition alone might not be sufficient to explain individual variances.

Conclusion:

In Conclusion, there is a complicated and multidimensional link between the gut microbiota and irritable bowel syndrome (IBS). There is mounting evidence that the pathophysiology of IBS may be significantly influenced by changes in the variety and composition of the gut microbiota. IBS patients have been shown to have dysbiosis, or an imbalance in the microbial community, with bacterial populations differing between IBS patients and healthy controls. IBS symptoms have been linked to certain microbial groups, including Firmicutes, Bacteroidetes, and species like Faecalibacterium prausnitzii.

Though a direct cause is still unknown, it is known that the gut microbiota can affect inflammation, visceral sensitivity, and gut motility—all of which are important aspects of IBS. IBS symptoms can also be caused by interactions between the microbiota and other factors including heredity, stress, and food. Though more study is required to completely understand the role of the microbiome and to create more effective, tailored medicines for IBS, personalized methods to therapy, such as dietary modifications, probiotics, and antibiotics, are showing promise.

A number of interesting techniques for controlling IBS are being investigated at the moment, including therapeutic treatments that target the gut flora. By restricting items that worsen bloating and pain, dietary interventions—in particular, the low FODMAP diet—have been shown to be useful in lowering

the symptoms of IBS. Although the benefits vary depending on the strain, probiotics like Bifidobacterium and Lactobacillus can help restore microbial balance and reduce symptoms. Prebiotics, which promote the growth of good bacteria, could also be helpful, but their usage must be carefully managed to prevent symptoms from getting worse. Fecal microbiota transplantation (FMT) is being researched as a possible therapy for people with refractory IBS, while antibiotics such as rifaximin have demonstrated promise in controlling IBS-D by lowering small intestine bacterial overgrowth (SIBO).

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THE IMPACT OF PASSIVE SMOKING ON CHILDREN

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ABSTRACT

Secondhand smoke (SHS), sometimes referred to as passive smoking, has become a major worldwide public health hazard with far-reaching effects on people of all ages. This review comprehensively evaluates the adverse effects of passive smoking on children's health outcomes, focusing on respiratory conditions, cardiovascular effects, cancer risk, growth retardation, and immune system compromise. A thorough analysis of existing literature reveals that exposure to tobacco smoke significantly heightens the risk and severity of lower respiratory tract infections in children. Additionally, passive smoking is associated withdecreased lung function and more respiratory issues, and impaired cognitive and behavioural development. These findings underscore the pressing requirement for efficient public health strategies to mitigate the impact of SHS on vulnerable populations, particularly children.This study aims to illuminate the multifaceted impact of passive smoking on children's health, emphasizing the critical importance of safeguarding young populations from the harmful consequences of tobacco smoke exposure.

Keywords: *secondhand smoke, cardiovascular effects,respiratory conditions,tobacco smoke exposure*

Introduction

The global prevalence of adult smoking in 2020 was 6.5% for women and 32.6% for men, according to the World Health Organization's important statistics on tobacco(Arafa1,2 2023).

When non-smokers breathe in cigarette smoke, smoke exhaled by smokers, or a combination of both, they are considered to be passive smokers. In public locations including restaurants, workplaces, shopping centers, parks, cars, and daycare centers, nonsmokers can breathe in secondhand smoke. The smoke was found to remain airborne for a few hours followingsmoking and to travel up to about 20 feet. Due to their developing bodies and faster breathing than adults, children are particularly susceptible to the harmful effects of secondhand smoke(Munther et al.2023).Although it affects people all across the world, passive smoking receives little attention. An estimated 600,000 avoidable deaths are attributedEvery year, people are exposed to secondhand smoke, according to a WHO (World Health Organization) research. According to the National Library of Medicine (NIH), despite the fact that smoking is not allowed in public places, around 30% of individualsare subjected to secondhand smoke, which results in 1.9 million deaths and 24 million disability-adjusted lives annually (Roopashree et al., 2024). Smoking has a detrimental effect on the cardiovascular and respiratory systems.Due to their developing systems and rapid breathing, children are more vulnerable.Asthma, ear infections, respiratory infections, low birth weight, and SIDS are all linked to SHS in kids.

This review assesses the health effects of passive smoking on kids, with an emphasis on immune system effects, growth retardation, respiratory disorders, behavioural problems, and cognitive impairment. The success of smoke-free policies is evaluated, along with its immediate and long-term effects. The importance of healthcare communication in lowering the dangers of SHS is also examined, as is parental preferences for smoking cessation messaging.

Impact of Passive Smoking on Children's Health

- **Respiratory Issues:** Secondhand smoke (SHS) exposure puts children at risk for respiratory conditions like pneumonia, bronchitis, asthma, and chronic cough. By irritating and inflaming their airways, SHS impairs lung function and makes them more prone to infections. In children in particular, passive smoking significantly raises the possibility of respiratory symptoms like dyspnea, wheeze, and coughing. Other respiratory conditions, including asthma, can also be made worse by secondhand smoking exposure.

- **Cardiovascular Diseases:** The risk of cardiovascular conditions, such as heart attacks, strokes, and coronary artery disease, is increased by passive smoking. Secondhand smoke exposure increases the risk of unexpected cardiovascular events among nonsmokers. These severe disorders are exacerbated by the harmful compounds in secondhand smoking, which damage blood vessels and induce inflammation.

- **Impaired cognitive development:** Cognitive deficits in children exposed to secondhand smoke (SHS) can include memory issues, attention span shortening, and poor reading and math performance. Cognitive abilities and scholastic achievement may suffer as a result of the harmful compounds in SHS, which can interfere with brain growth and function.

- **Growth Retardation:** Research indicates that kids who are exposed to SHS frequently had lower heights and bodily weights in comparison to their classmates who were not exposed. It's possible that the toxins in SHS are the cause of this growth impairment since they prevent the body from absorbing nutrients and developing normally.

- **Immune System Effects:** Due to compromised immune systems, children exposed to secondhand smoke (SHS) are more susceptible to infections and diseases. SHS can affect immune cell activity, which reduces the body's defenses against infections and increases the risk of respiratory infections and other illnesses (Barnoya et al. 2005).

- **Cancer risk:** Children who are in close proximity to secondhand smoke(SHS) have a much higher chance of developing cancer. Lung cancer and other cancers can arise later in life as a result of DNA damage caused by the dangerous chemicals in SHS. As they become older, children exposed to SHS have a higher probability of other health issues, which renders themfurther more susceptible to cancer. For their health, SHS exposure must be eliminated.

Table 1: Showing the rates of various health impacts on children due to secondhand smoke (SHS) exposure

Health Impact	Rate of Affectation (%)
Respiratory Infections	25%
Asthma	20%
Sudden Infant Death Syndrome (SIDS)	5%
Low Birth Weight	15%
Premature Birth	10%
Behavioural Problems	18%
Cardiovascular Diseases	8%
Cancer Risk	3%

Ways to reduce SHS exposure

- Make Your House and Vehicle Smoke-Free: Ensure that your home and vehicle are smoke-free.
- Promote Quitting Smoking: Assist friends and relatives who smoke in giving smoking.
- Steer Clear of Smoking Public Places: Don't let children near regions where smoking is permitted.
- Raise Awareness of the Risks: Inform kids about the risks associated with SHS and the significance of staying away from it.

Societal Implications

Healthcare Costs: Medical systems have a heavy financial burden from diseases linked to passive smoking. Children

exposed to secondhand smoking require medical care, including hospitalization, and regular visits with doctors. Passive smoking is an expensive public health issue because of these healthcare needs.

Educational Impact: Chronic health issues caused by passive smoking may increase a child's likelihood of missing school and performing poorly in school. Their academic performance is affected, and educational institutions are under additional strain as a result.

Public Health Initiatives: The harmful effects of passive smoking on children have prompted governments and health groups to implement a variety of public health initiatives. Laws that forbid smoking in public places are among them, as are programs for education and assistance in quitting smoking for parents and other caregivers.

Legal and Policy Measures: In several countries, laws have been implemented to protect children from secondhand smoke. These consist of regulations pertaining to smoke-free zones, bans on smoking in cars with children, and penalties for disobedience.

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THE IMPACT OF ORAL MICROBIOME IN MAINTAINING ORAL HEALTH AND PREVENTING DISEASE

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ABSTRACT

Among the vast array of microbial communities that inhabit in the human body ,one of the most diverse and complex microorganismsare oral microbes , including over 700 species of bacteria, numerous viruses, fungi and protozoa[1,2,3]. The oral microbiome plays an important role to maintains a normal oral physiological environment[4]. Dysbiosis of the oral microbiome has been linked to various oral and systemic diseases. The connection between the host and microorganisms significantly shapes the microbial composition which ultimately affecting the host's overall health.[5,6,7] Numerous studies have consistently indicates that the microbiome is closely linked to various types of cancer including oral cancer, prostate cancer, oral squamous cell carcinoma (OSCC) and also diseases like periodontal disease , diabetes and etc.[8] Also, research has demonstrated that cancer progression can, in turn, alter the composition of the human microbiome. The main risk factors for cancer of the oral cavity are the consumption of tobacco and alcohol. The effect of carcinogens on the oral mucosa creates an area prone to malignant transformation.[9,10,11] The immune-inflammatory response of the host in periodontitis is primarily marked by a physiological acute inflammatory reaction (gingivitis) to both supragingival and subgingival

plaque, maintained by innate immune system cells.[12] Fusobacterium plays an crucial role in the bacterial impact on OSCC where as it had the ability to co-adhere with other species and form an association network centered around itself in patients of the cancer group. [14,15] We will discuss the complex relationships between the oral microbiome and various oral health conditions and how these microbes are used to improve human health and the future aspects of these microbes in this paper.

Keywords: *Oral microbes, Oral disease, Periodontal disease, OSCC, Microbial Dysbiosis*

Introduction

Among the vast array of microbial communities that inhabit the human body, the oral microbes are one of the most diverse and complex microorganisms, including over 700 species of bacteria, numerous viruses, fungi and protozoa.The oral microbiome plays an important role to maintains a normal oral physiological environment. [1,2,3] It also influences the maintenance and development of oral tissues, including teeth and gums. Furthermore, it produces antimicrobial compounds and maintains the level of the oral ecosystem which contributes to the breakdown and digestion of food, modulation of the immune system, and protection against pathogens.[16] Here we have shown the examples of oral microorganisms through the figure given below.

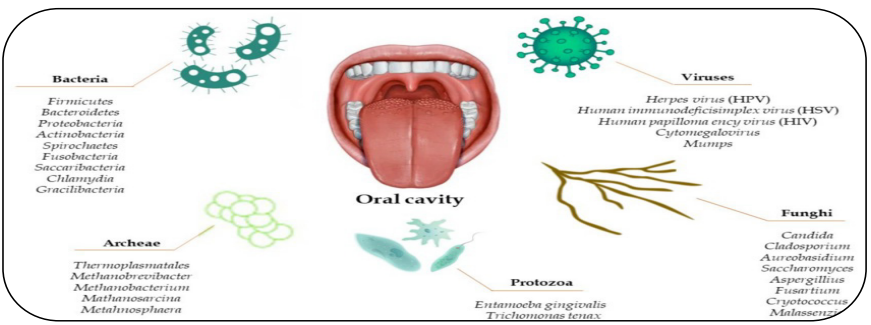

Fig: Diagrammatic representation of oral microbiome[48]

Dysbiosis of the oral microbiome has been connected to various oral and systemic diseases. The partnership between

the host and microorganisms significantly shapes the microbial composition which ultimately affecting the host's overall health. [17,18,] Numerous studies have consistently indicate that the microbiome is closely linked to various types of cancer including oral cancer, prostate cancer, oral squamous cell carcinoma (OSCC) and also diseases like periodontal disease, diabetes and etc. Furthermore, research has shown that cancer progression can, in turn, alter the composition of the human microbiome.The investigation of microorganisms as possible disease-fighting strategies has been prompted by the connection between the microbiome and cancer. [19,20,21] Particularlyseveral microbes have been recognized as:

1.Pathogenic agents, are microorganisms that aid in the onset and advancement of illnesses. For example -Porphyromonasgingivalis, is a type of bacteria that causes periodontal disease and other related diseases in mouth.[22]

2. Diagnostic markers, microorganisms that can be utilized to show whether a disease is present or is progressing. For instance, mouth samples can be used to detect the Human Papillomavirus (HPV), this type of virus can be used to diagnose disorders associated to HPV, including oral cancer. [23]

3. Therapeutic targets, microorganisms which can be used to stop or cure illnesses. For example, antifungal drugs can be used to treat the fungus Candida albicans.[24]

In this review we will talk about the complex relationships between the oral microbiome and various oral health conditions and how these microbes are used to improve human health and the future aspects of these microbes.

Oral Disease

The oral cavity contains the second most abundant collection of microorganisms in the body, next only to the gastrointestinal tract.The oral microbiome contributes to the balance of physiologic, metabolic, and immunological functions that help to maintain homeostasis through the formation of a mucosal barrier. Oral microbiome alterations have been observed in various conditions, such as Oral cancer,

Periodontal disease, Oral squamous cell carcinoma (OSCC), Diabetes Cardiovascular disease and etc.

Oral Cancer

Many of studies have been set aside to the significance of heredity in oral carcinogenesis. Several genes are involved in genetic tendency of oral cancer. Gene polymorphisms participating in the metabolism of xenobiotic factors which are the external, foreign materials that the body does not naturally create, For example cytochrome P450 1A1 (CYPIA 1) [25]and glutathione S-transferase mu 1 (GSTM1)[26,27] are blamed for the increase of relative danger in the carriers. Individuals with alcohol dehydrogenase 3 genotype are prone to the development of oropharyngeal cancers. .Oral cancer is becoming more common, especially in women and young people. Every year, between 350,000 and 400,000 new cases are diagnosed worldwide.The fundamental risk factors for cancer of the oral cavity are the consumption of tobacco and alcohol. The effect of carcinogens on the oral mucosa creates an area prone to malignant transformation. The risk factors are consumption of alcohol and tobacco use, cannot account for the variations in incidence, since oral cancer often develops in individuals without a background of alcohol or tobacco exposure[28,29]. Frequently, the disturbance of similar signalling pathways leads to the molecular pathogenesis of cancer of the oral cavity that promote oncogenesis which occursagainst a backdrop of tumor suppressor inactivation. In high frequency of oral cancer the inactivations of p53 and CDKN2A tumor sppressor happened. [30]

Periodontal Disease

Periodontal disease is a type of chronic inflammatory condition which affecting about 15–45% of people worldwide. The host'simmune-inflammatory response in periodontitis is primarily marked by a physiological acute inflammatory reaction (gingivitis) to both supragingival and subgingival plaque, maintained by innate immune system cells,.[12] During this stage, the resident cell population produces cytokines such as tumour necrosis factor (TNF)-α, interleukin (IL)-1β, and interleukin (IL)-6, which mainly aid in promoting

cell migration to infection sites, increasing the expression of neutrophil adhesion molecules on internal vessel surfaces, and increasing the synthesis of other proinflammatory cytokines. [31,32] The removal of plaque results in the gradual resolution of inflammation and the return to individual homeostasis. The activation of acquired immunity follows the clearance of plaque.This process, which is controlled by adaptive-immunity cytokines, includinginterferon (IFN)-γ, interleukin (IL)-2, and interleukin (IL)-4, occurs through antigen processing and presentation by lymphocytes, macrophages, and dendritic cells. The gradual breakdown of periodontal tissues are the cause in the loss of bone tissues and the deterioration of ECM. [33,34] Bone resorption occurs due to a changing balance between osteogenesis and osteoclastogenesis, tipping towards the latter, which is regulated by a complex pathway induced by inflammation that includes the receptor RANK, its ligand (RANKL), IL-1B, IL-6, and TNF-α [34]. The increased expression of 23 Zn2+- and Ca2+-dependent enzymes known as matrix metalloproteinases (MMPs) causes the destruction of extracellular matrix (ECM).[35] In healthy periodontal tissues, where they take part in specific physiological processes including tissue growth and turnover, these enzymes usually show a modest expression level. Additionally, MMPs contribute to pathological processes such as the degradation of connective tissue in periodontitis, which results in the breakdown of collagen in the gingival and periodontal ligaments[47].

Oral Squamous Cell Carcinoma

Oral squamous cell carcinoma is the most prevalent malignant epithelial tumour that affects the oral cavity. (OSCC)[36]. In the past few years, research hascentered on the involvement of oral bacteria in OSCC and their compositional alterations. Generally speaking, oral bacteria can contribute to the formation of OSCC by encouraging angiogenesis and cell proliferation, affecting normal apoptosis, aiding invasion and metastasis, and supporting cancer stem cells. The dorsum of the tongue and the palatal mucosa are viewed as low-risk zones, while the floor of the mouth and the ventrolateral tongue are regarded as high-risk areas.According to studies,

Fusobacterium may play a crucial role in the bacterial impact on OSCC because it can co-adhere with other species and create a self-centred association network in cancer patients.[14,16] Certain oral bacteria, like P. gingivalis and F. nucleatum, are involved in various pathways that aid in the development of OSCC. Additionally, inflammation might play a role in the process, with several inflammatory cytokines like IL-6, IL-8, TNF-α, and PGE influencing the tumor microenvironment, potentially facilitating tumor progression.[37]

Microbial Dysbiosis In Disease Condition

Dysbiosis is defined by an alteration in the microbiome that leads to an imbalance in the microbiota, modifications in their functional makeup and metabolic processes, or a variation in their local distribution. Dysbiosis in the oral microbiome may play a role in the onset of various oral and systemic conditions, such as dental caries [4,5], periodontitis [12], oral and other cancers [7,8,9], cardiovascular issues [38], and diabetes [39]. It is linked to a rise in harmful bacteria and a matching decline in helpful bacteria. This insight has prompted significant research aimed at interventions to rebuild microbial health and equilibrium in the oral microbiome, such as prebiotics, probiotics, and antimicrobial agents, serving as potential therapeutic methods for addressing and averting oral dysbiosis and related diseases.

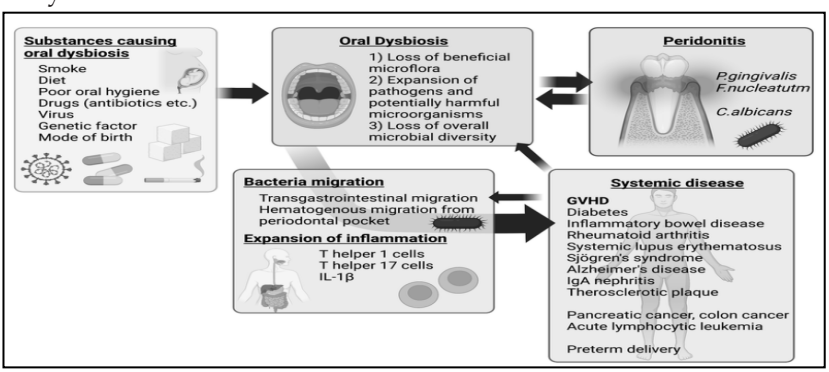

Fig: Oral dysbiosis [46]

Use of microbes to improve health

Numerous studies have reported that microbiota is made up of several microorganisms that reside in the gastrointestinal system and oral cavity.Many bacteria find that the oral cavity offers the best conditions for growth since it consistently maintains the right temperatureand humidity Saliva released into the mouth contains antimicrobial elements: agglutinins that adhere to oral bacteria like S. mutans and support phagocytosis by inducing bacterial clustering [40]; lysozymes that lead to cell lysis by disrupting the cell walls [41'42]; lactoferrin that hinders biofilm development by enhancing twitching, a unique surface movement, through iron chelation [59]; and peroxidase, which demonstrates antimicrobial properties by obstructing the bacteria's glycolytic pathway.To protect against infections brought on by new pathogens like the severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), oral sIgA may therefore be crucial[43,44].sIgA attaches to oral bacteria like Streptococcus mitis,[45] Streptococcus oralis, and S. mutans, which feature epitopes acknowledged by sIgA, thereby selectively attractingthese specific bacteria to the mucus layer on the mouth's mucosal surfaces.

The oral microbiota can affect how well the body digests specific foods. Additional variables include food extracts (such as red wine) and eating habits (vegetarian or not).[46] Here, we demonstrate the potential effects of food extracts and dietary patterns on body health. Yomo gyutto, or fermented Japanese mugwort, is a type of probiotic fermented milk (Batavito). Probiotic yoghurt, probiotic petit-suisse cheese, probiotic milk, and Italian Grana Padano (GP) cheese all controlled the oral microbiota or decreased the quantity of oral microorganisms, which prevented or improved dental caries and periodontal disease. Additionally, fermented milk reduced inflammation and changed the gut and mouth microbiome.

Conclusion

There are many different types of microorganisms that make up the oral microbiome, which includes bacteria, fungus, viruses, and archaea.When infections disrupt the symbiotic

connections that make up the oral microbiome and result in dysbiosis, a once-healthy state can turn unhealthy and raise the chance of comorbidities.The importance of the oral microbial community in preserving systemic and oral health is becoming more widely recognized. Additionally, contemporary lifestyle choices such as smoking, eating an unbalanced diet, and poor oral hygiene are major contributors to the disruption of the oral microbiome's delicate balance and the development of oral diseases like periodontal disease, particularly in people who are genetically and epigenetically susceptible. By creating oral probiotics and modifying the oral microbiota, we can improve dental health in future while also improving overall health. Also in future by using metagenomics we can identify more oral microbes in the early stage of disease an by analyzing the sequence further research like target specific oral microbes to treat disease and advance research between microbes and disease and probiotics. The intergration of metagenomics and oral microbes research will revolutionize to understand about the oral health and disease and also develop effective treatment and also helps to early identification of life threatening disease and etc.

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ANTIMICROBIAL STUDIES ON BHUMI AMLA (PHYLLANTHUS NIRURI)

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ABSTRACT

An international public health concern brought on by the abuse and overuse of antibiotics is antimicrobial resistance (AMR). It makes it harder to treat infections, which can result in fatalities, severe illness, and disability. Just 25% of the antibiotics on the market today have novel mechanisms against important diseases, indicating that antibiotic discovery is progressing slowly. Novel antibacterial chemicals may be developed from the secondary metabolites of traditional medicinal plants. Safe and efficient antibacterial chemotherapies can be developed using these substances, which frequently have potent antimicrobial action. In herbal medicine systems such as Traditional Chinese Medicine, Indian Ayurveda, and Indonesian Jamu, P. niruri has a long history. Among the conditions for which the entire plant is used as a treatment are dyspepsia, influenza, vaginitis, tumours, diabetes, diuretics, jaundice, kidney stones, and dysentery. In this study, Phyllanthus Niruri leaves were extracted using a variety of solvents, including petroleum ether, chloroform, acetone, ethanolic, and aqueous; physicochemical evaluation was determined; ash values were estimated; and phytochemical evaluation was conducted, involving the measurement of sugars, alkaloids, glycosides, tannins, flavonoids, steroids, proteins, and terpenes. The plant's water and ethanol extracts were made by the help of soxhlet apparatus, the solvent

extraction method. And its antibacterial activity against eleven clinical isolates was examined using the agar well diffusion method. This review highlights the present understanding of P. niruri's antimicrobial capabilities, emphasising its phytochemical makeup, modes of action, and effectiveness against a variety of infections. It seeks to provide light on its possible use as a natural antibacterial.

Keywords: *Phyllanthus Niruri, Antimicrobial resistance, Agar well diffusion method*

Introduction

The Indian herb Phyllanthus Niruri is frequently seen as a winter weed in warmer climates.1 In tropical and subtropical regions, it is a member of the Phyllanthus genus, which includes more than 600 species of trees, shrubs, and annual or biennial herbs.2 A member of the Euphorbiaceae family, Phyllanthus Niruri normally reaches a height of 60 cm. Its name, Phyllanthus, means "leaf and flower," referring to the way the fruit and bloom seem to blend into the leaf. Because of its hepatoprotective qualities, Phyllanthus Niruri, a common rainy season plant in wastelands and farmed areas, has attracted the interest of researchers.5Antimicrobial resistance (AMR) occurs when certain common antibiotics lose their ability to affect bacteria, fungus, parasites, or viruses. Genetic material changes with time, causing AMR, a normal mechanism in infections.3 The overuse and abuse of antibiotics in plants, animals, and people has sped up the emergence of infections that are resistant to them. The inability to treat infections due to AMR causes severe disease and morbidities, disability, and even death. In fact, AMR is regarded by the World Health Organisation (WHO) as one of the most dangerous dangers to global public health, and it was directly responsible for 1.27 million deaths globally in 2019. According to the World Bank, AMR is expected to cost the healthcare system USD 1 trillion by 2050, posing a serious financial strain.6It has long been known that traditional medicinal plants have antibacterial qualities. Bhumi Amla, or Phyllanthus Niruri, is a tropical plant that belongs to the Euphorbiaceae family. Recent research has demonstrated its promise against bacterial, fungal, and viral pathogens,

despite its traditional usage in treating liver ailments, urinary tract infections, and skin conditions.[7]

Botanical classification of Bhumi Amla:

KINGDOM	PLANTAE
DIVISION	Magnoliophyte
CLASS	Magnoliopsida
ORDER	Euphorbiales
FAMILY	Euphorbiaceae
GENUS	Phyllanthus
SPECIES	Niruri

Phytochemical Composition

The medicinal plant known as Bhumi Amla (Phyllanthus Niruri) is well-known for its diverse phytochemical makeup and potential for treatment. Alkaloids, flavonoids, lignans, tannins, polyphenols, terpenoids, and saponins are among the many different types of bioactive substances that are present in it.8 The plant's strong antioxidant qualities are attributed to its abundance of phenolic chemicals, which include gallic acid and ellagic acid. Important lignans with hepatoprotective properties include phyllanthin and hypophyllanthin. Bhumi Amla also includes a number of minerals, including calcium and potassium, as well as important vitamins, like vitamin C. Its anti-inflammatory, antibacterial, antiviral, and hepatoprotective properties are all attributed to these phytochemicals, which makes it an important tool in both conventional and alternative medicine.[9]

Table 1 -

Phytochemicals	**Compound**	**Application**
Alkaloids	Phyllanthine, Nirurine , Phyllanthin	to have antimicrobial and hepatoprotective properties

Glycosides	Niruriside , Phyllanthoside	composed of a sugar molecule and a molecule that isn't. They have anti-inflammatory, antioxidant, and anticancer qualities, among other health advantages.
Flavonoids	Quercetin , Kaempferol , Gallic acid	have antioxidant and anti-inflammatory properties. They may also help to protect against heart disease and cancer.
Tannins	Phyllanthusiin , Corilagin	contributing to wound healing and gut health.
Terpenoids	Phyllanthol , Niruradol	consist of essential oils and triterpenes with antibacterial, anti-inflammatory, and anti-cancer qualities.
Phenolic acids	Caffeic acid , Ferulic acid	enhance general health by aiding in the plant's anti-inflammatory and antioxidant properties.

Compounds including phyllanthin, hypophyllanthin, and quercetin have been discovered as important contributors to antimicrobial action by analytical methods like GC-MS and HPLC.

Antimicrobial activity

Bhumi Amla (Phyllanthus Niruri) has a well-established antibacterial effect that is ascribed to its diverse phytochemical makeup.4 Both Gram-positive and Gram-negative bacterial infections are among the several bacterial pathogens against which the plant demonstrates strong antibacterial activity. By breaking down bacterial cell walls, preventing enzyme function, and obstructing microbial DNA replication, bioactive substances like alkaloids, flavonoids, tannins, phenolics, and

saponins add to its antibacterial effectiveness.10 Extracts from Bhumi amla have been shown in studies to be very efficient against common bacterial strains, including Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli. According to these results, Bhumi Amla may be used as a natural supplement or substitute for synthetic antibiotics, particularly in light of the growing prevalence of antibiotic resistance.It has strong antibacterial qualities that make it a good option for wound healing and infection treatment.[11]

Its antibacterial effectiveness is attributed to bioactive substances such alkaloids, flavonoids, tannins, phenolics, and saponins, which break down bacterial cell walls, prevent enzyme activity, and obstruct microbial DNA replication. Research has shown that Bhumi Amla preparations are very efficacious against common types of bacteria, including Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli. Given the growing prevalence of antibiotic resistance, our results imply that Bhumi Amla may be used as a natural supplement or substitute for manufactured antibiotics. It is a potential option for the treatment of infections and the promotion of wound healing due to its strong antibacterial qualities.[12]

Antifungal activity

Bhumi Amla (Phyllanthus Niruri) has a wide range of bioactive substances, including tannins, alkaloids, flavonoids, and saponins, which are responsible for its strong antifungal properties. Together, these substances prevent the development and spread of many fungal infections. Ergosterol production, which is essential for preserving the integrity of fungal cell membranes, is mostly interfered with in the antifungal mechanism, which disrupts fungal cell walls and membranes.13 Furthermore, by causing the generation of reactive oxygen species (ROS), the plant's flavonoids and phenolic chemicals cause oxidative stress in fungal cells, which results in cell damage and death. Bhumi Amla preparations have been shown to be effective against common fungal pathogens like as Trichophyton rubrum, Aspergillus niger, and Candida albicans. Its antifungal potential indicates that

it can be used to treat fungal infections and that it can serve as a natural substitute for synthetic antifungal medications, especially when dealing with drug-resistant fungal strains.[14]

Interference with the synthesis of ergosterol, which is necessary for the integrity of fungal cell membranes.

Antiviral activity

Bhumi Amla prevents the virus from entering host cells by disrupting the viral surface proteins' ability to attach to host receptors,. Furthermore, it inhibits enzymes necessary for the manufacture of viral genomes, including as DNA polymerase and reverse transcriptase, which slows viral replication. Further supporting the removal of viral infections are the immunomodulatory qualities of the plant, which strengthen the human immune response. Research has indicated that it is effective against many viruses, including the human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV). Inhibiting viral protein synthesis and lowering HBV DNA levels highlight Bhumi Amla's potential as a natural antiviral drug, providing a viable substitute for treating viral infections.[15]

Methods of Extraction and Testing

A. Extraction Methods

Using a Soxhlet system, Bhumi Amla is extracted by putting the solvent in a round-bottom flask and the powdered plant material in a thimble. In order to extract phytochemicals, the solvent continuously circulates through the plant material, vaporising, condensing, and dripping back into the apparatus.[16] The extracted phytochemicals are obtained by collecting the extracted solution and evaporating the solvent. This technique provides better yield, less solvent consumption, and effective extraction.[17]

B. Antimicrobial Testing

The Disc Diffusion Method is commonly used for testing byprepare microbial cultures on agar plates. On the agar, place filter paper discs that have been impregnated with Bhumi Amla extract. Measure the zone of inhibition after

incubation.**18**

MIC stands for minimum inhibitory concentration. Make Bhumi Amla extract serial dilutions. To every dilution, add microbial cultures. After incubating, find the MIC.**19**

Conclusion

The study shows that Bhumi Amla, also known as Phyllanthus Niruri, has been shown to have strong antibacterial and antifungal effects against a variety of microorganisms and other microbes. Gram-positive and gram-negative bacteria, such as E. Coli, Staphylococcus aureus, and Pseudomonas aeruginosa, as well as a variety of fungal strains, including Candida albicans and Aspergillus niger, have all been discovered to be susceptible to the plant's effects. Because bioactive compounds like phyllanthin, nirurin, and corilagin have been shown to inhibit the growth of microorganisms by inhibiting cell wall synthesis, disrupting cell membrane integrity, and interfering with DNA replication and transcription, Bhumi Amla is said to have antimicrobial activity.Due to this, Bhumi Amla may be used as a natural preservative in food and cosmetic products to prolong their shelf life and prevent spoiling, as well as a potential natural remedy for a variety of infections, such as respiratory, urinary, and skin and wound infections. Overall, study on Bhumi Amla's antimicrobial activity is important, and more investigation is required to completely grasp its potential as a natural antibacterial agent.

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COMPREHENSIVE REVIEW ON STREPTOCOCCAL TOXIN SHOCK SYNDROME(STSS) AND MECHANISM OF ACTION OF CLINDAMYCIN AND IVIG

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ABSTRACT

An extremely dangerous side effect of group A Streptococcus infection is streptococcal toxic shock syndrome, which has a high fatality rate. When treating these individuals, prompt identification, early intensive care assistance, and surgical management are crucial. The care of patients with streptococcal toxic shock syndrome depends on early detection and interdisciplinary management, which includes prompt determination of the infectious source or sources, surgical surgery, and appropriate and intensive support of failing organs. The identification and reporting of necrotizing fasciitis and other extremely invasive group A streptococcal infections linked to organ failure and shock. Streptococcal toxic-shock syndrome has been the term used to describe such extreme occurrences. The potentially fatal infectioncalled necrotizing fasciitis damages the fat and fasciabeneath the skin.A deadly infection known as neonatal sepsis can develop within the first 28 days of a neonate's life or, in the case of preterm newborns, up to 4 weeks after the anticipated due date. Neonatal sepsis is linked to a few factors. These include immune system immaturity, low birth weight, and preterm.A consequence of an invasive Streptococcus pyogenes

infection (IGAS), streptococcal toxic shock syndrome (STSS) is characterized by hypotension and end-organ failure, frequently accompanied by immunological symptoms like rash[6].Though perspectives differ on whether clindamycin should only be used in cases of streptococcal toxic shock syndrome (STSS), it is currently generally advised for usage in conjunction with a beta lactam antibiotic for severe iGAS infections. Intravenous immunoglobulin (IVIG) therapy has been proposed as a treatment adjunct that can reduce mortality. Our goal is to further demonstrate the clinical effectiveness of IVIG therapy provided to well-defined STSS patients in a comparative observational analysis.Group A gram-positive, beta-haemolytic bacterium is Streptococcus (GAS). From minor ailments like pharyngitis and impetigo to more serious conditions like necrotizing fasciitis and streptococcal toxic shock syndrome [STSS], it can cause a wide range of illnesses.

Keywords: *Streptococcal Toxic Shock Syndrome, Intensive care assistance, Streptococcal infection, Necrotizing fasciitis, Neonatal sepsis, Clindamycin, IVIG, Group A gram-positive, Adverse Effects of Clindamycin and IVIG.*

1.Introduction:

Streptococcal toxic shock syndrome (STSS) is a serious, potentially fatal illness that exacerbates invasive streptococcal infections, primarily caused by group A streptococcus. Infections with streptococci are common and can cause a wide range of illnesses, from self-limited pharyngitis to serious conditions such bacteraemia, pneumonia, meningitis, endocarditis, sinusitis, arthritis, and deep soft tissue infections like necrotizing fasciitis and myositis. The STSS is a serious side effect of group A streptococcal infections, which are primarily invasive, however other streptococcal species can also cause it less frequently [**Schmitz, Roux, Huttner and Pugin et.al.2018**]. A severe consequence of a streptococcus infection, streptococcal toxic shock syndrome (STSS) typically develops quickly and leads to multi-organ failure. Even in healthy people, it has a high death rate and is commonly linked to group A streptococcus infection. High fever, hypotension, generalized erythematous rash, and numerous

organ dysfunction are among the clinical signs, which can quickly escalate to severe and uncontrollable shock. Although the exact mechanism underlying this illness is unknown, it is thought to be a mix of enterotoxins with superantigen activity and the host's reaction to the streptococcal infection[**Miguel Silva et.al.2022**]. In 40–50% of individuals, renal impairment occurs prior to hypotension (8). Upon admission and during the hospital stay, hypoalbuminemia and hypocalcaemia are linked. Deeper soft-tissue infections can be identified with the help of the serum creatinine kinase level. Emerging Infectious Diseases Necrotizing fasciitis or myositis are strongly correlated with elevated or growing levels. The mean percentage of immature neutrophils (including band forms, metamyelocytes, and myelocytes) is a startling 40% to 50%, even though the preliminary laboratory results only show mild leucocytosis[**L. Stevens et.al.1995]**. Because of laboratory findings showing potentially positive benefits, such as neutralization of superantigens and improved bacterial clearance, some specialists advocate Polyspecific intravenous immunoglobulin (IVIG) as an adjuvant treatment for STSS. Clinical evaluation of IVIG for STSS has been challenging, nevertheless; the sole randomized controlled study (RCT) was terminated early because of sluggish recruitment. The interpretation of these findings is confounded by the inherent risk of bias, the varying inclusion criteria, and the uneven use of clindamycin, which is commonly recommended as an adjuvant to penicillin, despite the tiny number of non-randomized studies that have been reported. We conducted a comprehensive evaluation of studies that assessed the use of adjunctive IVIG in STSS, both randomized and non-randomized[**Parks, Wilson, Curtis, Norrby-Teglund and Sriskandan et.al.2018**].

2. Diseases Caused by Streptococcal Toxin Syndrome:

Necrotizing fasciitis :

An acute, potentially fatal infection of the soft tissue and fascia beneath the skin is called necrotizing fasciitis. Depending on the bacteria involved, the microbiologic incubation duration can range from one to four days. Although necrotizing fasciitis can be brought on by a single or a combination of virulent aerobic

and anaerobic bacteria, Group A beta-haemolytic *Streptococcus pyogenes* is responsible for most of the infection's morbidity and mortality. GAS-induced necrotizing fasciitis, either by itself or in conjunction with other organisms, is categorized as Type II, while Type I necrotizing fasciitis is defined as the presence of at least one group of streptococci (apart from group A) along with facultative anaerobes, anaerobic species, and/or *Enterobacteriaceae sp.* member[**Larose-Pierre, J.Scrivens, Norwood and Rappa et.al.2002**].The affected area turns bluish purple and bullae appear, causing the skin and subcutaneous tissue to necrotize. Patients with multiple organ involvement, such as disseminated intravascular coagulation (DIC), acute renal failure, and liver failure, may advance to STSS [J.Cawley, Briggs, R.Haith, **J.Reilly , E.Guilday, R.Braxton and Patton et.al.1999].**

Neonatal sepsis:

The symptoms of neonatal sepsis include lethargy, fever, hypothermia, bradycardia, apnoea, abdominal distention, lethargy, and neutropenia. Neonatal sepsis can be categorized as either late-onset (4–90 days of life) or early-onset (0–3 days of life). While late-onset sepsis happens to infants who contract the bacterial pathogen via a healthcare worker or the hospital environment, early-onset sepsis happens to infants who contract bacteria from moms who are infected and those who are not. Even if neonatal sepsis mortality has decreased because of existing preventative strategies[**AI-Haqan, S.Boswihi, Pathan and E. Udo et.al.2020**]. Magnetic resonance imaging (MRI) and/or computed tomography (CT) scans are typically helpful in determining the soft tissue origins of GAS infection and in distinguishing between fasciitis and skin infections. When in doubt, a fascia biopsy should be used for surgical revision; in cases of infection should be used, respectively[**Schmitz, Roux, Huttner and Pugin et.al.2018**].

3.Adverse Events:

- **Neuroautoimmune Disorders :**

Headache, abducens nerve palsy, posterior reversible encephalopathy syndrome (PRES), aseptic meningitis, and seizures are among the neurological conditions linked to

immunoglobulin therapy.A headache after IVIG is a typical side effect.Headaches occur in over 50% of individuals following immunoglobulin treatment.

[L. Geller and Hackner et.al.2005]

- **Renal Impairment :**

Serious adverse effects from IVIG include haemolytic anaemia, thrombosis, arrhythmia, aseptic meningitis, renal impairment, and transfusion-related acute lung injury (TRALI). These negative effects are linked to individual variations and certain immunoglobulin formulations.

[L. Geller and Hackner et.al.2005]

- **Cardiovascular Disorder :**

Many investigations have documented arrhythmias that develop during or after immunoglobulin infusion.These arrhythmias can include bradycardia and supraventricular tachycardia, and most of these people had a history of heart illness. Due to the small sample size and potential bias in patient selection, the overall incidence IVIG-related headache in patients with a history of migraine was low among these studies.

[Darenberg, Ihendyane, Sjo¨ lin, Aufwerber, Haidl, Follin Andersson and Norrby-Teglund et.al.2003].

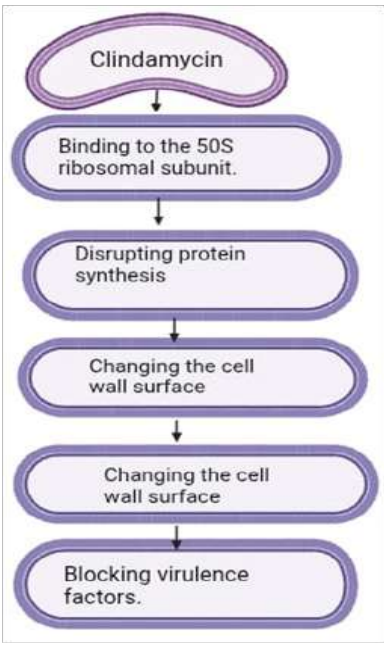
- **Haemolytic anaemia:**

Red blood cells can't be produced as quickly as they can be destroyed. The red blood cells' rupture.The spleen is where this most frequently happens, however it can also happen mechanically or in the reticuloendothelial system. Increased bilirubin excretion into the biliary tract might result from it, potentially leading to gallstones.Continuous free hemoglobin release has been connected to the onset of pulmonary hypertension, which in turn causes chest discomfort, syncope episodes, and increasing dyspnea.Eventually, pulmonary hypertension leads to right ventricular heart failure, which manifests as ascites and peripheral oedema.[**Schmitz, Roux, Huttner and Pugin et.al.2018**].

4. Mechanism Of Action:

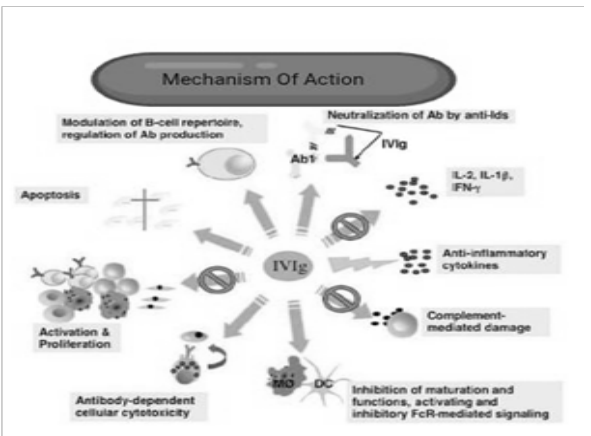
- **Clindamycin:**

Clindamycin suppresses the transcription and synthesis of several streptococcal proteins, including pyrogenic exotoxin superantigens, especially when given during early-log-phase growth. Clinical benefit in humans has only been demonstrated in retrospective case studies.As determined by the presence of STSS, length of hospital stay, and ICU admission, clindamycin-treated subjects were younger and had more severe disease than clindamycin-untreated cases. [Carapetis, Jacoby, Carville, Jeol Ang, Curtis and Andrews et.al.2014].A statistically significant decrease in mortality is linked to the delivery of adjunctive IVIG to patients with STSS receiving clindamycin. Importantly, our research separates the effects of IVIG and clindamycin, which has occasionally been troublesome [**Parks, Wilson, Curtis, Norrby-Teglund and Sriskandan et.al.2018**].



- IVIG :

Some specialists suggest Polyspecific intravenous immunoglobulin (IVIG) as an adjuvant treatment for STSS, in part due to laboratory studies showing potentially positive outcomes such as improved bacterial clearance and neutralization of superantigens. However, clinical evaluation of IVIG for STSS has been challenging; the sole RCT was terminated early because of sluggish recruitment . Even though there are only a few non-randomized trials available, the interpretation of these results is made more difficult by the possibility of bias, the varying inclusion criteria, and the uneven application of clindamycin, which is frequently recommended as a penicillin adjuvant. We conducted a comprehensive evaluation of studies that assessed the use of adjunctive IVIG in STSS, both randomized and non-randomized. [Parks, Wilson, Curtis, Norrby-Teglund and Sriskandan et.al.2018]. Intravenous immunoglobulin (IVIG) stimulates leukocytes, neutralizes superantigens and toxins, and lowers excessive immunological responses in streptococcal toxic shock syndrome (STSS).IVIg neutralizes the superantigens that the STSS-causing invasive streptococcus infection produces.T-cell proliferation and cytokine production are inhibited by IVIg. IVIg causes leukocytes to respond. IVIg has an all-encompassing anti-inflammatory action. [Carapetis, Jacoby, Carville, Joel Ang, Curtis and Andrews et.al.2014].



5.Conclusion :

A high death rate and extremely severe clinical condition, STSS is thought to be partially caused by group A Streptococcus's superantigen activity. This illness is deadly in part because of the patients' quick decline into multiple-organ failure, which necessitates intense care and organ support. Prompt antibiotic medication, surgical debridement, immunoglobulins, and hemoperfusion are some potential future therapeutic approaches that could help lower the high death rate.[**Miguel Silva et.al.2022**].IVIG seems to be a significant supplement to the usual treatment plan, even if the patient's favorable outcome was undoubtedly influenced by several clinical interventions and their young age. [**J.Cawley, Briggs, R.Haith, J.Reilly , E.Guilday, R. Braxton and Patton et.al.1999**]. Antibiotic resistance and immunological response (CRP) did not correlate. It advanced our knowledge of how virulence factors that cause newborn bacteraemia are carried in the central nervous system and antibiotic resistance**[Al-Haqan, S.Boswihi, Pathan and E. Udo et.al.2020**]. In summary, these findings imply that routine antibiotic prophylaxis for household contacts of cases is strongly supported, and that expanding the indications and putting policies in place to promote the use of clindamycin and IVIG in iGAS illness may lower mortality. **[Miguel Silva et.al.2022].**

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A COMPREHENSIVE STUDY - VAGINAL MICROBIOTAAND IT'S LINK TO PREGNANCY OUTCOMES

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ABSTRACT

Pregnancy outcomes are highly associated with the vaginal microbiome, which is essential for preserving reproductive health. This microbial community, which is primarily made up of Lactobacillus species in healthy people, produces lactic acid, hydrogen peroxide, and other antimicrobial substances that prevent the formation of harmful microbes and help to maintain an acidic vaginal environment. Negative pregnancy outcomes, such as preterm birth, miscarriage, and infections like bacterial vaginosis (BV), have been linked to dysbiosis, or an imbalance in the vaginal microbiota. New research emphasizes how important particular microbial patterns, immunological reactions, and metabolic interactions are in determining the course of pregnancy. Our understanding of these intricate interactions is being improved by developments in metabolomic profiling and sequencing technology, opening the door to new preventative, therapeutic, and diagnostic approaches that will maximize the health of mothers and newborns. To improve pregnancy outcomes globally, more research is needed to identify causal relationships and create therapies that target the microbiome.

This article highlights the concept of vaginal microbiota and its association with prevalence of microbial dysbiosis in pregnancy, preterm birth and gestational diabetes complications, screening methods for VMF, strategies for monitoring and restoring vaginal microbial balance and the need for standardized approaches for better understanding the relationship between the vaginal microbiota and pregnancy outcome.

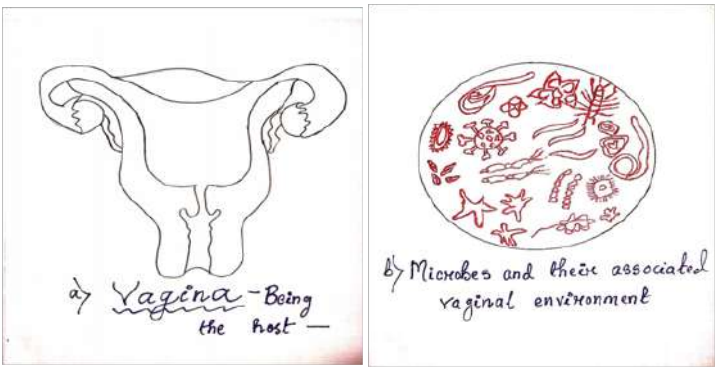

Fig1 (a and b) -Vaginal microbiota and its associated risk of causing dysbiosis

Keywords: - *Vaginal Microbiota, Microorganisms, Lactobacillus species, Dysbiosis, Preterm Birth, Miscarriage, Metagenomics, Probiotics*

Introduction to Vaginal Microflora and Its Species Community being Related to Pregnancy

The microbes that live in the vagina are referred to as the vaginal microbiota. These microbes have a major impact on a woman's overall and reproductive health. Lactobacillus species that produce lactic acid predominate in a low-diversity environment that is indicative of a healthy vaginal microbiome. Conditions like bacterial vaginosis can result from factors that upset the equilibrium of the vaginal microbiota, including the use of antibiotics, sexual activity, obesity, maternal age and hormonal changes. The vaginal microbiota's makeup shifts and becomes more significant during pregnancy, acting as a barrier to prevent infection for

both the mother and the foetus. Although the microbes that inhabit the vagina are significant, little is known about how variations in their diversity and composition may affect the course of pregnancy. This is particularly true for women who have a high Gardnerellavaginalis prevalence.

The approximately 1011 microorganisms, mostly bacteria, that reside on and within each person make up the human microbiota as a whole. (1) The vaginal microbial community (VMC), which is made up of all the bacteria that live in the vagina, is unique to women in terms of both composition and function. The digestive tract in humans has the greatest diversity and quantity of microorganisms, whereas the VMC is different in composition and typically exhibits a significantly lower diversity of microorganisms. (2) The health of women in general and women of reproductive age in particular is greatly influenced by the VMC. Beginning soon after birth and continuing until menopause, the vaginal consortium's makeup goes through several different stages. (3) Ovarian steroid hormone cycle causes changes in the vaginal environment of women of reproductive age, with estrogen being associated with higher glycogen availability (4,5). Because estrogen levels rise during gestation, these effects are exacerbated during pregnancy, and it has been demonstrated that the VMC during pregnancy differs markedly from that of women of childbearing age who are not pregnant. (6) The VMC community works with the host during pregnancy to give the growing foetus a barrier of defence against any infections. The pH of the environment can be lowered by converting the rich energy source from the breakdown of free glycogen by α-amylase into lactic acid. (7-9)Almost all of the species that produce lactic acid are members of the Lactobacillus genus. Culturing, microscopy, and molecular biology were initially explained by Döderlein (10) and then further developed by Rogosa et al. (11)

Techniques have revealed that members of the Lactobacillus genus (12) dominate the VMC. Lactobacillus species prevent colonization by other taxa, especially possible pathogens, by keeping their pH acidic below 4.5. High-throughput sequencing and other contemporary molecular approaches

revealed a clear structure in the VMC that was centred on Lactobacillus species. Using 16 s rDNA sequencing and analysis, Ravel et al. identified four main community sequence types (CSTs), with Lactobacillus crispatus (CST I), Lactobacillus gasseri (CST II), Lactobacillus iners (CST III), and Lactobacillus jensenii (CST V) predominating. A fifth CST was characterized by a comparatively higher proportion of anaerobic organisms, such as the species Gardnerella vaginalis (CST IV), and a lower proportion of bacteria that produce lactic acid. (13).

The frequency of Gardnerella vaginalis has long been thought to be a dysbiotic CST that is frequently linked to bacterial vaginosis (BV) and increased health concerns. One prevalent type of dysbiosis that affects women in their reproductive years is bacterial vaginosis. Reduced Lactobacillus abundance in the VMC, which raises pH > 4.5 and causes a corresponding rise in diversity (14), is a characteristic of BV. Most frequently, BV is linked to a high concentration of Gardnerella vaginalis in CST IV, which is frequently accompanied by an increase in anaerobes, Bacteroides, and Prevotella as well as a variety of other possible pathogens, such as Ureaplasma and Gram-positive Mobiluncus and Megasphaera. (15–17)

A baby born before 37 weeks of pregnancy is referred to as a preterm birth. Preterm births account for 11.1% of live births worldwide and are a major contributor to infant mortality and morbidity (18). Preterm birth problems are directly responsible for around one million fatalities among children under the age of five (19). Preterm infants may experience respiratory issues, feeding issues, and developmental delays, and they frequently need specialized medical care in neonatal intensive care units. Our knowledge of the cause of preterm delivery is still incomplete, with the exception of the 20% of preterm deliveries that are medically induced. A number of characteristics, such as numerous pregnancies, age, smoking, racial origin, high or low maternal BMI, and others, make it challenging to determine risk, but infections seem to be responsible for 30–40% of spontaneous preterm births (19). As previously mentioned, the vaginal microbiota's defence against infection is strengthened during pregnancy as

hormonal shifts lead to Lactobacilli's increased dominance (20). Along with its protective function, the VMC is probably also in charge of the newborn's first significant microbe colonization event, which happens during vaginal delivery; some of the initial components of the newborn's oral and intestinal microbiota are transferred through the birth canal (21,22). Contrary to the gut microbiota's strong diversity, it has long been believed that the ideal condition during pregnancy is one of low diversity and dominance by a single Lactobacillus species. However, it has recently been documented that the vaginal microbiota's structure changes and becomes more diverse in late-term pregnancy, which may help with this first colonization step (23).

In a mouse model, another study showed that the human VMC's composition had a discernible impact on the health and microbiota of the offspring (24). More focus has been placed on this community, its makeup during pregnancy, and its correlation with perinatal illnesses and premature birth because a number of VMC species are early colonizers of the neonatal microbiota at delivery. There is great interest in learning how changes in the composition of the VMC (25) are associated with the risk of adverse pregnancy outcomes, regardless of whether this is because of BV or another kind of disturbance to the normal vaginal microbiota. Certain organisms have been linked to an increased risk of preterm delivery, early amniotic membrane rupture prior to delivery and gestational diabetes.

Here, we delve deeply into how the vaginal microbiota, its diversity, and the frequency of various community types relate to the outcome of pregnancy.

Role of Lactobacillus in maintaining Vaginal Health

Because of their protective properties and dominance in the vaginal microbiome, Lactobacillus species are essential for maintaining vaginal health:

1. Lactic Acid Production: Lactobacilli produce lactic acid, which prevents the growth of harmful bacteria and fungi and keeps the vaginal pH acidic (between 3.8 and 4.5).

2. Antimicrobial Compounds: They generate bacteriocins and hydrogen peroxide ($H2O_2$), which further inhibit pathogenic microorganisms and ward against illnesses including yeast infections and bacterial vaginosis (BV).

3. Attachment to Vaginal Epithelium: By vying for attachment sites, Lactobacilli prevent harmful microorganisms from colonizing the vaginal environment.

4. Immunological Modulation: By influencing the generation of cytokines, lowering inflammation, and encouraging a well-rounded immunological response, they control the vaginal immune system.

5. Preserving Microbial Balance: Lactobacillus contributes to the stability and health of the vaginal microbiota by outcompeting other bacteria for resources and space.

6. Support During Pregnancy: A microbiota dominated by Lactobacillus is linked to improved pregnancy outcomes, such as a lower risk of infections and preterm birth.

Factors influencing composition of Vaginal Microbiota during Pregnancy

1. Hormonal Changes:Increased levels of estrogen and progesterone during pregnancy promote the growth of Lactobacillus species. These bacteria maintain a low vaginal pH (around 3.5–4.5), creating an environment unfavourable for pathogenic microbes.

2. pH Alteration: The acidic environment during pregnancy supports Lactobacillus dominance while inhibiting anaerobic and pathogenic bacteria, such as Gardnerella vaginalis and Prevotella.

3. Immune Modulation: Pregnancy involves changes in the immune system to prevent fatal rejection. This can influence microbial balance by reducing inflammation and allowing beneficial microbes to thrive.

4. Nutrient Availability: Increased glycogen levels in vaginal epithelial cells, driven by oestrogen, provide a food source for Lactobacillus, fostering their growth and metabolic activity.

5. Antimicrobial Peptides: The production of antimicrobial peptides, such as defensins, helps regulate the microbial community by limiting overgrowth of harmful bacteria.

6. Genetic Factors: Individual genetic predisposition can affect immune responses, vaginal epithelial function, and microbial composition.

7. Sexual Activity: Exposure to semen and other external factors during sexual activity can introduce new microbes, potentially influencing the vaginal microbiome.

8. Hygiene Practices: Use of vaginal douches or other hygiene products can disrupt the microbial balance by altering pH or removing beneficial microbes.

9. Antibiotic Use: Antibiotics prescribed during pregnancy can unintentionally reduce beneficial microbes, leading to dysbiosis.

10. Health Conditions: Conditions like gestational diabetes or bacterial vaginosis can significantly impact the composition and stability of the vaginal microbiota.

Pregnancy outcomes influenced by Vaginal Microbiota

1. **Preterm Birth:** - PTB is a leading cause of neonatal morbidity and mortality worldwide. While there are many factors that contribute to preterm birth, an emerging area of research has focused on the role of vaginal microbiota. The undermentioned mechanisms influence the dysbiosis phenomenon during pregnancy influencing the risk of preterm birth.

Mechanismsinvolved:

1. Inflammation and Vaginal Microbiota: Dysbiosis of the vaginal microbiota, such as reduced Lactobacillus dominance and increased anaerobic bacteria (Gardnerella vaginalis, Prevotella), triggers inflammation. These microbes produce metabolites and stimulate the release of pro-inflammatory cytokines (e.g., IL-6, TNF-α), leading to uterine contractions and preterm labour.

2. Infection Ascension: Pathogens from the vagina, facilitated

by a disrupted microbiota, ascend into the uterus and infect the amniotic fluid or placenta. This intrauterine infection activates inflammatory pathways, increasing the risk of fatal injury and preterm delivery.

3. Cervical Remodelling: Altered microbiota induces the production of matrix metalloproteinases (MMPs) through inflammation, which degrade cervical collagen. This premature cervical softening and dilation contribute to preterm labour.

4. Fetal MembraneWeakening: Bacterial byproducts and inflammation weaken the foetal membranes by degrading extracellular matrix proteins. Increased oxidative stress from dysbiosis further compromises membrane integrity, leading to preterm rupture of membranes (PROM).

5. Integrated Risk: The interplay between microbiota imbalance, immune activation, and tissue damage creates a cascade that culminates in preterm birth. Addressing vaginal dysbiosis may reduce these risks.

2. **Gestational Diabetes:** - It is a condition characterised by elevated blood sugar levels that develop during pregnancy in women who did not previously have diabetes, also influenced by the variation in composition of vaginal microbiota. It typically occurs in the second or third trimester and results from hormonal changes that impair the body's ability to effectively use insulin.

Mechanisms involved: -

1. Altered Microbial Composition: Pregnancy-induced changes in gut and vaginal microbiota, such as reduced diversity and increased pathogenic bacteria, disrupt glucose metabolism. Dysbiosis can impair gut barrier integrity, allowing microbial endotoxins (e.g., lipopolysaccharides, LPS) to enter the bloodstream and trigger systemic inflammation, promoting insulin resistance.

2. Inflammation: Dysbiosis-induced inflammation activates immune pathways, releasing cytokines like TNF-α, IL-6, and CRP. These cytokines interfere with insulin signalling in skeletal muscle, liver, and adipose tissues, exacerbating

glucose intolerance and insulin resistance.

3. Insulin Resistance: Elevated pregnancy hormones (e.g., human placental lactogen) already increase insulin resistance to ensure fetal glucose supply. Inflammation and altered microbial metabolites further impair insulin sensitivity, leading to hyperglycaemia.

4. Metabolic Changes: Changes in short-chain fatty acids (SCFAs) produced by gut microbes affect energy balance and insulin secretion. Dysbiosis reduces beneficial SCFAs, worsening insulin dysregulation and contributing to gestational diabetes.

5. Integrated Risk: The combined effects of microbial imbalance, inflammation, and metabolic disruption create a vicious cycle, increasing susceptibility to gestational diabetes. Addressing these factors can help mitigate risks.

3. Miscarriage: - The vaginal microbiota plays a significant role in pregnancy outcomes, including the risk of miscarriage; the spontaneous loss before 20 weeks of gestation. Several mechanisms influence this risk of miscarriage which is discussed under forth.

Mechanisms involved: -

1. Inflammation and Immune Dysregulation: - Dysbiosis reduces protective Lactobacillus dominance and promotes the overgrowth of pathogenic bacteria (e.g., Gardnerella vaginalis, Prevotella species). These pathogens trigger the release of pro-inflammatory cytokines (e.g., IL-6, TNF-α) and chemokines, leading to local inflammation in the uterus. Disruption of maternal-fetal tolerance, causing rejection of the embryo or fetus.

2. Infection and Ascension of Pathogens: - Pathogens from the vagina can ascend into the uterus, causing: a) Chorioamnionitis: Infection of the fetal membranes; b) Endometritis: Uterine lining infection.

These infections damage the uterine environment and trigger miscarriage through premature rupture of membranes or fetal compromise.

3. Cervical Remodelling and Weakening: - Vaginal dysbiosis increases the production of matrix metalloproteinases (MMPs), which degrade collagen in the cervix. Premature cervical softening or dilation leads to cervical insufficiency, increasing the risk of miscarriage.

4. Hormonal Disruption: - Dysbiosis alters vaginal pH and produces harmful metabolites, disrupting hormonal signalling critical for pregnancy maintenance, such as progesterone.

5. Oxidative Stress: - Pathogens from dysbiosis produce reactive oxygen species (ROS), causing oxidative stress in the uterine environment. This damages the placenta and fetal membranes, leading to pregnancy loss.

The disruption of vaginal microbiota influences miscarriage through a cascade of infection, inflammation, immune activation and tissue damage.

4. Pre-eclampsia: - It is a hypertensive disorder that occurs during pregnancy, typically after 20 weeks of gestation, characterised by high blood pressure and signs of damage to organs such as the kidneys and liver. This is also a result of the altered vaginal microbiota composition being influenced by various mechanisms.

Mechanisms involved: -

1. Systemic Inflammation: Dysbiosis, particularly the reduction of Lactobacillus species and overgrowth of pathogenic bacteria (e.g., Gardnerella vaginalis, Prevotella), triggers systemic inflammation. This results in the release of pro-inflammatory cytokines like IL-6, TNF-α, and CRP, which impair endothelial function and increase vascular permeability, key components in the pathogenesis of eclampsia.

2. Endothelial Dysfunction: Altered vaginal microbiota can contribute to endothelial dysfunction by stimulating the production of anti-angiogenic factors (e.g., soluble FM's-like tyrosine kinase-1 or sFlt-1). This disrupts normal placental development and blood vessel formation, causing poor placental perfusion and leading to the hypertension seen in

pre-eclampsia and eclampsia.

3. Oxidative Stress: Dysbiosis increases the production of reactive oxygen species (ROS) from pathogenic bacteria, which causes oxidative stress in the maternal circulation. Oxidative stress is a key mediator in vascular damage, endothelial dysfunction, and the development of hypertension, contributing to the onset of eclampsia.

4. Immune Activation and Pregnancy Complications:An imbalanced vaginal microbiota affects immune regulation, leading to an exaggerated immune response during pregnancy.This dysregulated immune response can result in heightened inflammation, placental damage, and increased risk of pregnancy complications like eclampsia.

5. Altered Hormonal Signalling:Vaginal dysbiosis affects the local immune environment and can disrupt the balance of hormones such as progesterone and estrogen, which are critical for maintaining vascular health.

Hormonal imbalances may exacerbate blood pressure dysregulation, contributing to the development of severe complications such as eclampsia.These mechanisms show how vaginal dysbiosis can increase the risk of eclampsia through inflammation, immune dysfunction, oxidative stress, endothelial damage, and hormonal disruption.

Analysing The Vaginal Dysbiosis During Pregnancy

The phenomenon of vaginal dysbiosis can be analysed mainly by carrying out Metagenomics study: -

Metagenomics in the context of analysing vaginal microbiota, refers to the study of the collective genetic material of all microorganism present in the vaginal environment. This approach provides a comprehensive understanding of the composition, diversity and functional potential of the vaginal microbial community without the need for culturing individual species.

Mechanism involved

The metagenomics process for analysing vaginal microbiota during pregnancy involves several systematic steps. Here's

an overview:

1. Sample Collection; Purpose: Obtain microbiota samples from the vaginal environment.

Method: Sterile swabs are used to collect vaginal secretions.

Samples are stored in appropriate media to preserve microbial DNA.

2. DNA Extraction; Purpose: Isolate microbial DNA from the collected samples.

Steps: Lysis of cells to release DNA.

Removal of proteins, lipids, and other contaminants.

Purification of DNA for further analysis.

3. Library Preparation; Purpose: Prepare DNA for sequencing.

Steps: Fragmentation of DNA into manageable sizes.

Addition of adapters or barcodes to each DNA fragment.

Amplification using PCR to ensure sufficient DNA quantities.

4. Sequencing; Purpose: Determine the genetic sequences of the DNA.

Technologies: 16S rRNA Sequencing: Targets bacterial ribosomal RNA genes to identify bacterial species.

Whole Genome Sequencing (WGS): Analyses the entire genetic content of all organisms present.

Platforms: Illumina, Oxford Nanopore, PacBio, etc.

5. Bioinformatics Analysis; Purpose: Analyse the sequencing data to identify microbial composition.

Steps: Quality control to filter out low-quality reads.

Alignment of sequences against reference databases (e.g., SILVA, Green genes).

Taxonomic classification and identification of microbial species.

Functional analysis to determine microbial roles.

6. Data Interpretation; Purpose: Relate microbial profiles to pregnancy health outcomes.

Aspects: Identification of beneficial and pathogenic microbes.

Correlation with gestational age, infections, or pregnancy complications.

7. Reporting; Purpose: Summarize findings for clinical or research purposes.

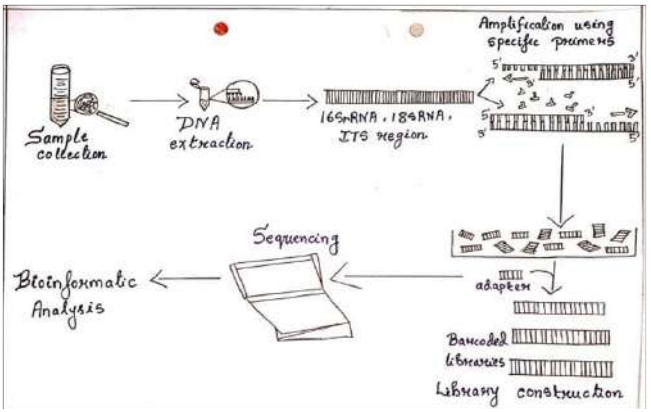

Fig 2 - The basic metagenomic process for analysing vaginal microbiota is carried out in the above outlined process

Application of Metagenomics on Vaginal Health during Pregnancy

1. Monitoring Vaginal Microbial Dynamics Role of Lactobacillus:

During pregnancy, a healthy vaginal microbiota is often dominated by Lactobacillus species, which maintain a low vaginal pH by producing lactic acid. Metagenomics can monitor the abundance of these beneficial bacteria.

Detecting Dysbiosis: Metagenomic sequencing can identify imbalances (dysbiosis) in the vaginal microbiota, such as overgrowth of pathogenic bacteria linked to bacterial vaginosis (BV) or yeast infections. These conditions may increase the risk of preterm birth or other complications.

2. Early Detection of Infection Risks

Preventing Preterm Birth: Dysbiosis has been associated with an increased risk of preterm labour. Metagenomics can identify specific microbial patterns or biomarkers linked to infection or inflammation, allowing for timely interventions.

Pathogen Identification: Metagenomics can detect and characterize pathogens such as Gardnerella vaginalis or Candida species, even if they are non-culturable, ensuring accurate diagnosis and treatment.

3. Personalized Probiotic and Prebiotic Strategies

Probiotic Development: By understanding the composition of a healthy vaginal microbiota, metagenomics can guide the development of targeted probiotics to restore or maintain a beneficial microbial balance during pregnancy.

Prebiotic Interventions: Insights from metagenomics can help identify dietary or supplemental strategies to promote the growth of beneficial bacteria like Lactobacillus.

4. Tailored Antimicrobial Treatments

Reducing Antibiotic Misuse: Metagenomics provides a detailed understanding of the microbial community, reducing the unnecessary use of broad-spectrum antibiotics, which can disrupt the vaginal microbiota and harm beneficial bacteria.

Specific Pathogen Targeting: Enables precise treatment of specific infections while preserving overall microbial diversity.

5. Supporting Maternal and Neonatal Outcomes

Foetal Development: A healthy vaginal microbiota during pregnancy reduces the risk of infections ascending into the uterus, protecting foetal development.

Birth Canal Microbiota: During vaginal delivery, the newborn is exposed to the mother's vaginal microbiota, which shapes their initial microbiome. Maintaining a healthy vaginal microbiota ensures optimal microbial seeding.

6. Research on Pregnancy-Associated Conditions

Preeclampsia and Inflammation: Metagenomics can explore potential links between vaginal microbiota and systemic conditions like preeclampsia, enabling new diagnostic and therapeutic approaches.

Gestational Diabetes: Emerging evidence suggests that vaginal and gut microbiota interact; metagenomics can uncover connections and offer insights into metabolic health during pregnancy.

By providing a detailed understanding of vaginal microbial ecosystems, metagenomics can revolutionize the management of vaginal health during pregnancy, ensuring better outcomes for both the mother and baby.

Therapeutic Interventions for maintaining Vaginal Health during Pregnancy

Maintaining optimal vaginal health during pregnancy is essential for reducing the risk of complications such as preterm birth, infections, and adverse neonatal outcomes. Therapeutic interventions focus on restoring and preserving a healthy vaginal microbiota dominated by Lactobacillus species. Here are key therapeutic approaches:

1. Probiotics

Purpose: Replenish beneficial Lactobacillus species that maintain a low vaginal pH and inhibit pathogenic microorganisms.

Examples:Lactobacillus crispatus, Lactobacillus rhamnosus, Lactobacillus reuteri

Delivery Methods:

Oral probiotics: Provide systemic support and gut-to-vagina microbial transfer.

Vaginal suppositories: Directly introduce probiotics to the vaginal environment.

Evidence: Probiotics have shown promise in reducing the recurrence of bacterial vaginosis (BV) and improving vaginal

microbial balance during pregnancy.

2. Prebiotics

Purpose: Stimulate the growth of beneficial bacteria by providing nutrients (e.g., inulin, fructoseoligosaccharides).

Mechanism: Prebiotics create a favourable environment for Lactobacillus species while inhibiting harmful pathogens.

Use in Combination: Often paired with probiotics for enhanced effectiveness (symbiotic therapy).

3. Antibiotic Therapy

Targeted Use:For treating bacterial vaginosis (BV) or sexually transmitted infections (STIs) like chlamydia and gonorrhoea.

Common antibiotics include metronidazole and clindamycin.

Precautions:Must be prescribed carefully during pregnancy to avoid disrupting the vaginal microbiota and causing antibiotic resistance. Probiotic co-administration is often recommended to restore microbiota post-treatment.

5. *Antifungal Therapy*

Purpose: Treat vaginal yeast infections (e.g., caused by Candida albicans).

Medications:Topical antifungals (e.g., clotrimazole or miconazole) are preferred during pregnancy.

Oral antifungals are usually avoided due to potential risks to the fetus.

Complementary Strategies: Probiotic use can help prevent recurrence.

5. Hormonal Regulation

Role of Oestrogen:Oestrogen levels during pregnancy promote the growth of Lactobacillus by increasing glycogen production in vaginal epithelial cells.

Hormonal therapies are generally avoided during pregnancy but may play a role postpartum.

6. Dietary and Lifestyle Modifications

Healthy Diet:Include fermented foods (e.g., yogurt, kefir) that naturally contain probiotics.High-fibre foods support overall gut and vaginal health.

Hygiene Practices:Avoid douching or using harsh chemicals that can disrupt the vaginal microbiota. Wear breathable, cotton-based undergarments.

Stress Reduction: Chronic stress can alter the immune response and microbiota balance.

7. Vaginal pH Modulation

Purpose: Maintain an acidic vaginal environment to deter pathogen growth.

Methods:Lactic acid gels or suppositories.Regular monitoring of pH during prenatal care.

8. Immunotherapy and Microbiota Transplantation (Emerging Therapies)

Vaginal Microbiota Transplantation (VMT):Involves transferring microbiota from a healthy donor to restore a balanced vaginal ecosystem. Still experimental but promising for women with recurrent dysbiosis.

Immune-Modulating Therapies:Focus on enhancing maternal immunity to support a healthy microbiota.

9. Regular Prenatal Care

Frequent monitoring; of vaginal health during prenatal visits ensures early detection and management of imbalances or infections.

Therapeutic interventions should always be tailored to individual needs, and all treatments must be prescribed under medical supervision to ensure safety for both the mother and foetus.

Conclusion

In conclusion, maintaining vaginal health during pregnancy is essential for ensuring maternal and foetal well-being,

as the vaginal microbiota plays a pivotal role in protecting against infections and maintaining reproductive health. The intricate interaction between host factors, such as hormonal changes, and microbial communities, particularly Lactobacillus species, highlights the importance of a balanced vaginal environment. Disruptions to this balance can lead to infections and complications, including preterm birth. The metagenomic study can be carried out on a proficient level for understanding the causal relationship of host-microbe interaction in the vaginal microbiota and implementing it's analysed data for therapeutic development and decreasing the risk of vaginal dysbiosis to occur.By adopting healthy practices, such as proper hygiene, a balanced diet rich in probiotics, and regular prenatal care, women can support the stability of their vaginal microbiota. Understanding the symbiotic relationship between the host and microbial communities emphasizes the need for proactive measures to preserve vaginal health and promote a safe pregnancy outcome.

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IMPLICATION OF ALTERNATIVE DRUG SOURCESTO COMBAT AGAINST FISH FUNGAL PATHOGEN SAPROLEGNIA PARASITICA

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ABSTRACT

Saprolegnia parasitica is an important fish fungal pathogen, causing a significant economic challenge in aquaculture. This present review shares insights into the development of effective pharmacological componentsto restrictSaprolegnia parasiticamediated infections. Pathogenic oomycetes can infect various plant and animal hosts, leading to several diseases that significantly impact the economy. Saprolegniasis is a condition that impacts young fish and eggs in hatcheries around the globe. This condition is brought about by the harmful oomycete known as Saprolegnia parasitica. As preliminary symptoms of the disease, greyish-white marks of filamentous mycelium appear on the different parts of the fish body, including fins. Such changes promote tissue damage and even death of the organism. Saprolegniasis can be managed with Malachite green, but due its harmful and cancer-causing effects on human its application was prohibited since 2002. However, ban of Malachite green has led to enhanced occurrence of saprolegniasis in fish affecting the entire fisheries industry once again, thus upsetting the fishery associated global economic status. Under such circumstances, exploration of alternative ways to manage

the pathogen Saprolegnia parasiticaseems to be relevant and crucial as well. Thus, the present reviewis an attempt to gather the available information on the topic keeping heed attention to discuss the probable management strategies and possible drug targets that can be brought into account to prevent Saprolegniasis.

Keywords: *Saprolegniaparasitica,Saprolegniasis, Oomycete, Fish Pathogen, Fungal Disease, Alternativedrug sources*

1. INTRODUCTION:

Oomycetes are a diverse group of filamentous organisms that were once classified as fungi due to their structural similarities and growth patterns. However, advancements in genetic and biochemical research have reclassified them under the group Stramenopiles, which also includes diatoms and kelp [**Kamoun, 2003**].The three subclasses of oomycetes, Saprolegniomycetidae, Rhipidiomycetidae, and Peronosporomycetidae, are capable of infecting a variety of hosts starting from vertebrate animals to economically significant plants [**van West P, 2006**]. While sexual and physical traits have historically been used to categorize the different species belonging to the genus(Saprolegnia), current molecular characterization using ribosomal DNA (rDNA) repeat revealedthephylogenetically divergent status of the genus. Species of Saprolegnia include S. parasitica, S. australis, S. ferax, and S. diclina [**Molina et al., 1995**]. Among these, S. parasitica is considered as a potential thread to the aquaculture industry. Frequently referred to as cotton mold, because of its unique characteristic white or, gray fibrous patchy appearance they form, Saprolegnia species are common water molds. The fish-pathogenic oomycete Saprolegnia parasiticais evident to cause a disease state known as saprolegniasis, that can affect fish, amphibians, and other aquatic species, leading to substantial financial losses in aquaculture that consequently declines wild fish populations and their worldwide distribution gets hampered. Saprolegniasis affects mainly incubating eggs and aquaculture broodfish where it is prevalent in the hatched salmon population. Estimated values further indicated that about 10% of the freshly hatched salmon succumb to the disease, causing significant losses to

the aquaculture industry that supplies a significant portion of fish worldwide for human consumption [**Molina et al., 1995**]. With over 80% of aquaculture production originating in Asia, saprolegniasis is common in tropical systems [**Karunasagar et al., 2003**]. In freshwater environments, Saprolegnia parasitica is a pervasive water mold and a major cause of fish disease. It can be found on both living and decaying organisms, often consuming saprophytes that decompose organic debris in water.Saprolegnia parasitica reproduces asexually through zoospores and sexually. Asexual stages are associated with saprolegniasis onset. Nutrient depletion triggers sporulation, forming sporangia at hyphal tips that release primary biflagellate zoospores, which infect host fish. These zoospores encyst into primary cysts, releasing motile secondary zoospores. Secondary cysts then produce laterally biflagellate zoospores, considered the infectious stage [**Andersson and Cerenius, 2002**].

Symptoms of saprolegniasis include sunken eyes, depigmented skin, hemorrhagic ulceration, cotton-like growths on the host's or eggs' skin and gills, and erosion of the skin, fins, gills, and muscles. Systemic mycosis affecting the liver, spleen, eyes, and kidney is also observed. S. parasitica has been evident to enter the body of adult fish through the epidermal tissues present either in the head or, in the fins and then spread throughout the entire body. However, in eggs it causes profuse mycelial development and enhances the probability of mortality of the fish [**van West et al., 2010**].Possible triggers for saprolegniasis include low water temperatures, skin injuries, crowded living conditions, contamination, and bacterial or viral infections.

Treatments for saprolegniasis include potassium permanganate, copper sulfate, new methylene blue, malachite green, benzalkonium chloride, and saltwater therapies. Azelaic acid, sulfonamide sulfamethoxazole, and chloramphenicol are also effective in inhibiting Saprolegnia parasitica growth [**Kumar et al., 2020**]. Additionally, antibiotics like Cladomarine from Penicillium coralligerum and Quellenin from Aspergillus sp. exhibit anti-Saprolegnia properties. Notably, Saprolegnia parasitica does not affect humans [**Kumar et al., 2020**]. Application of Malachite green

was found to be beneficial in restricting the growth of S. parasitica in aquaculture; however, concerns regarding its toxic and carcinogenic effects led to the ban of this chemical globally since 2002[**Torto-Alalibo et al., 2005**]. Thus, its high time to generate useful data for the better understanding of the physiology of the parasite and its modes of pathogenesis. Moreover, knowledge on its different developmental stages and host selection criteria will help in designing an effective way to restrict the progression of saprolegniasis. Now a days, plant-based extracts comprising specific phytochemicals, as a safer and eco-friendly alternative, are suggested as beneficial options due to their potent antifungal properties. In addition, knowledge on the immunological response of the host fish will encourage to develop S. parasiticaantibodies and may also contribute to the development of vaccines as early precautious measure to combat and detect saprolegniasis.

2. ROUTE OF INFECTION AND PATHOGENESIS OF *Saprolegnia parasitica:*

In freshwater fish, a fungal pathogen (Saprolegnia parasitica) is the primary host of the water mold that causes saprolegniosisprimarily in different fish species where it inducesepidermal deterioration and promotes macrophage recruitment[**van West et al., 2010**]. S. parasitica has been evident to produce an eicosanoid precursor, arachidonic acid, which is known to downregulate MH II by reducing fish macrophage activity [**Torto-Alalibo et al., 2005**]. Usually, the characteristic gray or, cottony white marks throughout the body includingfins, gills, and skin of fish confirms the infection of this virus. It is a major threat to aquaculture as well as wild fish populations, especially salmon and trout[**Kales et al., 2007**]. The pathogen Saprolegnia parasitica is known for its flexibility and wide-ranging dispersion because it can tolerate a wide range of water and temperature conditions. Reports suggested that, S. parasitica has been often found toact as a major pathogen, that rather being a secondary one.

Symptoms of Saprolegnia parasitica infections include organ failure, respiratory failure, and hemodilution. Most of the time, they can be fatal. When seen under a microscope, fish with morphology exhibit long, branching filaments known as

hyphae, as well as skin, gills, and fins coated with cotton-like white or, gray patches. It has the ability to reproduce both sexually and asexually. In asexual reproduction, movable spores called zoospores are created and released into the water. It is possible for these spores to infect new hosts. Oospores that are resistant to weather and have strong walls are produced by sexual reproduction [**Torto-Alalibo et al., 2005; Kales et al., 2007; Rezinciuc et al., 2018**].

Saprolegniosis is caused by pathogenicity, which primarily affects fish. The infection may cause significant mortality in fish populations, both wild and farmed. In its life cycle, the mold passes through stages where it attaches itself to the host, penetrates the tissues, and matures into sporangia that release zoospores to disperse the infection.In aquatic environments, Saprolegnia parasitica poses a serious threat due to its virulence. It adheres to stressed fish tissues, penetrates the skin, gills, and fins, and releasing enzymes like lipases and proteases to break down host tissues for deeper penetration and nutrient absorption.It can modulate the immune response of the host organism and thus, maintains the state of infection along with increasing disease severity[**Torto-Alalibo et al., 2005; Kales et al., 2007**]. This includes protection from being discovered and destroyed by the host's immune cells. It is more likely to infect more hosts as it possesses the capacity to disseminate swiftly in water because of the production of motile zoospores. Detailed ultramicroscopic studies on S. parasitica confirmed the presence of elongated hooked hair bundles (10.14±1.40 μm) in the cysts and cell wall that might have some implication in its pathogenesis [**Rezinciuc et al., 2018**]. Its resilience and spread across many aquatic environments are facilitated by its capacity to tolerate a wide range of temperatures and water conditions reproduction [**Rezinciuc et al., 2018**].

3. DIVERSE FUNCTIONAL PROTEINS OF *Saprolegnia parasitica:*

Sl. No.	Protein	Function	Reference
1	SpHtp1 RxLR effector protein Htp1	Binds to a tyrosine –o-fish cell surface ligand	Choudhury A, Kumar P, Nafidi HA. *et al.* (2024) Immunoinformatics approaches in developing a novel multi-epitope chimeric vaccine protective against *Saprolegnia parasitica*. Scientific Reports 14, 2260.
2	SpHtp3	Binds to the host cell	Trusch F, Loebach L, Wawra S. *et al.* (2018) Cell entry of a host-targeting protein of oomycetes requires gp96. Nature Communications 9, 2347.
3	SPRG19320 Spondin-like TSP1 domain-containing protein	It encodes a 936 amino acid protein	Jiang, R. H., de Bruijn, I., Haas, B. J., Belmonte, R., Löbach, L., Christie, J., ... & van West, P. (2013) Distinctive expansion of potential virulence genes in the genome of the oomycete fish pathogen Saprolegnia parasitica. PLoS Genetics, 9(6), e1003272.
4	SPRG_18636 Fibronectin type-III domain-containing protein	It encodes a 562 amino acid protein	Rezinciuc S, Sandoval-Sierra JV, Ruiz-León Y, van West P, Diéguez-Uribeondo J (2018) Specialized attachment structure of the fish pathogenic oomycete *Saprolegnia parasitica*. PLoS ONE 13(1): e0190361. https://doi.org/10.1371/journal.pone.0190361
5	SPRG_05384 Protein-tyrosine-phosphatase	It encodes a 6547 amino acid protein	Rezinciuc S, Sandoval-Sierra JV, Ruiz-León Y, van West P, Diéguez-Uribeondo J (2018) Specialized attachment structure of the fish pathogenic oomycete *Saprolegnia parasitica*. PLoS ONE 13(1): e0190361. https://doi.org/10.1371/journal.pone.0190361

6	SPRG_19863 GPS domain-containing protein	It encodes a 9440 amino acid protein	Rezinciuc S, Sandoval-Sierra JV, Ruiz-León Y, van West P, Diéguez-Uribeondo J (2018) Specialized attachment structure of the fish pathogenic oomycete *Saprolegnia parasitica*. PLoS ONE 13(1): e0190361. https://doi.org/10.1371/journal.pone.0190361
7	SPRG_03974 Fibronectin type-III domain-containing protein	It encodes a 5297 amino acid protein.	Rezinciuc S, Sandoval-Sierra JV, Ruiz-León Y, van West P, Diéguez-Uribeondo J (2018) Specialized attachment structure of the fish pathogenic oomycete *Saprolegnia parasitica*. PLoS ONE 13(1): e0190361. https://doi.org/10.1371/journal.pone.0190361

4. ROLE OF FUNCTIONAL PROTEINS IN PATHOGENESIS:

SpHtp 1 is known to bind to a tyrosine-o-fish cell surface ligand [**Choudhury et al., 2024**].Numerous proteins have been reported to participate in a cascade to exert the pathogenicity of the S. parasitica. Usually, S. parasitica translocate its RxLR ('x' encodes any amino acid)effector protein SpHtp1, a putative protein controlled by the host targeting protein 1 (Sphtp1), in the body of the host fish. Elevated expression of this SpHtp1protein has been noted prior to the initiation of the infection and during the initial stages of pathogenesis [**Epstein and Nicholson, 1997**]. Similarly, SpHtp 3 is evident to bind the host cell [**Trusch et al., 2018**], whereas SPRG19320 helps in the formation of the secretome [**Jiang et al., 2013**]. Secretome formation is an important stage for pathogenesis caused by S. parasitica,where five functional proteins have been evidenced to be upregulated relatively higher in the cysts in comparison to the mycelia. These five proteins viz. SPRG_18636, SPRG_05384, SPRG_19863, SPRG_03974 and SPRG_19320 are known to encode 562, 6547, 9440, 5297 and 936 amino acid containing proteins, respectively. In case of the first four proteins, the suggested catalytic domain possesses one or multiple domain(s) of ScanProcite fibronectin III;

while, SPRG_19320 consists of thrombospondin domain. However, investigation applying NetNGlyc tool suggested the presence of multiple (4-20) sites for N-glycosylation in all the five putative proteins [**Rezinciuc et al., 2018**]. On the other hand, further studies on the host-parasite interaction revealed that specific molecules released from the oomycetes helps S. parasitica to attach to their host surface [**Hardham, 2001; Mendgen et al., 1996; Epstein and Nicholson, 1997**]. In such regards, presence of bundles of elongated hooked hair-like structures in the cysts also facilitate the pathogen to attach the fish body [**Rezinciuc et al., 2018**]. Despite of the fact that numerous attempts have been made to explore the mechanism of attachment of the pathogen to the host body, but the specific functions of these specialized structures remained unexplained.Such information is of eminent importance as it may help the researchers to develop a therapeutic agent to inhibit the attachment of the parasite to the host, thus, may restrict it pathogenesis in the aquatic organisms. In such regards, plant extract may serve as a natural remedial measure with less or, no toxicity in the host organism.

5. PLANT EXTRACTS AS ALTERNATIVE DRUG SOURCES:

Sl. No.	Name	Scientific name	Parts used	Reference
1	Denaee thyme	*Thymus daenensis Celak*	Leaves	Pirbalouti AG, Taheri M, Raisee M, Bahrami HR, Abdizadeh R (2009) In vitro antifungal activity of plant extracts on *Saprolegnia parasitica* from cutaneous lesions of rainbow trout (*Oncorhynchus mykiss*) eggs. Journal of Food, Agriculture and Environment 7, 94-96.

2	Khuzestani thyme	*Thymus khuzestanicum*	Leaves	Pirbalouti AG, Taheri M, Raisee M, Bahrami HR, Abdizadeh R (2009) In vitro antifungal activity of plant extracts on *Saprolegnia parasitica* from cutaneous lesions of rainbow trout (*Oncorhynchus mykiss*) eggs. Journal of Food, Agriculture and Environment 7, 94-96.
3	Mint	*Mentha longifolia (L.) Hudson*	Leaves	Pirbalouti AG, Taheri M, Raisee M, Bahrami HR, Abdizadeh R (2009) In vitro antifungal activity of plant extracts on *Saprolegnia parasitica* from cutaneous lesions of rainbow trout (*Oncorhynchus mykiss*) eggs. Journal of Food, Agriculture and Environment 7, 94-96.
4	Common myrtle	*Myrtus communis L.*	Leaves	Pirbalouti AG, Taheri M, Raisee M, Bahrami HR, Abdizadeh R (2009) In vitro antifungal activity of plant extracts on *Saprolegnia parasitica* from cutaneous lesions of rainbow trout (*Oncorhynchus mykiss*) eggs. Journal of Food, Agriculture and Environment 7, 94-96.

5	Tansy	*Tanacetum parthenium (L.) Schultz-Bip*	Flower	Pirbalouti AG, Taheri M, Raisee M, Bahrami HR, Abdizadeh R (2009) In vitro antifungal activity of plant extracts on *Saprolegnia parasitica* from cutaneous lesions of rainbow trout (*Oncorhynchus mykiss*) eggs. Journal of Food, Agriculture and Environment 7, 94-96.
6	Bakhtyari savory	*Satureja bachtiarica Bunge*	Leaves	Pirbalouti AG, Taheri M, Raisee M, Bahrami HR, Abdizadeh R (2009) In vitro antifungal activity of plant extracts on *Saprolegnia parasitica* from cutaneous lesions of rainbow trout (*Oncorhynchus mykiss*) eggs. Journal of Food, Agriculture and Environment 7, 94-96.
7	Hyssop	*Hyssopus officinalis L.*	Leaves	Pirbalouti AG, Taheri M, Raisee M, Bahrami HR, Abdizadeh R (2009) In vitro antifungal activity of plant extracts on *Saprolegnia parasitica* from cutaneous lesions of rainbow trout (*Oncorhynchus mykiss*) eggs. Journal of Food, Agriculture and Environment 7, 94-96.

8	Galanga	*Alpinia galanga*	Rhizome	Udomkusonsri P, Trongvanichnam K, Limpoka M, Klangkaew N, Kusucharit N (2007) In vitro efficacy of the antifungal activity of some Thai medicinal-plants on the pathogenic fungus, Saprolegnia parasitica H2, from fish. Kasetsart Journal (Natural Science), 41, 56-61.
9	Betel Vine	*Piper betel* Linn	Leaf	Udomkusonsri P, Trongvanichnam K, Limpoka M, Klangkaew N, Kusucharit N (2007) In vitro efficacy of the antifungal activity of some Thai medicinal-plants on the pathogenic fungus, Saprolegnia parasitica H2, from fish. Kasetsart Journal (Natural Science), 41, 56-61.
10	-	*Rhinacanthus nasutus* Linn	Leaf	Udomkusonsri P, Trongvanichnam K, Limpoka M, Klangkaew N, Kusucharit N (2007) In vitro efficacy of the antifungal activity of some Thai medicinal-plants on the pathogenic fungus, Saprolegnia parasitica H2, from fish. Kasetsart Journal (Natural Science), 41, 56-61.

11	-	*Kaempferia galanga* Linn	Leaf	Udomkusonsri P, Trongvanichnam K, Limpoka M, Klangkaew N, Kusucharit N (2007) In vitro efficacy of the antifungal activity of some Thai medicinal-plants on the pathogenic fungus, Saprolegnia parasitica H2, from fish. Kasetsart Journal (Natural Science), 41, 56-61.
12	-	*Kaempferia galanga* Linn	Root	Udomkusonsri P, Trongvanichnam K, Limpoka M, Klangkaew N, Kusucharit N (2007) In vitro efficacy of the antifungal activity of some Thai medicinal-plants on the pathogenic fungus, Saprolegnia parasitica H2, from fish. Kasetsart Journal (Natural Science), 41, 56-61.
13	Black caraway	*Nigella sativa*	Seeds	Mostafa AAF, Al-Askar AA, Yassin MT (2020) Anti-saprolegnia potency of some plant extracts against *Saprolegnia diclina*, the causative agent of saprolengiasis. Saudi Journal of Biological Sciences 27(6), 1482-1487.

14	Thyme	*Thymus vulgaris*	Leaves	Mostafa AAF, Al-Askar AA, Yassin MT (2020) Anti-saprolegnia potency of some plant extracts against *Saprolegnia diclina*, the causative agent of saprolengiasis. Saudi Journal of Biological Sciences 27(6), 1482-1487.
15	Pomegranate	*Punica granatum*	Peels	Mostafa AAF, Al-Askar AA, Yassin MT (2020) Anti-saprolegnia potency of some plant extracts against *Saprolegnia diclina*, the causative agent of saprolengiasis. Saudi Journal of Biological Sciences 27(6), 1482-1487.
16	Ginger	*Zingiber officinale*	Rhizome	Mostafa AAF, Al-Askar AA, Yassin MT (2020) Anti-saprolegnia potency of some plant extracts against *Saprolegnia diclina*, the causative agent of saprolengiasis. Saudi Journal of Biological Sciences 27(6), 1482-1487.

Apart from these information, several other plant extracts with established antifungal propertieshave been reported **[Emara et al., 2020]** such as- (i)Terpenoids present in plants such as eucalyptus (Eucalyptus globulus), tea tree (Melaleuca alternifolia), and neem (Azadirachta indica); (ii) Flavonoids present in plants such as turmeric (Curcuma longa), ginger (Zingiber officinale), and garlic (Allium sativum); (iii) Alkaloids present in plants such as nightshade (Solanum spp.) and papaya (Carica papaya); and (iv) Phenolic compounds present in thymesuch as (Thymus vulgaris), cloves (Syzygium

aromaticum), and cinnamon (Cinnamomum verum). Surprisingly, their mode of action against Saprolegniasis and the underlying molecular mechanism are yet to be explored and requires detailed research.

6. CONCLUSION:

As an offshoot of the international ban of Malachite Green in 2002, the need to develop alternative methods for the control of saprolegniasis has arisen with greater urgency. Genomic and proteomic studies of S. parasitica and other pathogenic oomycetes provided some good resources regarding the molecular underpinnings of saprolegniasis. Control of saprolegniasis is essential for continued advancement in the aquaculture industry, particularly in Asian tropical aquaculture systems, where more than 70-80% of fish produced by aquaculture originates from these regions. For an industry responsible for about 30% of fish consumed worldwide, the molecular processes underlying saprolegniasis in S. parasitica-host fish interactions must be investigated thoroughly. In such regards, discovery of plant extracts, antifungal agents, biocontrol methods, and genomic strategies should be promoted to develop effective treatments against saprolegniasis. Identification of plant extracts with strong antifungal activity against Saprolegnia parasitica will lead to the development of an eco-friendly, non-toxic treatment for saprolegniosis. Good environmental management practices will help reduce the pathogen's prevalence. A step toward sustainable aquaculture practices by reducing reliance on synthetic antifungal agents. It will lead to the promotion of biodiversity by using natural plant resources. Reduction in environmental and health risks associated with conventional treatments. Furthermore, ongoing research and collaboration between researchers, aquaculture professionals, and regulatory bodies are essential for the development and implementation of effective control strategies. Through research, the goal is to create sustainable and safe solutions that protect fish health and enhance aquaculture productivity, ultimately benefiting both the industry and the ecosystems involved. Exploring the combination of plant extracts with other biocontrol agents may enhance the overall efficacy

of treatments. The advancement of genomic tools and bioinformatics will aid in identifying more targeted, efficient, and sustainable treatment options. The collaboration between researchers and aquaculture professionals will be necessary for complete eradication of the pathogen from the ecosystem.

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DRUG-INDUCED LIVER TOXICITY: MECHANISMS AND THERAPEUTIC TARGETS

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ABSTRACT

Drug-induced liver injury (DILI) remains a critical challenge in clinical pharmacology, posing significant health risks and limiting the therapeutic potential of numerous drugs. This review explores the underlying mechanisms of DILI and highlights emerging therapeutic strategies aimed at mitigating hepatotoxicity. The pathogenesis of DILI involves intricate interactions between drugs and hepatic cellular structures, leading to oxidative stress, mitochondrial dysfunction, immune-mediated damage, and apoptotic pathways. Key molecular mediators, including cytochrome P450 enzymes, reactive oxygen species (ROS), and pro-inflammatory cytokines, are critically examined. Additionally, the limitations of current diagnostic biomarkers in predicting DILI are discussed. Emerging therapeutic approaches focus on modulating oxidative stress, enhancing mitochondrial protection, and regulating immune responses. Potential targets such as nuclear factor erythroid 2-related factor 2 (Nrf2), peroxisome proliferator-activated receptors (PPARs), and microRNAs (miRNAs) offer promising avenues for reducing hepatocellular injury. Furthermore, the role of personalized medicine and genetic predisposition in DILI

susceptibility is explored, emphasizing the need for tailored interventions. This review underscores the importance of advancing research to develop reliable biomarkers and innovative therapeutics, ultimately improving patient safety and drug efficacy.

Keywords: *Drug- induced liver injury, diagnosis, therapeutic*

INTRODUCTION

The liver, being the primary organ responsible for the metabolism and detoxification of drugs, is particularly susceptible to injury from various pharmaceutical agents, herbal products, and environmental toxins. Drug- induced liver injury (DILI) can manifest as a spectrum of liver abnormalities, ranging from mild, transient elevations in liver enzymes to severe, potentially life-threatening conditions such as acute liver failure.The mechanisms underlying DILI are diverse and complex, involving metabolic bioactivation leading to direct hepatocellular damage and immune-mediated responses resulting in liver inflammation. Despite advances in understanding the pathophysiology of DILI, diagnosing and managing this condition remains a challenge. Early detection and prompt cessation of the offending drug are crucial for preventing severe liver damage and improving patient outcomes[1-7].

The widespread use of medications and the continuous development of new pharmaceutical agents has raised growing concerns regarding their safety. One of the most frequent and severe adverse drug reactions (ADRs) is Drug-Induced Liver Injury (DILI) [8]. DILI can be triggered by various types of medications, including prescription and over-the-counter drugs, biological agents, traditional medicine (TM), natural medicines (NM), health products (HP), dietary supplements (DS), as well as their metabolites and excipients [9]. Despite extensive research, there are no specific diagnostic markers for DILI, making its diagnosis highly challenging. Globally, many countries and regions have developed empirical therapeutic strategies for DILI, supported by comprehensive guidelines. DILI is typically classified into three distinct clinical patterns—hepatocellular,

cholestatic, or mixed injury—based on the serum ALT and ALP ratio derived from the first available biochemical tests [10]. The therapeutic approaches for DILI, especially with regard to drug treatments, are often tailored to these three patterns of liver injury.

CLINICAL PRESENTATION AND DIAGNOSIS

Drug-induced liver injury (DILI) can present with a wide range of clinical manifestations, from asymptomatic elevations in liver enzymes to more severe conditions like jaundice, coagulopathy, and acute liver failure. Patients often exhibit symptoms such as fatigue, nausea, abdominal pain, and jaundice. Diagnosing DILI is particularly challenging due to its reliance on a combination of clinical history, exclusion of other potential causes of liver disease, and a temporal relationship with drug exposure. In certain cases, a liver biopsy may be performed to determine the extent of liver damage and to exclude other liver diseases. While ALT and AST are commonly used biomarkers to monitor liver function, there is a growing recommendation for the development of more specific biomarkers for predicting and early diagnosing DILI [11-13].

RISK FACTORS

The risk of developing DILI is influenced by both patient-related and drug-related factors. Patient-related factors include age, sex, genetic predisposition, pre-existing liver diseases, and comorbid conditions. For example, elderly individuals and those with pre-existing liver conditions such as hepatitis or non-alcoholic fatty liver disease (NAFLD) are at a higher risk. Genetic variations in drug-metabolizing enzymes and immune-related genes also play a role in determining susceptibility to DILI. Drug-related factors include the chemical structure of the drug, dosage, duration of therapy, and interactions with other medications. Certain drug classes, such as antibiotics, anticonvulsants, and non-steroidal anti-inflammatory drugs (NSAIDs), are more frequently associated with DILI [14-15].

DILI- INDUCED PHYSIOLOGICAL COMPLICATIONS

1. Cytochrome P450 Enzyme-Mediated Metabolism: One of the primary mechanisms underlying DILI is the metabolism of drugs by the liver's cytochrome P450 (CYP450) enzymes. These enzymes catalyze the biotransformation of drugs into metabolites, which, in some cases, can be highly reactive and toxic. The generation of reactive metabolites often leads to oxidative stress and cellular damage. For example, acetaminophen (paracetamol), a commonly used analgesic, undergoes CYP450-mediated metabolism to form a highly reactive intermediate, N-acetyl-p-benzoquinone imine (NAPQI). In high doses, NAPQI depletes intracellular glutathione, resulting in hepatocellular damage and necrosis [17-18].

2. Oxidative Stress and Mitochondrial Dysfunction: Oxidative stress is a pivotal factor in the pathogenesis of DILI. Drugs that generate reactive oxygen species (ROS) can lead to lipid peroxidation, protein oxidation, and DNA damage. ROS accumulation disrupts cellular integrity, leading to mitochondrial dysfunction and hepatocellular apoptosis. Mitochondria play a crucial role in cellular energy metabolism, and their dysfunction is a hallmark of many forms of liver injury, including DILI. Disruption of the mitochondrial membrane potential and release of cytochrome c can trigger apoptotic pathways, exacerbating liver damage [19].

3. Immune-Mediated Mechanisms:In addition to direct hepatotoxicity, DILI may involve immune-mediated processes, where the immune system erroneously targets and destroys hepatocytes. The role of T lymphocytes and cytokines in DILI is gaining recognition, with studies indicating that certain drugs may induce immune responses that lead to liver damage. In some cases, this immune reaction can be attributed to a drug's ability to bind covalently to proteins in the liver, forming neoantigens that provoke an autoimmune-like response. The immune response can be both specific and nonspecific, with the activation of pro-inflammatory cytokines such as TNF-α and IL-1 contributing to the inflammatory cascade in DILI [20].

4. Apoptotic and Necrotic Pathways: The liver's response to toxic insults can trigger both apoptosis (programmed cell death) and necrosis. Apoptosis is typically mediated through the intrinsic pathway, involving mitochondrial dysfunction, and the extrinsic pathway, where death receptors on the hepatocyte surface trigger cell death. In cases of severe liver injury, necrosis can occur, leading to hepatocyte rupture and inflammation. These processes contribute to the clinical manifestation of liver injury, including jaundice, hepatomegaly, and elevated liver enzymes [21].

COMMON DRUGS ASSOCIATED WITH LIVER INJURY

TYPES OF DRUG	USED FOR	MODE OF ACTION	PHYSIOLOGICAL COMPLICATIONS
1. Uricosuric agent	Gout, hyperuricemia	Inhibits uric acid reabsorption in renal tubules, promoting uric acid excretion.	Hepatotoxicity, Kidney Stones, Liver toxicity, Headache, Gastrointestinal Symptoms [15-18]
2. Acetylcholinesterase inhibitor	Alzheimer's disease.	Inhibits acetylcholinesterase and also modulates nicotinic receptors, increasing acetylcholine release in the brain, enhancing cholinergic function.	Vomiting, Liver toxicity, Muscle weakness, Excessive salivation, Nausea, Diarrhea, [20-22]
3. Imidazopyridine	Anxiety disorders	Selectively binds to GABA-A receptors, increasing GABAergic activity, which helps induce sleep. It has a rapid onset and short duration of action.	Drowsiness, Dizziness, Headache, Memory Impairment, Daytime Sedation, Liver toxicity [19]

4. Antifungal	systemic and superficial fungal infections	Binds to ergosterol in fungal cell membranes, forming pores that disrupt membrane integrity, leading to leakage of cellular contents and fungal cell death.	Headache, Liver Toxicity, Skin Rash, Allergic Reactions [17-18]
5. Antibiotic	Infections	Inhibits DNA gyrase and topoisomerase IV, enzymes responsible for DNA replication and repair, leading to DNA strand breakage and bacterial death.	Allergic reactions, Liver toxicity [11]

Therapeutic Strategies to Combat Drug-Induced Liver Toxicity (DILT)

1. Antioxidants

Oxidative stress plays a crucial role in DILT, as many drugs generate reactive oxygen species (ROS) that damage liver cells. N-Acetylcysteine (NAC) is a widely used antioxidant, particularly in treating acetaminophen overdose, which depletes glutathione and results in hepatotoxicity. NAC replenishes glutathione levels, neutralizing ROS and preventing further liver damage. Beyond acetaminophen toxicity, NAC is being explored for other forms of DILT, with promising preliminary results [10, 17].

2. Immunosuppressive Agents

In certain DILT cases, liver injury is immune-mediated, such as in isoniazid-induced liver toxicity. In these cases, drugs such as corticosteroids (e.g., prednisone) are employed to suppress the immune response. Corticosteroids reduce inflammation and inhibit the activation of immune cells, offering protection against further liver damage. However, their use is limited by potential side effects, including immunosuppression and

metabolic disturbances [21].

3. Liver Support:

For patients who develop acute liver failure due to DILT, liver transplantation is the definitive treatment when the liver is no longer able to function. Although it is life-saving, liver transplantation comes with risks such as immune rejection and limited donor availability. Artificial liver support systems are also under investigation as temporary measures to support liver function while awaiting transplantation [22].

4. Enzyme Inhibitors

Some drugs undergo metabolic activation by cytochrome P450 enzymes (CYP450), leading to the formation of toxic metabolites. CYP450 inhibitors can block the enzymes responsible for metabolizing certain drugs, preventing the formation of harmful intermediates. This strategy holds promise in reducing DILT by limiting the bioactivation of toxic metabolites, though further research is required to establish its clinical effectiveness [22].

MANAGEMENT AND PREVENTION

Effective management of DILI involves early recognition, discontinuation of the offending drug, and providing supportive care. In cases of severe liver injury, hospitalization and close monitoring of the patient may be necessary. Preventive measures include the careful selection of medications, particularly in patients at risk, and regular monitoring of liver function during treatment with drugs known to have hepatotoxic potential. Ongoing research aims to develop safer drugs with reduced hepatotoxicity and to identify biomarkers for the early detection of DILI [19-20].

CONCLUSION

Drug-induced liver injury remains a significant challenge in clinical pharmacology. Understanding the complex mechanisms involved in DILI, improving diagnostic biomarkers, and developing targeted therapeutic strategies are crucial for minimizing hepatotoxicity and improving patient safety. Advancements in molecular biology, genetic

research, and personalized medicine hold great promise for the future management of DILI. As research continues to explore the underlying pathways and identify novel therapeutic targets, the hope is to develop more effective and personalized approaches to prevent or treat this potentially life-threatening condition.

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ADVANCEMENTS IN PROBIOTIC ISOLATION AND CHARACTERIZATION: METHODS, MICROBIAL DIVERSITY, AND HEALTH BENEFIT

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ABSTRACT

Probiotics are live microbes that when administered in small amounts confer some health benefits to the host. Several different types of products which are formulated as drugs or other dietary supplements, originate from live microbes called probiotics. The majorities of these organisms belong to the LAB (Lactic Acid Bacteria) group. The LAB predominantly includes organisms which belong to the genera of Lactobacillus and Bifidobacterium but from varied species and strains which altogether is considered as potential probiotic. The aim of this review is to comprehensively explore the methodologies and advancements in the isolation and characterization of probiotics. This review investigates different methods for separating probiotics from a range of sources, including fermented foods, the human microbiome, and natural settings. It highlights the importance of cutting-edge methods like molecular biology, metagenomics, and whole genome sequencing in the identification of new probiotic strains, the

description of their genetic and functional characteristics, and the evaluation of their possible health advantages.

Keywords: *probiotics, isolation, characterization, microbiome, lactobacillus*

1. Introduction

Probiotics have attracted a lot of attention in the last several years because of their possible health advantages and range of uses in the food, pharmaceutical, and agricultural industries. Probiotics are live bacteria that, when given in sufficient quantities, boost the host's health. Probiotics have the potential to be used therapeutically because of their capacity to alter gut microbiota, boosting immunity, and having antimicrobial properties. The notion of probiotics originated in the early 1900s, when Elie Metchnikoff said that lactic acid bacteria-containing fermented dairy products could extend life and improveshealth [20]. Subsequent research has clarified the various pathways via which probiotics achieve their benefits on human body. Probiotics were once primarily linked to digestive health, but their uses have since broadened to include dermatological, oral health, and even mental wellness [35].

Probiotics come from many different sources, each of which has its own special strains with certain health advantages. Probiotics are mostly found naturally in fermented foods and drinks such kombucha, yogurt, kefir, sauerkraut, and kimchi. Lactic acid bacteria (LAB) and other helpful microorganisms process these traditional meals, populating the gut microbiota after ingestion and supporting digestive health and general well-being [24]. To find probiotic strains with desired health-promoting qualities, it is essential to isolate and characterize the organisms. By employing selective culture conditions and biochemical assays, probiotic bacteria were traditionally identified from fermented foods and human faecal samples. Probiotic identification and characterisation, however, has been completely transformed by advances in genomics and molecular biology. The genetic makeup, metabolic pathways, and possible modes of action of probiotic strains can be fully characterized by the use of techniques including

transcriptomics, metagenomics, and whole-genome sequencing.

Probiotics' ability to support digestive health is one of its main advantages. They assist in preserving a balanced population of gut bacteria, which helps lessen the symptoms of digestive problems such diarrhoea brought on by antibiotic use, inflammatory bowel diseases (IBD), and irritable bowel syndrome (IBS)[13]. Additionally, probiotics' immunomodulatory effects have been researched. These effects may improve immune responses and lessen the frequency and severity of allergies and respiratory infections. Furthermore, they could enhance lipid profiles and glucose metabolism, which could help manage diseases like type 2 diabetes and obesity by promoting metabolic health [31]. This review paper examines how probiotics are isolated and characterized, covering traditional and modern methods. The study highlights the potential of probiotics to promote health and prevent disease by discussing their significance in food and medicine, among other applications. It also discusses future research paths that will improve the safety, efficacy, and customized uses of probiotics in healthcare.

1. Sources of probiotics

It has long been known that fermented foods are a great source of probiotics. One of the most popular foods high in probiotics is yogurt, which include strains of Lactobacillus acidophilus and Bifidobacterium lactis, which support immune system and digestion and hence promote gut health [19]. Along with helping with lactose digestion, kefir, a fermented milk beverage, has a rich probiotic profile dueto the wide community of bacteria and yeasts it contains. Traditional fermented foods like kimchi, kombucha, and sauerkraut include a range of probiotic strains, including Saccharomyces boulardii and Lactobacillus plantarum, which are well-known for their immunomodulatory and antibacterial qualities [30].

The microbiota found in the faeces of humans is a storehouse of various microbial communities and a useful resource for the discovery of probiotics. The therapeutic potential of transplanting beneficial bacteria from healthy donors to

recipients suffering from gastrointestinal illnesses has been highlighted by research on faecal microbiota transplantation (FMT) [40]. Beyond FMT, research is still being done to find and describe new probiotic strains from human sources that have health-promoting qualities, like immune modulation and maintenance of gut barrier integrity. Probiotics obtained from natural sources, like plants, soil, and aquatic environments, increase the variety of microbial strains that can be used for both nutritional and medicinal purposes. For example, the ability of soil bacteria to withstand adversity and offer health advantages similar to those of typical probiotics found in fermented foods has been studied [27]. Probiotics that have been isolated from marine settings also have special adaptations and bioactive substances that may have positive effects on health, such as anti-inflammatory and antioxidant qualities.

Table 1: Some common probiotic strains and their sources

Species	**Source**	**Reference**
Lactobacillus acidophilus, *Bifidobacterium bifidum*	Kefir	[22]
Lactobacillus rhamnosus	Kombucha	[27]
Lactobacillus paracasei, *Lactobacillus casei*	Fermented soybeans	[36]
Lactobacillus plantarum, *Lactobacillus brevis*	Kimchi	[42]
Lactobacillus lactis, *Lactobacillus plantarum*	Idli batter	[33]
Lactobacillus acidophilus	Miso	[14]
Lactobacillus helveticus	Parmigiano cheese	[4]
Lactobacillus plantarum, *Lactobacillus bulgaricus*	Koumiss	[29]

Lactobacillus casei, *Lactobacillus plantarum*	Kalari cheese	[21]
Lactobacillus brevis	Sauerkraut	[19]
Streptococcus thermophilus, *Lactobacillus bulgaricus*	Yoghurt	[12]
Lactobacillus lactis	Buttermilk	[2]
Lactobacillus casei, *Bifidobacterium breve*	Tempeh	[5]

2. Techniques for isolation and characterization of probiotics

The isolation and characterization of probiotics is a key step in microbiology and biotechnology. Microbial strains from a variety of ecological niches, such as fermented foods, the human microbiota, and natural settings, are systematically explored and chosen for this purpose. The use of selective media and conditions to isolate specific probiotic species, is essential in traditional culture-based approaches [10]. Simultaneously, the utilization of novel species and thorough microbial profiling has been made possible by the development of molecular tools like 16S rRNA gene sequencing and metagenomics [38]. These approaches are essential for clarifying the mechanisms of action of probiotics as well as their capacity to alter the makeup of the gut microbiota, strengthen the immune system, and generate bioactive metabolites that are vital to human health.

2.1 Traditional culture-based methods for isolation

Conventional culture-based techniques are used to carefully isolate probiotics from a variety of natural sources, namely fermented foods, and human microbiota. To guarantee the identification and selection of advantageous microbial strains, traditional culture-based techniques involve several crucial procedures. Usually, the first step in these procedures is the extraction of samples from sources. Following that, the samples are selectively enriched in specialized growth medium that promote the development of the intended

probiotic species while inhibiting the growth of rival bacteria. Formulations for selective media, such as Bifidobacterium-selective media or MRS agar for lactobacilli, are created with certain nutrients and pH levels that promote the growth of probiotic strains [6]. Following identification of isolated colonies based on their physical traits, biochemical assays, such as carbohydrate fermentation profiles and enzyme activity, are used to confirm the isolates. Moreover, for accurate species identification and phylogenetic analysis, contemporary methods like PCR and sequencing of conserved genes (such 16S rRNA) are used [39]. Because of their ease of use, dependability, and affordability in identifying potential candidates for additional research and use in functional foods and therapeutic interventions, traditional culture-based methods are still valuable for the initial stages of probiotic discovery and strain characterization, even in light of the advancements in molecular and genomic approaches.

2.2 Molecular techniques for isolation and characterization

Molecular methods for isolating probiotics have transformed biotechnology and microbial ecology by making it possible to precisely identify, characterize, and utilize advantageous microbes. In contrast to conventional culture-based techniques, molecular approaches provide a thorough understanding of the diversity and functionality of microbes. The direct analysis of genetic material from environmental samples has been made possible by methods like metagenomics. It has produced novel probiotic candidates from a variety of environments, including the human gut and natural habitats [16]. Moreover, 16S rRNA gene sequencing has been a fundamental instrument for taxonomic categorization and microbial community characterization, making it easier to identify certain probiotic strains and their quantity. Along with these, methods like whole genome sequencing (WGS) reveal the complete genetic composition of probiotic bacteria and throw light on their detailed metabolic pathways.

3.2.1 16s rRNA Sequencing

A crucial molecular technique for the isolation and identification of probiotics, 16S rRNA sequencing offers information on the

diversity of microorganisms and taxonomic classification according to sequence differences in the 16S rRNA gene, a region that is universally conserved in bacteria. Researchers can analyse complex microbial communities, including those containing potentially probiotic species, using this approach [25]. 16S rRNA sequencing makes it easier to precisely identify particular bacterial strains and their abundance in intricate microbial ecosystems for probiotic research. Using taxonomic classification and functional predictions obtained from genomic data, this technique has proven useful in the discovery of novel probiotic candidates and the assessment of their potential health effects [7]. Moreover, 16S rRNA sequencing offers a basis for understanding the ecological functions of probiotics in diverse settings, microbial community dynamics, and probiotic-host interactions.

3.2.2 Metagenomics

Metagenomics is a powerful approach in microbiology that involves the direct study of genetic material recovered from environmental samples, bypassing the need for microbial cultivation. This technique allows researchers to explore the collective genomes of entire microbial communities, including previously unculturable species, thereby providing a comprehensive view of microbial diversity and functionality [39]. In the context of probiotics, metagenomic studies have been instrumental in identifying novel microbial candidates from diverse environments such as soil, marine habitats, and the human gut microbiota. These studies not only expand the scope of study of potential probiotic species but also offer insights into their ecological roles and functional properties.

3.2.3 Whole genome sequencing (WGS)

Whole genome sequencing (WGS) has emerged as a key component in the isolation and characterisation of these organisms because of its ability to provide a thorough understanding of the genetic makeup, activity, and possible health advantages of probiotics. WGS offers a thorough analysis of the complete genome of microbial strains, including genes encoding for metabolic pathways, virulence factors, and probiotic features [1]. This is in contrast to conventional

culture-based methods and partial sequencing techniques like 16S rRNA sequencing. The first step in the WGS method is to harvest high-quality genomic DNA from the target probiotic strain. Then, using cutting-edge technologies like Illumina or PacBio, which produce enormous volumes of sequence data, this DNA is broken up and libraries are ready for sequencing. The entire genetic code of the probiotic strain can then be examined by researchers by using bioinformatics methods to put these sequences together into complete genomes[16].

3.2.4 Functional genomics and transcriptomics

Functional genomics and transcriptomics are pivotal in the isolation and characterization of probiotics, offering detailed insights into their genetic and functional attributes. These methodologies enable the comprehensive study of gene expression profiles, metabolic pathways, and regulatory networks within probiotic microorganisms. The methodical investigation of gene activities and their connections with the genome is known as functional genomics. Genes encoding probiotic characteristics like resilience to environmental stressors, adhesion to host tissues, and antimicrobial synthesis can be identified using methods like comparative genomics and whole genome sequencing (WGS) [28]. Researchers can forecast the possible health-promoting qualities of probiotic strains and maximize their utility in therapeutic applications by studying their complete genetic composition.

Transcriptomics is concerned with the analysis of the transcriptome, which is the whole collection of RNA transcripts generated by the genome at particular growth stages or environmental circumstances. Researchers can measure the levels of gene expression, find genes that are differentially expressed, and discover the regulatory networks that mediate probiotic actions by using RNA sequencing [23]. This method makes it easier to create probiotic therapies that are suited to certain medical diseases by offering dynamic insights into how bacteria react to different environmental stimuli and interact with host cells.

2.3 Techniques for biological and phenotypic characterization

Probiotics are biologically characterized by assessing physiological characteristics such as resistance to gastrointestinal disorders, adhesion to intestinal epithelial cells, and tolerance to acid and bile. The ability to survive and colonize in the gut environment is essential for probiotic efficacy. In vitro assays, flow cytometry, and microscopy are among the methods used to evaluate probiotic survival and adherence in simulated gastrointestinal environments [15]. The study of probiotic metabolic processes, such as the synthesis of vitamins, short-chain fatty acids, and antibiotic chemicals, is included in phenotypic characterisation. Their possible health-promoting qualities and interactions with the host microbiota are facilitated by these metabolic outputs [3].

In biological characterisation, the following physiological characteristics are crucial in determining the survivability and usefulness of probiotics in the gastrointestinal tract:

i. Acid and Bile Tolerance: For probiotics to be effective, they must be able to withstand exposure to bile salts in the intestines and acidic environments in the stomach. Probiotics can be assessed by subjecting them to artificial stomach and intestinal fluids in order to gauge their viability and tolerance [13].

ii. Adherence to Intestinal Epithelial Cells: In order for probiotics to colonize and remain in the gut mucosa, they must adhere to intestinal epithelial cells. This allows them to engage with the host immune system and affect the makeup of the microbiota [15]. Adhesion assays are used to evaluate the adhesion properties of probiotics utilizing intestinal cell lines or cell culture models.

Phenotypic characterization explores the metabolic activities and functional behaviours of probiotics that contribute to their health-promoting effects:

i. Formation of antimicrobial compounds: A variety of probiotics generate compounds that inhibit pathogenic bacteria and improve gut health, including bacteriocins,

organic acids, and hydrogen peroxide [8]. Antimicrobial activity tests against certain pathogens are used to evaluate these substances.

ii. Study of metabolic routes: Knowledge of the metabolic routes of probiotics, such as the fermentation of dietary fibres to short-chain fatty acids (SCFAs), sheds light on how these microbes contribute to better nutrition metabolism and gut barrier function [18].The profiles and activity of metabolic pathways are revealed by metabolic profiling methods like mass spectrometry and NMR spectroscopy.

2.4 Techniques for functional and clinical characterization

Through clinical trials and in vivo models, functional characterisation evaluates the unique health advantages of probiotics. Studies like this assess the effectiveness of probiotics in treating ailments like immunological regulation, allergies, and gastrointestinal diseases. The processes behind probiotic effects on human health are explained by measuring biomarkers of host response, makeup of the gut microbiota, and metabolic profiles [41].Clinical trials are conducted on humans to evaluate the effectiveness of probiotics in treating gastrointestinal problems such as diarrhoea and inflammatory bowel disease, avoiding infections by pathogen competitive exclusion, and reducing allergy symptoms via immunological modulation [9].Probiotic goods must adhere to regulatory standards, and safety assessments guarantee this by highlighting the GRAS (Generally Recognized as Safe) status of the products[18].These studies add to the body of evidence supporting the use of probiotics in personalized medicine and functional foods by examining their safety profiles as well as their effects on general immunological and metabolic health.

3. Future scope and challenges

Innovative research approaches and developing technology are expected to produce considerable breakthroughs in the isolation and characterization of probiotics in the future. It is anticipated that isolation techniques would advance beyond conventional culture-based procedures to integrate high-throughput screening techniques, like robotic automation

and microfluidics, allowing for the quick identification and production of a variety of probiotic strains from intricate microbial ecosystems [34]. Probiotic discovery is expected to be revolutionized by metagenomic and single-cell genomics techniques, which will enable researchers to investigate unculturable microbial ecosystems and identify particular beneficial bacteria with focused health effects.

Molecular, cellular, and community-level understanding of probiotic functioning requires characterization techniques to depend more and more on multi-omics approaches that integrate data from genomes, transcriptomics, proteomics, and metabolomics [27]. As metabolomics advances, it will become clearer how probiotics metabolically function, particularly how they produce bioactive substances like vitamins and short-chain fatty acids that are essential for immune regulation and gut health. Understanding the gene expression patterns and protein profiles of probiotics under different physiological situations through transcriptomics and proteomics will shed light on their methods of action in relation to adhesion, colonization, and host cell interaction [32].

Probiotics are also anticipated to find more use in the treatment of neurological conditions, metabolic diseases, and customized nutrition, in addition to gastrointestinal health issues. Thorough clinical trials utilizing cutting-edge molecular techniques will help to confirm the effectiveness of probiotics, adjust dosing schedules, and create safety profiles specific to certain demographics and medical conditions [26].

Despite technical developments, there are still significant hurdles in the isolation and characterisation of probiotics. Robust techniques are needed to recognize and isolate probiotic strains appropriate for a range of demographics due to the cultural variety in gut microbiota [13]. To maintain consistency in quality and health benefits, probiotic formulation standardization and efficacy testing are still essential. Strict adherence to worldwide criteria and comprehensive clinical trials are required to meet regulatory requirements for safety and efficacy [11]. To overcome these obstacles and create guidelines for the creation and marketing

of probiotic products that adhere to strict safety and efficacy criteria, cooperation between academic institutions, business, and regulatory agencies is crucial.

4. Conclusion

Although there has been progress in the field of isolating and characterizing probiotics, more research and technical developments are expected to drive further development and application in a range of medical and health concerns. Because of advancements in molecular techniques such as metagenomics and single-cell genomics, probiotic strains may now be isolated from a wide range of habitats, a departure from the old culture-based procedures. Our knowledge of probiotic activity at the molecular level has been expanded by characterization techniques that span from morphological and physiological evaluations to omics technologies including proteomics, metabolomics, transcriptomics, and genomes. The functions of probiotics in immunological control, metabolic regulation, gut health, and other areas have been clarified by these finding. Their potential therapeutic applications in the management of gastrointestinal illnesses, immune function enhancement, and possible influence on systemic health conditions demonstrate the growing clinical relevance of these microorganisms. But issues like population-specific variations in probiotic efficacy, legal requirements for supporting health claims, and moral questions about probiotic promotion and consumer education still need to be addressed.

Future avenues in research should focus on customized probiotic treatments based on each person's unique microbiome composition, utilizing developments in precision medicine and analytics. Understanding how probiotics interact with the gut microbiota and host physiology can help to maximize the safety and effectiveness of probiotics. Moreover, overcoming these obstacles and successfully integrating research findings into clinical practice would need cooperative efforts across scientific fields, regulatory agencies, and industry sectors. In conclusion, continued research and interdisciplinary collaborations promise to advance the field and pave the way for individualized, evidence-based probiotic interventions that improve health

outcomes globally, even though there is still much to learn about the full therapeutic potential of probiotics and their wider impact on human health.

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HYPERTENSION MANAGEMENT: TACKLING THE CLINICAL CHALLENGES OF OMAPATRILAT AND ITS ANALOGUES (SAMPATRILAT AND SAMASP) IN HYPERTENSION THERAPY

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ABSTRACT

Vasopeptidase inhibitors have shown great efficacy in treating hypertension patients over decades. VPIs like omapatrilat effectively target both neprilysin (NEP) and angiotensin-converting enzyme (ACE) for blood pressure control but suffer from severe side effects due to off-target activity. Omapatrilat inhibits NEP, nACE, and cACE, but itleads to excessive accumulation of bradykinin and substance P, which contribute to adverse effects such as angiodema. This review explores the dynamics of this drug and its analogue molecules, addressing the issues behind its stop in clinical usage and how adjusting the inhibitor design can reduce the off-target effects,mitigating bradykinin-related side effects.

Keywords:*Vasopeptidase, hypertension, Omapatrilat, Sampatrilat, off-target, angiodema.*

Introduction

Hypertension is the major chronic, non-communicable disease in the world, with high morbidity and mortality

related to it. The number of people aged 30 to 79 with hypertension in 2019 is approximately 1.28 billion, which is a surprising doubling of the 1990 number (Verdecchia et al., 2022). Although conventional antihypertensive drugs have significantly improved outcomes, there are still huge gaps in the majority of the patients who reach blood pressure control. Additionally, chronic HTN appears to greatly contribute to both cardiovascular and renal morbidity, highlighting the need for further exploration of alternative treatment strategies.

Human angiotensin-1-converting enzyme(ACE) is a zinc dipeptidyl carboxypeptidase that plays a pivotal role in vascular health by controlling blood pressure (Cozier et al., 2018).It is a component of the renin-angiotensin-aldosteronenetwork that transforms angiotensin I to angiotensin II, which is a vasoactive peptide. ACE is made up of two domains- 612 amino acids in N-terminal domain and a C-domain of 650 amino acid residues. Although the substrate specificity of both domains is expansive, certain biochemical characteristics vary (Tzakos&Gerothanassis, 2005).

Omapatrilat was the first-in-class dual inhibitor of both neutral endopeptidase (NEP) and angiotensin-converting enzyme (ACE) among vasopeptidase inhibitors (VPIs) (Packer et al., 2002; Campese et al., 2016). Vascular homeostasis is modulated and vasodilation is encouraged by the dual mechanism. But negative side effects including angioedema have raised questions about their clinical progress in the medical field (Sharma et al., 2020).

Current State of Omapatrilat and Its Analogues

Omapatrilat, although highly effective, was discontinued due to the high incidence of angioedema observed in trials. Researchers have since focused on analogues like sampatrilat and SamASP, which aim to retain its efficacy while minimizing adverse effects. Sampatrilat, in particular, has shown improved performance in selective inhibition, reducing the accumulation of bradykinin (Rajni et al., 2016). SamASP, while less potent, still provides insight into structural modifications that could improve drug design (Sharma et al., 2020).

The key challenge is to develop compounds that maintain antihypertensive benefits while mitigating angioedema risk (Maki et al., 2003).In addition, computational modeling has aided in the design of next-generation vasopeptidase inhibitors that refine molecular interactions and improve enzyme specificity.

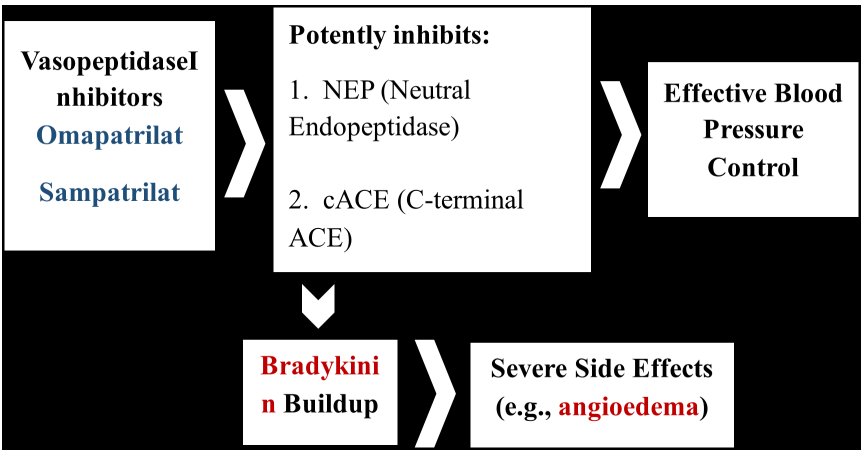

Fig 1: A schematic diagram of how these VPIs control blood pressure as well as increase side effects

Advantages Over Conventional Therapies

Compared to traditional antihypertensive drugs, vasopeptidase inhibitors present unique advantages. While ACE inhibitors or angiotensin receptor blockers (ARBs) primarily reduce angiotensin II levels, they fail to significantly influence natriuretic peptide activity i.e. vasodilation. By targeting NEP in addition to ACE, vasopeptidase inhibitors augment vasodilation and natriuresis while reducing systemic vascular resistance(Campese et al., 2001). This dual mechanism results in pronounced reductions in systolic and diastolic blood pressure.

Moreover, studies suggest that vasopeptidase inhibitors can attenuate arterial stiffness, enhance cardiac output, and reduce vascular remodeling(Mitchell et al., 2002). These properties are especially valuable for patients with comorbidities like

heart failure, where vascular and myocardial adaptations are critical. By contrast, dual inhibitors like omapatrilat enhance natriuretic peptide effects, improving vasodilation and sodium excretion (Campese et al., 2001)

Path of Action and Pharmacodynamics

Omapatrilat and its analogues exhibit a unique mechanism of action through the simultaneous inhibition of two pivotal enzymes: angiotensin-converting enzyme (ACE) and neprilysin (NEP). In a dose-based research, angiotensin converting enzyme function was suppressed by over 80%, 24 hours following the dosage of omapatrilat (Liao et al., 2003). Compared with placebo, omapatrilat increased 24-h urinary excretion of ANP at doses of 10 mg and higher on days 1, 3, and 10 in the multiple dose study and at doses of 7.5 mg and higher in a single-dose study.**Sampatrilat** increased plasma aldosterone levels, decreasing plasma ACE concentration at days 28 and 56 (Reza Tabrizchi,2003).Urinary cGMP release was elevated by all three dosages of sampatrilat after 10 days ($P < .05$ compared to control for 100 mg and 200 mg sampatrilat; P = NS for the 50 mg dosing).Administered in doses varying from 50 mg to 200 mg, it exhibited minimal antihypertensive efficacy following the initial dosage (Wallis et al., 1998).

Pharmacokinetics

In 3–4 days, steady-state plasma concentrations for omapatrilat were attained, indicating consistent existence (Liao et al., 2003). After oral administration, it is well-absorbed, reaching peak plasma concentrations approximately 2 to 4 hours post-dose. The drug exhibits high plasma protein binding (~95%) and is widely distributed throughout the body, including the central nervous system, though its concentration in the brain is lower compared to peripheral tissues. Omapatrilat is heavily metabolized in the liver, primarily by the cytochrome P450 pathway (particularly CYP2C9), which produces inactive metabolites.When treating cardiac failure and hypertension, its stimulation of ANP, a protein having vasodilatory effects, may be more advantageous than using an ACE inhibitor(Vesterqvist et al., 1999). Its elimination is primarily renal, with a half-life of around 11 hours, which can

be prolonged in patients with renal impairment, necessitating dosage adjustments.

Similar to Omapatrilat, Sampatrilat is a dual inhibitor of ACE and NEP, used to manage hypertension and heart failure. It is absorbed efficiently after oral administration, reaching peak plasma concentrations within approximately 2 hours. The drug is highly protein-bound (~95%) and distributed throughout the body, with limited penetration into the central nervous system. Sampatrilat undergoes extensive hepatic metabolism, primarily via the cytochrome P450 enzymes, and is metabolized into inactive compounds. The drug is eliminated primarily through the kidneys, with a half-life of around less than 24 hours.

SamASP (also known as **Samapril**) is an ACE inhibitor used in the treatment of hypertension. It is moderately absorbed after oral administration, with peak plasma levels occurring within 2 to 4 hours. The drug exhibits low bioavailability (~30%) and is widely distributed throughout the body, with high plasma protein binding (~80-90%). It undergoes hepatic metabolism, converting into active metabolites, although it experiences considerable first-pass metabolism. The elimination of **SamASP** occurs primarily through renal excretion, and both the parent compound and its metabolites are eliminated via urine. The half-life of **SamASP** is approximately 10 hours, and its clearance is slower in patients with renal impairment, requiring dose adjustments to prevent accumulation.

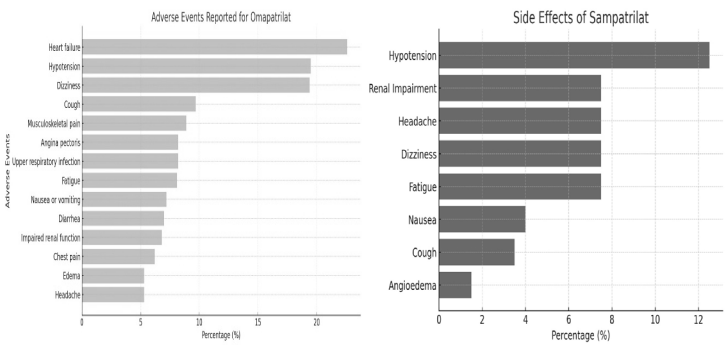

Fig 2: Adverse events reported in Omapatrilat and % of Side effects of in Sampatrilat.

Ligand Interactions

The efficacy of vasopeptidase inhibitors such as omapatrilat, sampatrilat, and SamASP is deeply rooted in their ligand interactions with the active sites of ACE and NEP.

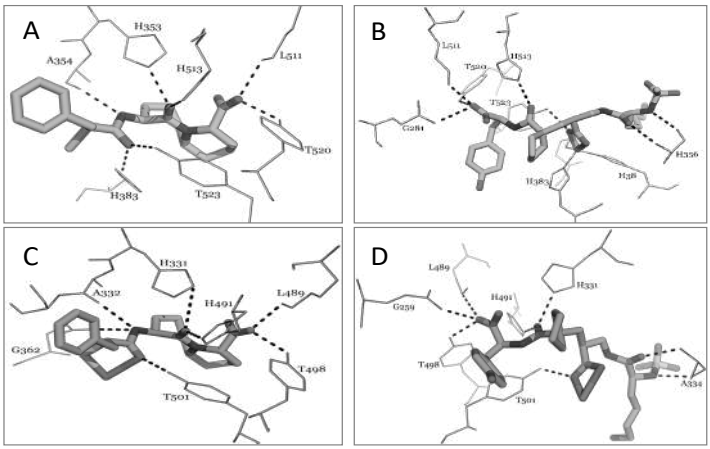

Fig 3: Omapatrilat in carbon domain of ACE enzyme (PDB ID: 6H5W). B.Sampatrilat in carbon domain of ACE enzyme (PDB ID: 6F9T).C. Omapatrilat in nitrogen domain of ACE enzyme (PDB ID:6H5X). D. Sampatrilat in nitrogen domain of ACE enzyme(6F9V).

Omapatrilat is designed as a tripeptide mimic, enabling its simultaneous binding to the catalytic sites of ACE and NEP. It establishes bidentate coordination with zinc ions present in both enzymes' active sites. In NEP, omapatrilat occupies the S1', S2', and partially the S3' subsites, with its bicyclic group extending towards non-prime regions. Its C-terminal carboxylate group interacts with residues like Glu584 in NEP and Glu411 in ACE, stabilizing the binding. These strong interactions contribute to high affinity and slow dissociation rates, which underlie omapatrilat's potency but exacerbate bradykinin accumulation.

Sampatrilat shows a unique binding pattern, demonstrating selectivity for ACE's C-domain over the N-domain. Its lysine side chain forms hydrogen bonds with Glu748 and Glu1008

in the S1 and S2 subsites of ACE's C-domain. This selectivity reduces bradykinin buildup, offering a safer profile compared to omapatrilat. In NEP, sampatrilat binds to S1′ and S2′ subsites with slightly weaker interactions than omapatrilat, balancing its potency across both enzymes.

SamASP binds to NEP and ACE similarly to sampatrilat but with reduced affinity due to weaker electrostatic interactions. Its C-terminal modifications increase steric hindrance, limiting its access to key residues like Lys461 and Asp422 in NEP. Additionally, its lack of domain selectivity for ACE reduces its therapeutic potential while increasing the likelihood of side effects such as angioedema (Sharma et al., 2020).

Key Ligand-Residue Interactions

Ligand	Enzyme	Key Binding Residues	Subsites Occupied	Zinc Interaction	Binding Affinity
Omapatrilat	ACE	Glu411, His383, His387	S1, S1', S2'	Bidentate	High
	NEP	Glu584, Lys421, Asp642	S1', S2', S3'	Bidentate	High
Sampatrilat	ACE	Glu748, Glu1008, Lys511	S1, S2	Monodentate	Moderate
	NEP	Lys461, Asp422, Glu460	S1', S2'	Monodentate	Moderate
SamASP	ACE	Glu748, Asp422	S1, S2	Weak	Low
	NEP	Asp422, Lys461	S1', S2'	Weak	Low

Comparison of Binding

Compound	Ki for NEP (nM)	Ki for ACE (nM)	Ki for nACE (nM)	Ki for cACE (nM)	Selectivity (cACE/nACE)
Sampatrilat	8	1.2	171.9	13.8	12.4
Omapatrilat	8.9	0.5	0.015	0.016	1.06
SamAsp	Not available	8.7	10.6	7.4	1.4

Omapatrilat binds extensively across the S1′, S2′, and S3′ subsites of NEP, forming strong interactions with zinc ions and catalytic residues (Sharma et al., 2020).Sampatrilat inhibited both cACE and nACE in the lownanomolar range, but it was 12.4-foldmore selective for cACE (Ki of 13.8 and 171.9nMrespectively) (Cozier et al., 2018). H-bondinteraction betweenthe lysinesidechainof sampatrilatandnACE (due toGlu403of cACEbeing replacedbyArg381 in nACE),andalessextensivehydrophobicnetwork.The comparison of the N1/C1 and N2/C2 poses indicates that the lysine side chain of sampatrilat exhibits considerable flexibility within the S2 pocket of both nACE and cACE, while demonstrating more precise interactions in the S1 pocket (Rajni et al., 2016). On the other hand, in SamASP, the aspartate group replaces the lysine chain of sampatrilat, showing non-specific and low micromolar affinity (Cozier et al., 2018).

Lipinski's Rule of Five

Lipinski's Rule of Five (Ro5) serves as a fundamental guideline in drug discovery, helping researchers evaluate the potential of a compound for oral bioavailability. It provides insights into whether a molecule is likely to be well-absorbed and distributed in the human body, making it a valuable tool in pharmaceutical development. By applying

these criteria early in the drug design process, scientists can improve the chances of selecting compounds with favorable pharmacokinetic properties while minimizing failures due to poor absorption. According to the rule, an orally active drug should not exceed five hydrogen bond donors, ten hydrogen bond acceptors, a molecular weight of 500 Daltons, and a logP value greater than five, ensuring a balance between solubility and permeability. Although some drugs may deviate from these parameters, Ro5 remains an essential reference point in medicinal chemistry, guiding the development of effective and bioavailable therapeutic compounds (Karami et al., 2022).

Table: Lipinski's Rule of Five Analysis for Omapatrilat, Sampatrilat, and SamASP

Comparison Table of Lipinski's rules: Omapatrilat vs. Sampatrilat

Parameters	*Lipinski's Threshold*	*Omapatrilat*	*Sampatrilat*	*Compliance*
Molecular Weight	≤ 500 daltons	492.52 daltons ✅	563.63 daltons ❌	Omapatrilat complies; Sampatrilat exceeds the threshold, possibly reducing membrane permeability.
Hydrogen Bond Donors	≤ 5	2 ✅	7 ❌	Both compounds comply with the rule for hydrogen bond donors.
Hydrogen Bond Acceptors	≤ 10	6 ✅	13 ❌	Both compounds comply with the rule for hydrogen bond acceptors.
LogP (Lipophilicity)	≤ 5	2.47 ✅	2.90 ✅	Both compounds have favorable lipophilicity, supporting good absorption potential.

Discussion

Omapatrilat is consistent with many criteria for productive drug development, though its high polar surface area may adversely affect permeability and ultimately bioavailability. In contrast, Sampatrilat complies most with Lipinski's rules, generating better pharmacokinetic characteristics and higher clinical future potential. In opposing, SamASP is deficient for its high molecular weight and polar surface area, indicating

that structural modifications are required for SamASP to be used as a drug candidate.These findings underscore the need for iterative optimization to enhance the drug-like properties of next-generation vasopeptidase inhibitors.

Comparison of Binding

Omapatrilat binds extensively across the S1′, S2′, and S3′ subsites of NEP, forming strong interactions with zinc ions and catalytic residues (Sharma et al., 2020).Sampatrilat inhibited both cACE and nACE in the lownanomolar range, but it was 12.4-foldmore selective for cACE (Ki of 13.8 and 171.9nMrespectively) (Cozier et al., 2018). H-bondinteraction betweenthe lysinesidechainof sampatrilatandnACE (due toGlu403of cACEbeing replacedbyArg381 in nACE),andalessextensivehydrophobicnetwork.The comparison of the N1/C1 and N2/C2 poses indicates that the lysine side chain of sampatrilat exhibits considerable flexibility within the S2 pocket of both nACE and cACE, while demonstrating more precise interactions in the S1 pocket (Rajni et al., 2016).On the other hand, in SamASP,the aspartate group replaces the lysine chain of sampatrilat, showing non-specific and low micromolar affinity (Cozier et al., 2018).

Challenges of Bradykinin Accumulation

Bradykinin, while essential for vasodilation, poses a dual-edged sword in the context of ACE and NEP inhibition. Excessive accumulation increases vascular permeability and risk of angioedema. Recombinant full-length ACE have shown that the Km of ACE for bradykinin is substantially lower than for Ang I, reflecting a greater affinity for metabolism of bradykinin than for the production of Ang II (Corti et al., 2001).

Future Directions in Vasopeptidase Inhibitor Design

Emerging technologies, including AI-driven drug discovery and advanced docking simulations, are accelerating the design of next-generation inhibitors. Combining vasopeptidase inhibition with complementary pathways, such as endothelin receptor antagonism, is being explored to enhance efficacy further. Additionally, biomarker-guided approaches are

improving patient stratification, tailoring therapies to individual needs.C-domain–selective ACE inhibitors offer hope for a new generation of ACE inhibitors with improved safety but are unlikely to offer improved efficacy unless combined with other drugs (Arendse et al., 2019).

Conclusion

Vasopeptidase inhibitors, spearheaded by omapatrilat, represent a transformative approach to hypertension management. Despite challenges, advances in analogues like sampatrilat highlight their potential to overcome existing limitations. Through innovative design and rigorous clinical evaluation, these agents are poised to redefine hypertension treatment paradigms, offering hope for improved patient outcomes. The utilization of contemporary structure-based drug development techniques on established therapeutic regions will yield more selective molecules targeting ACE.

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The effectiveness of the Rotigotine Transdermal Patch in Parkinson's Disease along with Clinical Cases

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ABSTRACT

Rotigotine, a non-ergot dopamine receptor agonist targeting D3/D2/D1 receptors, is administered through a transdermal delivery system which has been studied for managing idiopathic Parkinson's disease.

Two surveys made, has shown that if patients diagnosed with preliminary Parkinson'sstages is treated with a monotherapy of rotigotine it has given positive results – with significant greater decrease of Parkinsonian symptoms when measured in scale of Unified Parkinson's Disease Rating Scale Scores, in comparison to placebo receiving patients. Notable, marked positive outcomes were observed when rotigotine patches of 30 cm2 and 40 cm2 were used during studies.

For patients with advanced Parkinson's, rotigotine used alongside levodopa led to clinically meaningful reductions in "off" time from baseline in two well-conducted clinical trials. However, one trial reported a substantial placebo effect, thus it was observed that in primary outcome no significant

difference was observed in rotigotine treatment and placebo treatment for patches of size 20, 40 and 60cm2. Nevertheless, a more recent study has produced remarkable results and shown reductions ($p \leq 0.003$) in "off" time for patients receiving 40 cm^2 and 60 cm^2 rotigotine patches compared to placebo.

Across clinical trials, rotigotine is found to be tolerant in both cases – either singly as a monotherapy or in combination with levodopa. Reported adverse effects were mostly mild to moderate in severity.

Keywords: *levodopa, monotherapy, receptors, placebo, rotigotine*

Introduction

Parkinson's disease was 1st described by James Parkinson in 1817, and is defined as a progressive neurodegenerative disease. It primarily affects older adults with primary symptoms occurring in early to mid-60s. The disease is prevalent among all ethnic groups and has been found in greater occurrence among men than in woman. Almost 1% of individuals having a age above 60 is found to be show symptoms of Parkinson.

The dopaminergic neurons are the 1st to be affected in Parkinsons disease showing degeneration in the mid brain region particularly from SNpc-Substantia Nigra Pars Compacta to a part of basal ganglia the caudate nucleus and putamen together known as the striatum (Dauer & Przedborski et al., 2003). However though initially symptoms are not visible, they surface or clinically become eminent when already 60–80% of striatal terminals and 40–50% of nigral neurons have been degenerated (Fearnley & Lees, 1991). The core symptoms of Parkinson's disease include rigidity, stiffness, tremor in resting state, and bradykinesia, which are typically asymmetric and respond well to dopaminergic therapies. In addition to these motor symptoms, patients may also experience other symptoms which can be categorized under nonmotor symptoms that includes autonomic dysfunction, cognitive and psychiatric issues, sensory disturbances, and sleep disorders (Chaudhuri et al., 2006).

Levodopa has long been effective basis for treatment of Parkinson's disease. Levodopa is a precursor to dopamine and thus when enters body forms dopamine thereby alleviating symptoms for most patients (Olanow et al., 2009). To reduce peripheral conversion of levodopa into dopamine and minimize associated side effects, it is often administered with a dopa-decarboxylase inhibitor (Cotzias et al., 1969). However, prolonged use of levodopa is associated with complications such as dyskinesias and motor fluctuations, including "wearing-off" and "on-off" episodes (Obeso et al., 2000).

Dopamine entering the body quickly binds with the dopamine receptors and stimulates thembut are slightly less effective than levodopa but have a lower risk of causing dyskinesias (Stowe et al., 2008). The advent and effectiveness of these medications has put them as primary contenders in monotherapy for treating early stages of Parkinson's disease or under severe condition they are used as a adjunctive therapy with levodopa so that the clinical cases can prolonged "on" time and reduce the required levodopa dose (Schapira et al., 2009).

This review focuses on rotigotine (marketed as Neupro®), a dopamine agonist. The delivery of the drug is obtained through transdermal patch which in turn is an effective treatment procedure for Parkinson's disease. Rotigotine is used both as monotherapy and as an adjunct with levodopa for early-stage disease advanced-stage disease respectively(Watts et al., 2007). Rotigotine is also effective against restless leg syndrome as well as devised as a nasal spray for managing severe Parkinson's syndrome (Oertel et al., 2010). However so the application protocols are beyond the scope of this discussion.

Note: Neupro® is a trade name for rotigotine which is administered as transdermal system, is used for identification purpose solely and not for endorsement

Pharmacodynamic Properties

Rotigotine, formerly referred to as N-0923, belongs to a class of non-ergot aminotetralin dopamine stimulating receptor which mostly binds to target receptors like D3, D2, and D1. (Schneider et al., 1998). Dopamine agonists alleviate parkinsonian symptoms by mimicking the effects of dopamine on postsynaptic receptors in the substantia nigra which is a part of midbrain and a portion of basal ganglia (Jenner, 2003). Most of the pharmacodynamic data on rotigotine comes from in vitro studies or animal research, with many findings only published as abstracts or posters (Cawello et al., 2009).

Rotigotine's receptor binding affinity and Activation

The receptor binding affinities of rotigotine have been examined in vitro. The D2 and D1 receptors mentioned above, which are studied in bovine caudate membranes was found to have high-affinity equilibrium constants with values 0.3 nmol/L and 3 nmol/L, respectively, showing a 10:1 superiorityof D2 receptor in comparison to D1 receptor (Laduron et al., 1994). On the other hand, D2 and D1 receptorslow-affinity constants were measured as 79 nmol/L and 1160 nmol/Lrespectively, indicating a 15:1 preference for D2 in comparison to D1 (Perachon et al., 2000). Studies conducted on recombinant rat models harboring D1, D2, and D3 receptors expressed in CHO cells measured affinity constants of 364, 11, and 0.94 nmol/L, respectively, preferring a D2 over D1 with a selectivity ratio of 33 (Newman-Tancredi et al., 2002). This binding profile closely mirrors that of natural dopamine (Millan et al., 2002).

Rotigotine is found to bind with other receptor types like α2-adrenergic receptors as well as serotonin receptors. The dissociation constant which is an effective measure of binding potential, was measured as 110 nmol/L in case of a α2-adrenergic receptors found on vas deferens of a rat model.Serotonin receptors like 5-HT1A and 5-HT2 showed dissociation constant values of 102 and 3400 nmol/L, respectively when observed on bovine caudate membranes. (Schneider et al., 2003).

The effectivity of rotigotine was further illustrated in an experiment where aslow-release dose of 0.5mg/kg rats when administered showed a reduction of 20% extracellular dopamine levels after three to four hours which persisted for at least 48 hours. The dopamine level drops were calculated in rotigotine administered rats in comparison against controls rats receiving only the vehicle (utilized for rotigotine delivery) were in comparison.This reduction in dopamine levels for such long duration, suggested sustained stimulation of dopamine receptors (Cawello et al., 2009).

Rotigotine against Parkison and Restoring Motor Activity:

Rotigotine was found to play effective role in restoring both motor and nonmotor functions in 6-hydroxydopamine (6-OHDA)lesioned rats. 6-hydroxydopamine is commonly used to induce Parkinsons condition in healthy animal. 0.03 µmol/kg, was found to be an effective mode of treatment for half the animal population under study. In fact the response is also dose specific which sustains for an hour (Schneider et al., 2003).

Rotigotine if used continuously, does not seem to create dyskinesia in animal model. This was supported by the fact that, there was no sign of involuntary movements when mild rotigotine was subcutaneously administered @1mg/kg every 48 hour for over 10 days (Jenner et al., 2005). A study showed similar outcome where rotigone failed to enhance locomotory activity in rat model lesioned by 6-OHDA (Cenci et al., 2007).

Both subcutaneous and intramuscular rotigotine administration improved motor and nonmotor performance in animal models of motor deficits in a dose-dependent manner (Pearce et al., 2015). Subcutaneous doses of rotigotine ranging from 0.019 to 0.3 mg/kg enhanced motor activities and showed a decrease in multifactorial disability scores in organism closely related to monkey treated with systemic methylphenyltetrahydro pyridine (MPTP) (Bezard et al., 2001). Motor and non motor task performing ability increased in hemi-lesioned monkey if rotigotine was administered through intra-muscular pathway (Fox et al., 2012). The motor effects of rotigotine were blocked by the administration of the

D1 receptor antagonist SCH 23390 (Gerlach et al., 1996).

Few other effects observed in single dose rotigotine administered rats included decreased consumption of palatable foods along with yawning behavior in rats (Perachon et al., 2000). Intraperitoneal rotigotine administeredat doses of 1.0 and 5.0 μmol/kg reduced food intake, which was result of reverting back the activity of D2 receptor antagonist nemonapride (YM-09151-2) (Millan et al., 2002). At a lower dose (0.5 μmol/kg), rotigotine increased yawning and forepaw stretching, which is mainly caused due to effects of reversal of the D2/D3 receptor antagonist UH 232 (Newman-Tancredi et al., 2002).

Neuroprotective Activity

Rotigotine showed neuro protective effects in Parkison'disease induced rat model when administered subcutaneously (Bezard et al., 2001). Rotigotine effective against degenerating neurons (Schneider et al., 2003) at dosage of slow-release formulation @ 3mg/kg administered along with methyl phenyl tetra hydropyridine (MPTP) or separately at 16 hours prior to MPTP. Additionally, successive treatment with slow-release rotigotine at doses of 0.3, 1, or 3 mg/kg for 7 days following MPTP exposure prevented the degeneration of striatal terminals in a dose-dependent manner (Jenner et al., 2005).

Pharmacokinetic Properties

Rotigotine is delivered via a silicone-based adhesive matrix transdermal patch, ensuring a consistent release of the drug over a day (Cawello et al., 2009).

In a clinicaltrial which was comprised of around 8 volunteers, when administered with 4.5 gm of rotigotine through a 10 cm2 transdermal patch showed an averaged sized peak for concentration of plasma (Cmax) of 0.215 ng/mL, reached 16 hours after application. The graph area which covered the area between the axes of plasma concentration and time curve (AUC) up to the last measurable concentration (AUC0–tz) was 3.94 ng•h/mL. After 24 hours when the patch was removed, rotigotine plasma levels declined with a median

terminal elimination half-life (t½) of 6.82 hours (Wolff et al., 2007).

Plasma concentrations when checked on 70 patients increased proportionately with proportionate increase of rotigotine. The dose specificity was checked in case of early-stage Parkinson's disease who were administered with rotigotine, starting with a 10 cm^2 patch and titrating up to a 40 cm^2 patch (containing 18 mg of rotigotine) over 24 days. The mean steady-state plasma concentration with the 40 cm^2 patch applied to the abdomen was 0.79 ng/mL,and when checked for variations in different application site showed similar result (Cawello et al., 2013). When a new patch was put to use, there was a slight decrease in plasma levels, with a lag of approximately three hours, but concentrations remained stable throughout the 24-hour period (Elshoff et al., 2012).

Rotigotine often faces greater extent of metabolism. The former compound is metabolized through conjugation (via sulfation and glucuronidation) and through phase I metabolism by cytochrome P450 (CYP) isoforms, forming two metabolites, which are followed by combining to form a functional unit (Cawello et al., 2015).

An important parameter of any drug is its clearance from body. The pharmacokinetic parameters of rotigotine, including AUC, Cmax, and t½, gave rise to a common outcome among healthy individuals and patients with very littleto severe renal impairment which was measured through creatinine clearance levels – that accounted between ≥30 to <50 mL/min (mild impairment) or <30 mL/min (severe impairment) (Cawello et al., 2010). However, when it comes renal clearance of rotigotine, severe renal impaired patients showed 50% lesser efficacy than healthy patients.Patients with final stages of renal disease undergoing hemodialysis, further showed that rotigotine concentrations remained stable, and the drug was not detected in dialysis fluid (Wolff et al., 2010).

Potential Drug Interactions

Studies comprising human liver microsomes in invitro condition and cells with human cDNA suggest that rotigotine and its metabolites have a lesser binding potential low

potential with other drugs metabolized by CYP enzymes, including CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 (Cawello et al., 2010). Furthermore, no significant inhibition of rotigotine metabolism was observed when selective CYP isoform inhibitors were used (Wolff et al., 2007). An in vivo study in cynomolgus monkeys confirmed that neither rotigotine nor its phase I metabolites significantly inhibit CYP enzyme activity (Elshoff et al., 2012).

In a study involving 12 healthy male volunteers, coadministration of 400 mg of cimetidine twice daily with a 10 cm^2 rotigotine patch did not affect the steady-state maximum concentration of plasma (Cmax) or the area under the plasma concentration-time curve (AUC) of rotigotine (Cawello et al., 2013).

No interaction pharmological was observed between transdermal rotigotine (9 mg/day) and oral levodopa/carbidopa (100 mg/25 mg twice daily) in 23 patients with restless legs syndrome. The steady-state Cmax and AUC values of levodopa, carbidopa, and rotigotine were comparable whether administered separately or together (Oertel et al., 2008).

Therapeutic Efficacy

The efficacy of rotigotine waschecked for primary stages of Parkinson's as only drug for treatment, as well as adjunctive therapy with levodopa for treating advanced-stage disease through four large, randomized, double-blind, placebo-controlled, multicenter trials (Watts et al., 2007; Poewe et al., 2007; Trenkwalder et al., 2011; LeWitt et al., 2013). These studies typically included a screening/run-in phase, a dose-titration phase lasting 3-5 weeks, and a maintenance phase of 7 to 24 weeks.

Administration and Patch Sizes: The rotigotine transdermal system was applied once daily. Patch sizes ranged from 10-40 cm^2 for early-stage Parkinson's disease and 20-60 cm^2 for advanced-stage disease (Schapira et al., 2013).

Early Parkinson's Disease

Individuals reported with primary stages Parkinson's disease

show a H-Y stage of ≤3 (Poewe et al., 2007). In a trial , patients were treated continuously with fixed doses of selegiline, amantadine, or acetylcholine receptor antagonists if maintained for at least 28 days before baseline and throughout the trial (Watts et al., 2007).

The completion pointin some studies was taken as the change in the Unified Parkinson's Disease Rating Scale (UPDRS) parts II and III scores (activities of daily living and motor subscales) from baseline to the end of treatment (LeWitt et al., 2007). The percentage of patients achieving ≥20% improvement in these scores was also evaluated (Schapira et al., 2013).

Patients using rotigotine patches had better results regarding improvement compared to those on placebo. In a reported trial, patients using 30 cm^2 or 40 cm^2 patches showed marked improvements within 4 weeks of treatment in UPDRS scores. These improvements persisted through week 11 (Poewe et al., 2007). A dose-response relationship was observed, with benefits plateauing between 30 cm^2 and 40 cm^2 patches (Trenkwalder et al., 2011).

In another trial with a maintenance phase of 6 months, patients treated with rotigotine (up to 30 cm^2) showed an average improvement of 5.3 points in UPDRS scores compared to placebo (LeWitt et al., 2013). In comparison to placebo group, greater proportion of patients in the rotigotine group achieved a response (Schapira et al., 2013).

Advanced Parkinson's Disease

Trials in advance-stage Parkinson's disease included patients with a H- Y stage of 1-5 scoreor those which gave inadequate responses when tried to be controlled with levodopa. In this study the participants were continuously on levodopa.The primary completion point, in these trials was marked by reduction in "off" time per day.

Rotigotine patches significantly reduced "off" time in advanced-stage patients when studied with two large sample. In the formerstudyplacebo and rotigotine groups showed similar reductions in "off" time during the 7-week maintenance period due to a substantial placebo effect.

However, a later study demonstrated significant reductions in "off" time with rotigotine (40 cm^2 and 60 cm^2 patches) compared to placebo after 24 weeks of treatment.

Tolerability

General studies conducted regarding tolerance of Rotigotine treatment during different stages of Parkinsons disease showed that no adverse effects were shown up to 7 months in early stages as well as in advance stages where it was used as an adjunct to levodopa.(Poewe et al., 2007; LeWitt et al., 2007).

Application-Site Reactions: One of the most frequent adverse effects of treatment with rotigotine was reactions specific to application site. It was as frequent as 39-44% rotigotine-treated patients.In fact 12-21% placebo recipients also showed this kind of adverse effects(Trenkwalder et al., 2011). Though these reactions are frequent, they are usually mild to moderate in effect and very easily cured over time. In another study due to this adverse discomfortfewer than 5% of patients discontinued treatment (Schapira et al., 2013).

Other Adverse Events: Adverse effects consistent with dopamine receptor stimulation, such as nausea, somnolence, insomnia, and dizziness, were reported (Watts et al., 2007). The incidence of these effects appeared dose-related in some trials (LeWitt et al., 2013).

Serious Adverse Events: Treatment with rotigotine showed serious adverse effects in about 4-5% of the patients under trial, and this adversity was also observed in 0.2-2% of placebo recipients (Elshoff et al., 2012).

Treatment Discontinuation: Adverse effect caused discontinuation of trials in approximately twice as many patients receiving rotigotine compared to placebo (10-14% versus 4-6%) (Poewe et al., 2007).

Rotigotine had no significant effects on laboratory parameters, physical examinations, or vital signs. Mild ECG changes and occasional reports of tachycardia were noted (Schapira et al., 2013).

Administered Dosages for treatment:

Rotigotine treatment was made by administering rotigotine through transdermal position with varying patch size to administer different dosage:

Table 1: **Rotigotine dosage for Early- Stage Parkinsons Disease according to Schapira et al., 2013; Watts et al., 2007**

Sl. No	Patch Size	Rotigotine quantity (Total Drug Content)	Dosage /24 Hour
01.	10 cm^2	4.5 mg	2mg/24 hours
02	20 cm^2	09 mg	4 mg/ 24 hours
03	30 cm^2	13.5 mg	6 mg /24 hours
04	40 cm^2	18 mg	8 mg /24 hours

For advanced Parkinson's disease, patch sizes of 20, 40, and 60 cm^2 (27 mg; 12 mg/24 hours) were used as adjunctive therapy to levodopa (Poewe et al., 2007; Trenkwalder et al., 2011).

Current Status

Rotigotine, delivered via the transdermal system, has demonstrated clinical efficacy as monotherapy for early-stage Parkinson's disease and as adjunctive therapy with levodopa in advanced-stage disease (Poewe et al., 2007; LeWitt et al., 2007; Trenkwalder et al., 2011). It showed very less adversity in both conditions, unfortunately application-site reactions the most common adverse effect(Schapira et al., 2013; Watts et al., 2007). Regulatory evaluations for these indications are underway in Europe and the US (Elshoff et al., 2012).

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CARDIOVASCULAR DISEASES ASSOCIATED WITH IMMUNE RESPONSE: A COMPREHENSIVE REVIEW

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ABSTRACT

Immune checkpoints naturally help to protect the heart and blood vessels by controlling T-cell activity. They reduce inflammation, promote the development of protective regulatory T-cells and help to maintain stability in blood vessels plaques. When immune checkpoint inhibitor blocks these checkpoints, T-cells can cause inflammation and damage. In some cases, ICIs have also been linked to autoimmune reactions, where the immune system mistakenly attacks heart cells. Normally monoclonal antibodies are the immune checkpoint inhibitors. After escaping from the immune checkpoints, the tumour cells promote T-cell activation which allows them to proliferate. The monoclonal antibodies bind to immune checkpoints and their ligands to prevent inhibition of T-cell activation by tumour cells multiple immune checkpoints inhibitors are developed specially targeted to cytotoxic T-lymphocyte antigen 4 (CTLA 4), programmed death protein 1 (PD-1) and its ligand (PD-L1) and lymphocyte activation gene-3 (LAG-3). This therapy has demonstrated powerful anti-tumour effects and significantly enhanced prognosis for cancer patients. But due to systematic effects, the therapy causes immune related adverse events (irAEs) like atherosclerosis, cardiomyopathy

and myocarditis. These events affect mainly cardiovascular system, liver and lungs and skin. This review highlights the mechanisms through which immune checkpoints protect the cardiovascular system and how blocking them can lead to damage. By understanding this process researchers can identify new ways to prevent or treat cardiovascular irAEs without reducing the effectiveness of cancer treatment. This knowledge could lead to better outcomes for patients by balancing cancer control with good heart health. This simplified overview aims to provide insights into the complex relationship between immune checkpoint inhibitors, immune system regulation and cardiovascular health.

Keywords: *Cardiovascular diseases, CTLA 4, immune response, LAG-3*

Introduction:

Immune checkpoint inhibitors have revolutionized oncotherapy due to their ability to active T cells against cancer cells and protect other cells against damage from cancer. They have secured a crucial place in various cancer treatment. The mechanism of immune checkpoint inhibitors involves the initial recognition and processing of cancer cell antigens by APC (antigen presenting cell) like macrophages, B cells and dendritic cells. These antigens are then presented to T-cells through the MHC (major histocompatibility complex),which initiates the immune response against the cancer cells. There are some crucial secondary co-stimulatory signals which are associated with the interaction between CD80 or CD86 on APCs and CD28 on T cells.Theinhibitory molecules such as CTLA-4, PD-1 and its ligand PD-L1 play a crucial role in regulation of T-cell activation and inhibit the excessive immune responses. The function of ICIsis blocking these co-inhibitory molecules, thereby enhancing T-cell mediated anti-tumour responses. Despite their efficiency, ICIs are associated with immune related adverse events (irAEs) affecting various tissues, including thyroid,lungs, colon. The role of PD-1,PD-L1,CTLA-4 pathways has been well documented, but precise mechanism of cardiac irAEs remain unclear.A meta-analysis report states that 8.32% incidence of cardiovascular events among over 21,000 patients who are treated with

ICI in randomized clinical trials (Salem.et. al;2018), (Xavier et.al;2022). irAEs pose a significant threat, ranging from mild arrhythmias to myocarditis, the latter having up to 50% mortality rate. Another meta-analysis states that while cardiovascular irAEs were among the least common (8%), they were the second leading cause of death (25%)(Wang et,al,2018). Upon diagnosis current guidelines recommend temporary or permanent cessation of ICI therapy and immediate immunosuppressive treatment. However, not all patients respond to immunosuppressives and in some critical cases the risk of death may increase by this treatment. In this review we aim to provide a overview about the mechanism of the IC and a possible treatment of cardiac irAEs. (Yousif L. I.et al.2022)

Immune checkpoints:

Immune checkpoint has a regulatory mechanism which regulates the immune response. These checkpoints have a significant role to prevent overactivation of immune cells, which could damage the healthy tissues, it involves proteins such as PD-1, CTLA-4 which are situated on T-cells. These proteins interact with their specific ligands such as PD-L2 TO supress T-cell activity. The T-cell receptor and co stimulatory signals CD28 binds to CD80/ CD86. This binding recruit (PI3R) phosphoinositide 3-kinase which activates the PI3K/Akt pathways. The pathway promotes proliferation differentiation and anti-apoptotic signals in T-cells. After activation the CD8 cells divides into two types of cells cytotoxic T cells and CD cells.

PD-1 is located on the surface of T-cells. It binds only with is two ligands PD-L1 and PD-L2(Ghiotto M.et al.2010). The PD-L1 is situated on hematopoietic cells and tissue cells in different organs. PD-L2 found on macrophages dendritic cells. Binding of PD-L1 and PD-L2 inhibits the T-cell activation through dephosphorylation of P13K-Akt pathway, results low cytokine production, which is associated with inflammation. CTLA- 4 is situated on the surface of activated T-cells CTLA-4 binds to CD80 and CD86 to suppress T-cell activation by down regulating the P13K pathway. In comparison with PD-1, CTLA- 4 inhibits T- cell activation more rapidly(Parry R V.et

al.2005). LAG- 3 is expressed on the surface of activated Tcells, natural killer cells, B cells and dendritic cells. LAG-3 specially binds to galactin-3 (GAL-3), fibrinogen like protein(FGL-1) and primary ligand MHCII. It gives protection against atherosclerosis by inhibiting excessive T-cell activation.

Immune checkpoint inhibitors in cardiovascular disease and immune response:

Immune checkpoint inhibitors such as pembrolizumab and ipilimumab have transformed cancer treatment by altering immune responses.Pembrolizumab, which inhibits programmed death (PD-L1) and ipilimumab, a blocker of cytotoxic T lymphocyte associated antigen-4, enhance T-cell activity directed against tumours. Though they have endless positive impact on oncology, they may affect the cardiovascular health by altering the immune mediated pathways which causes heart related diseases.Studies of safety records have found possible heart problems from ICIs includes atherosclerosis,cardiomyopathy, myocarditis, heart rhythmic issue(heart block), blood vessel infection (vasculitis), irregular heartbeats, blood clots in veins heart attacks.Pembrolizumab has shown effectiveness against advanced cancers but it carries 1% risk of immune mediated myocarditis which could be fatal if not treated properly. On the other hand, ipilimumab has been associated with cardiac inflammation. Other ICIs likenivolumab (a PD-1 inhibitor) and atezolizumab (targets PD-L1) also have associated cardiac risks. Uses of ICIs can show an arise of irAEs which often require immunosuppressive treatments.

- **Atherosclerosis:** The interaction between PD-1 and PD-L1 suppresses inflammatory T-cell activities and contributes to plaque stability by enhancing Treg activity and decreasing pro- inflammatory cytokines [Vuong J T.et al.2022] Inhibition or removing PD-1/PD-L1 results in heightening inflammation, enlarged plaques which leads to adverse vascular damage. The PD-L1 on on endothelial cells defence against the immune related damage by the T-cell function and lower the chances of apoptosis in endothelial cells.CTLA-4 plays a important role in regulating T-cell activation and inflammation, thereby decreasing plaques size and slowing

or lowering the risk of atherosclerosis (Poles K.et al.2020). In the absence of CTLA-4, lesions increase in size which increase the risk of deposition of fatty material on the inner wall of heart. LAG-3 supports the survival of endothelial cells through MAPK /Erk and PI3K/Akt / mTOR signalling pathways (Hemon P.et al.2011).

• **Myocarditis:** Myocarditis is the inflammation of the heart muscle and is frequently associated with immune checkpoint inhibitor therapies.Although it is uncommon, it carries a high risk of mortality(50% mortality) especially in severe cases (Salem J E.et al.2018). Factors that increase the risk include the specific type of ICI used (anti PD-1/PD-L1 is more risky than anti CTLA-4), combination therapies and radiotherapy,chemotherapy. The breakdown of immune tolerance causes T-cell activation,resulting myocarditis. The PD-L1 expression on endothelial cells offers protective immune separation, which disrupt by ICI treatment. ICIs stimulatesT-cells and inflammatory cytokines which increases cardiac inflammation.

• **Cardiomyopathy:** it is a disease in which heart loses its ability to pump blood effectively leads to heart failure arrhythmias and other complications. PD-1 and PDL1 are proteins that help to regulate the immune system and prevent excessive immune responses (Kushnareva E.et al.2022). In mice, lacking PD-1, researchers observed dilated cardiomyopathy (DCM), a condition where the heart becomes enlarge and weak. This was linked to an autoantibodies(immune proteins attacking the body's own heart proteins) but not to immune cell infiltration, suggesting information and altered heartcell behaviour as the Cause (Tay W T.et al.2020). In humans, PD-L1 was found in damaged heart tissues, suggesting it helps to protect the heart by reducing immune activity and inflammation.A genetic variation in the CTLA-4 gene, which is involved in immune regulation, was found more often in DCM patients, hinting that autoimmunity plays a role in the disease, though this variation didn't affect disease progression after one year

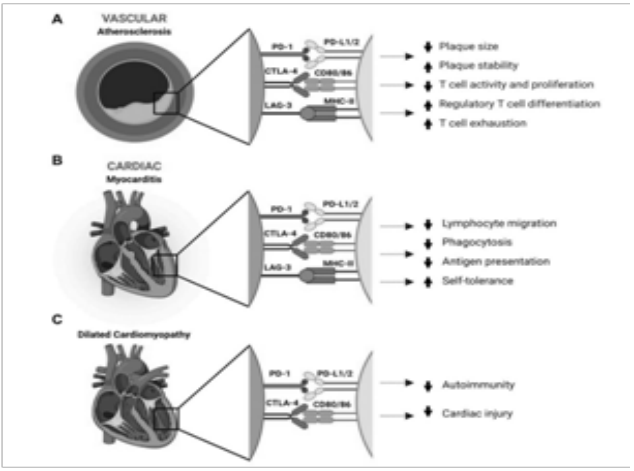

Figure: the role of immune checkpoints PD-1,CTL-4 and LAG3 in mitigating atherosclerosis, dilated cardiomyopathy, and myocarditis

PATHWAY SPECIFIC THERAPEUTIC TARGETS: Exploring alternatives to immunosuppressive drugs for managing cardiovascular immune-related adverse events(irAEs) linked to immune checkpoint inhibitors presents possible therapeutic targets in downstream pathways (Matzen E.et al.2021). Although corticosteroids are currently the first line treatment, there is a growing need for additional therapies due to the diverse patient responses and the potential for tumour progression during recovery. The JAK/STAT pathway which is affected by PD-L1/PD-L2 and CD80/CD86 signalling, is a key focus of research (Kubo S.et al.2014) . JAK inhibitors such as Tofacitinib,have demonstrated potential in managing ICI- induced myocarditis allowing for rapid patient recovery without significant side effects.Nonetheless, caution is warranted as JAK/STAT3 inhibition may promote tumour growth (Henderson Berg M. H.et al2022).Additionally, pathways like MAPK/Erk/PI3K/Akt/mTOR arebeing investigated.Inhibiting mTOR in conjunction with anti PD-L1 therapy has shown advantages in maintaining anti-tumour

efficacy while minimising toxicity (Esfahani k.et al.2019)At the same time MNK1/2 might lower oncogenic activity but could also elicit pro-inflammatory cytokines.Finally targeting the tryptophan pathway influenced by CD80/86 signalling, especially through the enzyme indoleamine 2,3-dioxygenase 1and (IDO1) represents another potential strategy for cancer immunotherapy (Tang k.et al.2021). Current clinical trials involving IDO inhibitors are showing potential across various cancers, although challenges persist, particularly following a failed phase III trial involving pembrolizumab and IDO inhibitors.

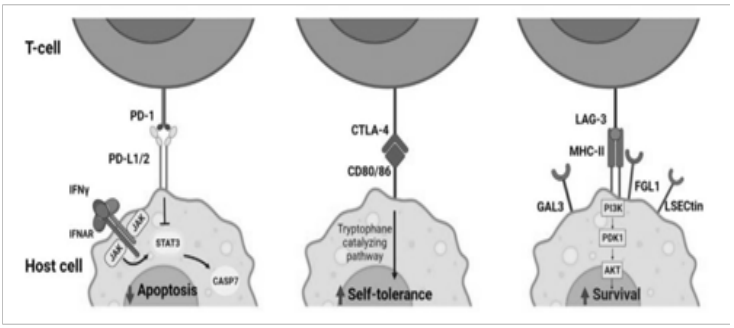

Figure: downstream signalling and effect of immune checkpoint ligands

Perspective:PD-1, PD-L1 and LAG-3 are presently recognised as therapeutic targets in the treatment of cancer. However, approximately 10% of patients receiving immune checkpoint inhibitors (ICIs) experience potentially life threatening cardiovascular immune related adverse events (irAEs) (Xavier et. al.,2022). With the increasing number of ICI being authorised and utilised in clinical settings, the occurrence of cardiovascular irAEs is anticipated to rise. Immune checkpoints down regulate the immune response by influencing various pathways involved in T cell activation, differentiation, and survival, thus diminishing inflammation and fostering tolerance. Moreover, the ligands for immune checkpoints have been found to possess additional intracellular signalling mechanisms that decrease

apoptosis and encourage self-tolerance in target cells such as endothelial cells and cardiomyocytes. The precise mechanisms responsible for the 3 diseases induced by ICIs-atherosclerosis, myocarditis and cardiomyopathy are not fully understood common necessitatingfurther research. Presently, the management of irAEs with corticosteroids does not yield benefits for every patient, and there is an urgent need for downstream targets.There have been recorded cases of successful treatments for ICI-induced myocarditis such as abatacept and randomised trials are in progress. Furthermore, additional research concentrating on the downstream pathways of immune checkpoint ligand host cells, including the JAK/STAT,MAP/Erk/mTOR, PI3K/Akt pathways and IDO1,as potential therapeutic targets is essential. while is crucial to comprehend the role of human checkpoints in the cardiovascular system, monitoring and prevention are equally significant in clinical practice. It is recommended to conduct serial monitoring using eco-cardiography (ECG) electrocardiograms and biomarkers (like troponin) for patients undergoing immunotherapy, chemotherapy and radiotherapy(Banfill et. al.,2021). According to the latest all patients treated with ICIs should have an ECG and troponin levels assessed baseline with extra ECG evaluation for those with elevated baseline troponin levels(Lyon et al.,2022). In instances of newly occurring abnormalities in ECG readings, changes in biomarkers, or cardiac symptoms at any stage during ICI treatment the guidelines recommend prompt cardio oncology assessment, including additional ECG for evaluating left ventricular ejection fraction and strain analysis. Cardiac magnetic resonance imaging is advised when myocarditis is suspected.

Conclusion:

Immune checkpoint inhibitor therapies have offered successful treatment options and enhance prognosis for a significant number of cancer patients. However, their interactionwith the cardiovascular system can be harmful. The blockade of immune checkpoint ligand interactions has been shown to hasten the onset and progression of atherosclerosis heighten inflammation and lead to myocardial infiltration, which can

result in life threatening myocarditis.

Additionally, it is suggested that these therapies made trigger autoimmunity and cause electrophysiological changes in cardiomyocytes, resulting in cardiomyopathy. Understanding the mechanisms related to immune checkpoint and cardiovascular immune related adverse events is crucial to :

1)identify preexisting risk factor for improved patient selection

2)exploretreatment option for cardiovascular irAEs that ideally maintain prolonged anti-tumour ICI effectiveness and

3) highlight potential new targets for cancer treatment.

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Bdellovibrio Bacteriovorus : A NATURAL SOLUTION FOR CONTROLLING HARMFUL BACTERIA

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ABSTRACT

Bdellovibrio bacteriovorus is a gram-negative predatory bacterium it is prey on harmful gram-negative bacteria. Their predatory nature is supported by various structural, biochemical, physiological and genetic adaptations. From their genetic studies it was found that their genes are specifically involved in prey recognition, chemotaxis, motility and to produce enzymes those are involved in nutrient acquisition and degradation of prey. Some important proteolytic enzymes and peptidoglycan hydrolases biochemically produced by them this can be used in biotechnological industries. Due to the presence of their distinct LPS various in vitro and in vivo studies shows that their use is safe and it can elicit minimal inflammatory responses in mammalian systems. They have some antibacterial properties including reduction of Klebsiella pneumooniae, Salmonella enteritidis like pathogens. Some significant characteristics of B.bacteriovorus makes them a non-toxic and novel alternative to the traditional antibiotics.

Keywords: *Antibacterial property, Bdellovibrio bacteriovorus, predatory bacteria*

Introduction

Bdellovibrio bacteriovorus is a small delta proteobacterium, it has a unique mode of their survival by invading the periplasm

of gram-negative bacteria, they consume the cytoplasmic materials of their respective prey and destroys them totally (Negus et al,2017). In terrestrial and aquatic environment, they are widely distributed (Varon et al,1980, Jurkevitch et al,2000). They are also present in mammalian feces, suggesting their presence in mammalian gut (3B). Field Emission Scanning Electron Microscopy (FESEM) demonstrated that this predatory bacterium can also prey on gram positive Staphylococcus aureus through an epibiotic mechanism (Lebba et al,2014). It is important to find alternatives to antibiotics and asses the vulnerability of pathogenic prey to B.bacteriovorus predation (Nrgus et al,2017). This predatory bacteria also prey on multidrug-resistance gram negative bacteria A. baumanni and K.pneumoniae studied under in vitro condition(Kadouri DE et al,2013). From the in vivo studies it was found that this bacteria can reduce K.pneumoniae load in rat lungs (Shatzkes K et al,2016). It helps to overcoming eye infections and burn wounds, lung infections in cystic fibrosis patients and periodontal infections (Shanks et al,2013; Lebba et al,2014; Bonfiglio et al,2020).This bacterium can be used as a veterinary medicine to target Moraxella bovis a causative agent of conjunctival and corneal infections in cattle, it also preventsSalmonella enterica infections in chicks (Atterbury et al,2011). In agricultural purpose this is effective to prevent bacterial soft rot in potatoes (Youdkes et al,2020) and bacterial blight caused by Pseudomonad glycibea (Scherf et al, 1972). B.bacteriovorus whole genome is sequenced to identify the metabolic products produced by this bacteria and to explore their potential use in biotechnological field (Bratanis et al ,2020). This review focused on the predation strategies and potential application of Bdellovibrio sp. in various fields. It also paving the way to use Bdellovibrio sp.in the field ofcancer treatment, in food preservatives and as a conventional antibiotic thus reducing the use of chemicals.

Bdellovibrio sp.Characteristics:

These bacteria have various important characteristics that enable it to act as a predator those are as follows –

Structural characteristics: -

1.Unique Cell Shape: - It has a spiral or vibroid (comma shaped) shape,which helps it to move rapidly and efficiently. (Lambert et al. 2006)

2.Cell Wall Modifications: - The activity of Penicillin-Binding Proteins (PBPS) in B. bacteriovorus which acts on prey cell wall is important.PBP4 proteins (DacBs, Bd0816 and Bd3459) act as endopeptidases, breaking D-amino acid containing peptide cross links in prey cell walls to round and relax them for predator entry (Lerner TR et al,2012), but it has a self-protection protein which inhibits the function of PBPS proteins when Bdellovibrio sp. Is insight the predator and in this way, they can protect their own cell wall (Lambert C et al,2015).

Physiological Characteristics:

1.High Motility:It has high motility rate;their swimming speeds range from 35µm/s for strain 109 J to 106 µm/s for the HD100 strain (Rendulic et al. 2004).Motility is essential which helps to generate forces required for attachment or penetration of prey cell,researchers proposed that active,motile 'drilling' of the Bdellovibrio sp.was responsible for prey entry (Burnham et al.1968).

2.Chemotaxis: Bdellovibrio sp. exhibits chemotaxis activity,due to the presence of this feature this bacterium moves towards chemical signals secreted by prey bacteria.

Biochemical Characteristics:

1.Proteolytic Enzymes: Bdellovibrio sp. produces some proteolytic enzymes that helps the bacteria to get entry in prey by penetrating and breaking down the prey's cell wall(Sockett et al.2009).

2.Peptidoglycan Hydrolases: Bdellovibrio sp.secrets peptidoglycan hydrolases that assist in degrading the prey's cell wall (Sockett et al.2009).

Genetic Characteristics :-

1.Predation Genes: Bdellovibrio sp. contains some genes which

predisposing this bacterium towards predatory nature,such genes actually codes for some proteolytic enzymes and toxins(Kuru et al.2017).

2.Gene Regulation: Bdellovibrio sp.regulates their genes in this way which enhance their predatory nature, those genes are basically related to motility and prey recognition being up regulated during this process (Karun Ker et al.2013).

Classification of Bdellovibrio sp. based ontheir predation nature:On the basis of predation Bdellovibrio sp. fall into three main groups those are as follows –

I. Endobiotic,in this case predatory organism invades prey and multiplies there.

II. Epiobiotic,here predatory organism attaches and kill prey from outside.

III. Transbiotic where lytic proteins secreted by the predator and they feed cellular contents released from the prey (Seccareccia et al,2015).

Endobiotic Predation of B.bacteriovorus :-

The predatory activity involves an attack phase (AP),during which free-living Organisms locates its prey, where it detects a specific signal in the prey's cell wall, and it becomes transfer from the attack phase to the growth phase (GP). Using type IV pilus predator attaches to form the pre cell wall,and predator releases some hydrolytic enzymes in response to another cellular signal that create a channel in the cell wall. Via this channel predator get entry in the periplasm of prey (Kuru E, et al 2017).After entering into the periplasm, it triggers the development of a spherical structure known as a 'bdelloplast',utilise the prey cell's contents to grow into multicellular filament, undergoes septation, and finally lyses the prey cells.

Epibiotic Predation: B. bacteriovorusand B.exovorus:

B.exovorus is an epibiotic predator and surprisingly, B.bacteriovorusepibiotically attack S. aureus in both planktonic suspensions (Pantanella F et al,2018; Iebba V et al,2014). Electron microscopy reveals B.bactriovorus can

externally attached to the partially disrupted S. aureus, indicates that it can destroys the cell externally and extracts all the contents in a characteristics epibiotic fashion (Novick et al.2021).

Transbiotic Predation Bdellovibrio Sp.:This type of predators is not of clinical interest but some of their products play a major important role but further research is necessary to get the information on transbiotic predation.

The Predatory Life Cycle Of Bdellovibrio bacteriovorus :-

Bdellovibriobacteriovorus attack gram negative bacteria Escherichia coli, a typical prey cell. The life cycle of similar in both Bdellovibrio bacteriovorus strain the smaller (0.35×1.1μm) vibroid shaped B. bacteriovorusHD100 (complete genome sequence identified already) and the larger (0.5×1.5μm) rod shaped B. bacteriovorus 109 J (most biochemical studies happen on it). B. bacteriovorus HD100 has slightly greater than the larger B. bacteriovorus 109J.

The life cycle of a typical Bdellovibrio bacteriovorus consists of eight phases,those are as follows-

1.Attack-Phase:Bdellovibrio sp. use active flagellar motility to move towards the E.coli ,their chemotaxis in nature helps them to find bacteria-rich areas where they randomly collided with prey.

2.Attachment(5-15 min.):-After colliding with a prey cell Bdellovibrio sp. either detached within few seconds (abortive attachments) or permanently attached with prey E.coli cells.

3.Invasion(12-25 min.):-Bdellovibrio sp .secrete some cell wall degrading enzymes to invade prey cells. The flagellar motility ceases as Bdellovibrio sp. penetrates the prey's (E. coli) periplasm.

4.Establishment:Bdellovibrio sp. settles down within the periplasm and also get attached to the cytoplasmic membrane of a prey (E.coli) cell.

5.Growth In Bdelloplast (25-180 min.):-Cell wall remodeling happen in this phase thus the prey's cell appears rounded, and a starts growing and simultaneously replicates its DNA co-

encytically within the periplasm, thus a significant structure Bdelloplast is formed.

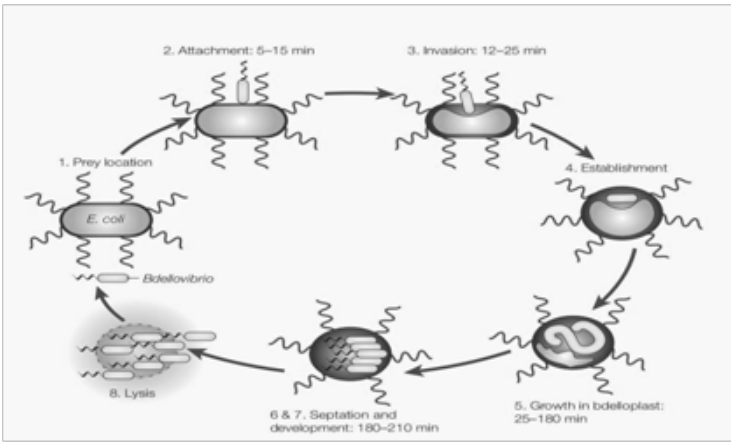

Fig 1. **The predatory life cycle of Bdellovibrio bacteriovorus HD100 and Bdellovibrio bacteriovorus 109 J** (Refference:- Sockett et al .2004)

6.&7. Septation And Development (180-210 min):-In septation phase Bdellovibrio sp. recognize that the cytoplasmic resources of prey are exhausted this time they divide into individual cells. At the development phase Bdellovibrio sp. Produce flagella and swims within the depleted Bdelloplast.

8.Lysis :-At last prey's outer membrane get ruptured and releasing Bdellovibrio sp.in the environment that they can back to the attack phase to hunt new prey (Sockett et al,2004).

In this way an Bdellovibrio sp. complete their predatory life cycle in their corresponding prey bacteria.

- PREY RANGE:- Bdellovibrio sp.have broad spectrum of pray range Pseudomonas sp. was resistance against Bdellovibrio sp. White Vibrio sp.was found to be most susceptible against Bdellovibrio sp.(Jurkevitch et al,2000).It is reported that Bdellovibrio bacteriovorus was prey on the strains those are belonging to the genera Pseudomonas sp. , Chromatium sp. ,Spirillum sp. and Escherichia sp.(Martin et

al,2002).

Recent studies found that Bdellovibrio bacteriovorus is able to attack someSpecies those are belonging to the genera Bardetella sp., Citrobacter sp., Acinetobacter sp., Burkhalderia sp., Escherichia sp., Listonella sp., Enterobacter sp., Klebsiella sp., Proteus sp., Salmonella sp., Shigella sp., Yersinia sp., Aeromonas sp., Marganella sp.,Pseudomonas sp.,Serrati asp., and Vibrio sp.(Dashiff et al,2011). Bdelovibrio bacteriovorusactually prey on several gram negative bacteria but it does not show any type of predatory effect on itself, even if they are grown axenically as prey and co-cultured with another predator strain, because their outer membrane is different from the other gram-negative bacteria (Hobley L et al,2012). Schwdke et al. found that in the lipid A of Bdellovibrio bacteriovorus, shows mannose substitution in place of phosphates.Due to this alternation negative charge is typically reduced in polar head group which enabling closer interaction with the other membrane of a typical prey organisms (Schwudke D et al,2003).This modification distinguishes Bdellovibrio sp.as non-prey thus reducing their susceptibility to cationic antibiotics .It also exhibits greater sensitivity to Triton X-100 compared to the other gram-negative bacteria (Tudor J.J et al,2008; Negus D et al,2017).Recently we found an information that Bdellovibrio bacteriovorus also interacts with gram-positive bacteria ,including Staphylococcus aureus (Iebba V et al,2014; Pontanella F et al,2018)

- Isolation Of Bdellovibrio bacteriovorus From Environmental Samples:-Soil samples and waste water samples are collected to isolate Bdellovibrio bacteriovorus from the environment.

- Isolation Process From soil sample :-

BdellovibriobacteriovorusIsolation Process from The Soil Samples Described Here -

1. Initial Culture Preparation: E. coli MG1655 culture was inoculated in 50 ml LB broth and incubated overnight aerobically at 37°C. After that this culture was centrifuged at 4000 rpm for 5 minutes and the pellet was resuspended in 50

ml HM buffer.

2. Enrichment Culture: This resuspended sample was added to a 250 ml Erlenmeyer flask containing 100 mg of soil sample to make enrichment culture. This enrichment cultures was incubated aerobically for 48 hours at 30°C with shaking condition at 150 rpm.Turbidity depletion checked periodically to identify predation happen or not.

3. Filtration And Dilution: After 48 hours, the culture was centrifuged at 300 xg for 10 minutes to remove sediment and debris. This supernatant was filtered by 0.45 µm PES syringe filter (Gene Bio Systems, Burlington, ON, Canada) to exclude any E. colicells. Upto 105 dilutions were prepared using HM buffer.

4. Double-Layer Agar (DLA): Assay:In 15 ml glass tubes 100 ml of filtered dilution, 250 ml of E. coli MG1655, and 4 ml molten YPSC top agar were combined, and vortexed it into a petridish solidified YPSC bottom agar in petridishes. Those plates were incubated 3-10 days aerobically or anaerobically at 30°C to observe Plaques (Indicative Of Predation)

5. Plaque Purification: Individual plaques were picked by using inoculation loop and transferred it to a 15 ml conical containing 5 ml of HM buffer and 500 ml of a overnight E. coli MG1655 culture resuspended in HM buffer (Approx OD600 of 1.0).This co-culture were subjected to another round of DLA plating for purification.

This process was repeated until pure isolates of Bdellovibrio bacteriovorus were obtained.

6. Cryopreservation: Once turbidity reduction was observed , 200 ml aliquotes were mixed with 2 ml of skim milk freezing media in a 2 ml cryovial, gently mixing it and preserve it at -80°C for further use.

7. Microscopic Verification: The presence of Bdellovibrio bacteriovorus cells were verified using phase contrast microscopy.10 ml aliquotes of enrichment culture or filtered samples were plated onto the microscopic slides under 100X magnification on a Leica DM200 (Leica Microsystem,Wetzlar,Germany).

If Dark, small, vibrio-shaped cells were observed it indicates successful isolation of Bdellovibrio bacteriovorus.

From Wastewater Sample:

Raw sewage samples (36 samples) were collected from December 2017 to March 2019 from North municipal wastewater treatment plant at Isfahan in Iran.

1. Sample Collection: Raw sewage samples were collected with a PH of 7.4, BOD =55 mg per liter, and COD=274 mg per liter.This samples were collected in clean bottles and transported immediately to the laboratory after collection (Oyedara OO et al,2016).

2. Centrifugation: This samples were centrifuged at 500 Xg for 5 minutes at 4°C. After centrifugation pellet was discarded and the supernatant was further centrifuged at 20,000 Xg at 4°C for 10 minutes. Then the final pellet was resuspended in 4 millilitres of cold HEPSE buffer (Oyedara OO et al,2016).

3. Filtration: This suspension was filtered through a 1.2 µm micropore filter to remove larger bacteria and debris (Oyedara OO et al,2016).

4. Dilution And Cultivation: Serial dilution of the filtrate were done using HEPES buffer. This dilutions were mixed with 200 ml of E. coli host suspension (1×109CFU/ml) and finally it is spreading over broth DNB bottom agar (1.5%) plates (Oyedara OO et al,2016; Feng S et al,2016)

5. Inoculation: Those plates were incubated at 30°C for 3-6 days (12,13 E) with the addition of 3 mM MgCl2 .6H2O and 2 mM CaCl2.2H2O. After incubation plaques were observed over the DNB agar plates (Oyedara OO et al,2016; Feng S et al,2016).

6. Identification Of Bdellovibrio Bacteriovorus :- After incubation period Bdellovibrio sp. plaques were separate out from the DNB plate using scalpel and resuspended it in HEPES buffer,after that this plaque containing buffer solution passed through a 0.45 µm syringe filter and were further used for molecular analysis and microscopic examination to identify Bdellovibrio sp.strains(Oyedara OO et al,2016).

Whole genome sequencing of Bdellovibrio bacteriovorus isolates:-

After isolation of this predatory bacteria we have to know the gene sequence of them to get information of their phylogenetic position,pathogenicity, antibiotic resistance tracking and to develop any diagnostic tools from them.The process of whole genome sequencing are described below-

1. Illumina whole genome sequencing of B.bacteriovorus isolates:-Minimum 5ng/µl concentration of genomic DNA(gDNA) was extracted from B.bacteriovorus .For gDNA extraction and concentration analysis Qiagen QIAamp powerFecal pro kit (modified)and Qubit dsDNA HS assay kit were used respectively.Based on their availability at the time of experiment isolates were selected and send them to the Microbial genome sequencing center for Illumina-based whole genome sequencing. Illumina DNA prep kit and IDT 10bp UDI indices were used to prepare DNA libraries. After that sequencing was performed on an Illumina Next seq200 platform using 2×151bp paired-end reads. Demultiplexing,quality control and trimming was performed with bcl-convert software(v3.9.3).Trimmomatic, MEGHIT and proKKa was used for trim reading, assemble contigs and genome annotation respectively (Greg Higgins,2024).

2. Nanopore whole genome sequencing of B.bacteriovorus isolates:-At first genomic DNA was prepared using the Oxford nanopore SQK-LSK109 native ligation sequencing kit after that multiplexing was achieved using EXP-NBD104 kit.The sequencing library was run on a MIN106D flow cell using a MinION MK1C device.Fast guppy model was used to perform live basecalline via Minknow software. Porechop was used for adapter removal. NanoFilt was used to remove low-quality reads (<200bp) and apply a minimum quality score threshold of 8 (Greg Higgins,2024).

3. Hybrid genome assembly and analysis of B.bacteriovorus isolates genome sequences:-Hybrid assembly and Illumina-only assembly were used to process each isolate.ProKKa was used for genome annotation.Genome quality was evaluated using checkM, it ensures completeness and accuracy. After

that potential exogenous microbial contamination was removed using JGI RQC filter pipeline.Then RAST tool was used to identify biological subsystem distributions across B.bacteriovorus and P.exovorus JSS genomes. Further ANI analysis were conducted using FastANI and ANI cluster map to compare isolate genomes and reference strains (Greg Higgins,2024).

Genetic mechanisms that govern Bdellovibrio sp predation strategies:

- Gene expression during life cycle phases:Following gene oligonucleotide microarrays,gene expression analysis and reverse transcription qPCR it was found that approximately 40% of genes among the whole genome potentially involved in the predatory life cycle of B.bacteriovorus (Lambert et al .2010). B.bacteriovorus co-cultured with E.coli to further characterise the Attack phase and Growth phase genes by using Illumina sequencing (Karunker et al.2013).From this studies authors found that 67% of genes expressed by B.bacteriovorus during growth phase (1557 genes) were active,15% were active during the attack phase (353 genes)and 18% were expressed in both growth phase and attack phase.

- Attack phase genes: In Bdellovibrio bacteriovorus 59% genes encode hypothetical proteins and 41% genes were involved in their motility, chemotaxis and their cell surface composition.

- Growth phase genes:Growth phase genes mostly coded for cell division, ribosome biogenesis, DNA polymerase, chromosome portioning and energy metabolism.

- Attack phase:-In attack phase non-replicating bacterial cells use a single sheathed flagellum for high-speed prey hunting (Lambert et al.2006). In this phase upregulation of bd108 gene happen which control the extrusion or retraction of type IV a pilus (Rotem et al.2015). Also upregulation of merRNA was happen in this phase.

- Transition to Growth phase:When B.bacteriovorus irreversibly binds to a prey cell its gene expression become

shifts to the growth phase. As a result this predatory bacteria creates a pore to the prey cell wall to enter in the periplasmic space and this time they secretes peptidoglycan-modifying enzymes to seal the pores that forms earlier than bdelloplast formation takes place(Lambert et al.2010; Lerner et al.2012). These are some peptidoglycan-modifying enzymes are present those are D-ala-D-ala carboxypeptidase, lytic murein transglycosylase, a polysaccharide de-acetylase which helps in the modification of peptidoglycan of prey cell wall during bdelloplast formation and those enzyme also helps to lyse prey cell when progenyget matured.

- Growth phase:When Bdellovibrio sp. enter into the growth phase this time upregulation of some genes happened those encoding some hydrolytic enzymes, regulatory factors and energy metabolism proteins(Rotem et al.2015). But this time σ 28 regulon, merRNA and bdelloplast forming genes remains silenced.

Study of host immune responses to predatory bacteria:

If we want to choose this predatory bacterium as a alternative to antibiotics to treat multidrug-resistance bacterial infections,we have to understand their interaction with mammalian cells and the immune responses stimulated by them.

- Immune response in human:-B.bacteriovorus is a gram-negative bacteria but due o the presence of unique lipopolysaccharide (LPS) it does not elicit strong inflammatory response like other pathogenic bacteria E.coli (Schwudke at al,2003).Their LPS contains α-D-mannose instead of phosphate groups, which results in a lower binding affinity for LPS receptors on human cells and inflammatory responses is also reduced .From the studies it was found that B.bacteriovorus HD100 which contains neutrally charged LPS triggers 100 time less tumor necrosis factor alpha(TNF-α) production in human mononuclear cells compared to an E.coli k12 control.

- In vitro studies: Two experiment was done in laboratory to study human responses the presence of Bdellovibrio bacteriovorus , those are as follows :-

1st Experiment: Monappa Ak uses four human epithelial cell lines (Intestinal HT29, Caco-2, T84 cells and Lung NuLi-1 cells) and murine microphage cell line (RAW 264.7) to Bdellovibrio strains HD100 and BY1 for in vitro studies. After 24 hours of incubation (without B.bacteriovorus) epithelial cell line viability is reduced slightly, while the murine macrophages were not affected at all. (Monappa et al,2016).But in the presence of B. bacteriovorus an increase in the production of TNF-α and IL-12 inflammatory cytokines observed from the RAW 264.7 macrophages after 6 hours exposure. However, the levels of these produced in response to E. coli, which reveals that the membrane components of B. bacteriovorus are relatively harmless.

2nd Experiment:(Gupta et al,2016)

The findings of Gupta et al. further confirmed the first experiment result. In their study, they tested three types of human epithelial cell lines (HaCaT, HepG2 and HK-2) and two human immune cell lines (Blood-derived monocytes and splenic monocytes, THP-1) by exposing them to Bdellovibrio bacteriovorus 109J and HD100. Their study basically measures cell line viability and cytokine responses.

After 24 hours of exposure, no deleterious effect was observed in any of the cell lines which is determined by cell imaging and cytotoxicity assays. Additionally, there was no significant elevation of several key cytokines [GMCSF (Granulocyte Macrophage Colony-Stimulated Factor] interferon gamma (IFN-ϒ), IL-10, IL-12p70, IL-1β, IL-2, IL-6, IL-8 and TNF-α] in four of the five human cell lies (HaCaT,HepG2, HK-2 and Spleen monocytes) over the same time period.

But, upon exposure of THP-1 macrophages to the predatory bacteria show increased level of two cytokines (IL-1β and IL-6) at 4 hours duration and additional increase in GM-CSF, IL-10, IL-12p70 and TNF-α at 24 hours duration. But still, the levels of IL-1β and TNF-α were lower than those seen in control cells when exposed to harmful E. coli 0157:H7. However, an anti-inflammatory role played by IL-10 which may help to balance the immune response.

- In Vivo Studies: Two experiments was conducted in laboratory to study mouse immune responses in the presence of B. bacteriovorus, those are as follows-

1st Experiment: In a mouse infection model, after administering predatory bacteria (B. bacteriovorus 109J) intranasally or intravenously an mild inflammatory responses was observed.

Intranasal Administration: Within 1 hour administration of B. bacteriovorus 109J, proinflammatory cytokines (IL-1β, IL-23 and TNF-α) increased by fivefold or more in the lungs of mouse. But this cytokine levels returned to normal within 24 hours (58).

Intravenous Administratrion:At 3 hours post-inoculation significant level TNF-α and IL-6 were observed in the kidneys, liver and spleen, but it become dropped to baseline by 18 hours . Similarly in the blood IL-1β, IL-6, IL-10, IL-12,CXCL-1,INF-Y' and TNF-αcytokines was observed (Shatzkes K et al,2015).

2nd Experiment: A high dose of B. bacteriovorus strains HD100 and 109J was administrated intranassaly as a result threefold or higher induction of CXCL-1, IL-6 and TNF-α was detected in the lungs of rat within 1 hour. But it returned to baseline levels within 24 hours. Similarly, IL-1βand IL-13 was detected at 24 hours of post-inoculation w3hich returned to baseline by 48 hours (Shatzkes K et al,2016).

3rd Experiment: Shank et al. showed that no cytotoxicity was found in human corneal-limbal epithelial cells upon exposure of predatory bacteria and no significant increase in pro inflammatory cytokines IL-8 and TNF-α was observed compared to P. aeruginosa positive controlat 4 and 24h post-exposure (Romanowski EG et al,2016).

Antibacterial Activity Of Bdellovibrio bacteriovorus:

From the In vitro and In vivo studies, we have found that Bdellovibrio bacteriovorus also shows significant antibacterial activity.

In-Vitro Antibacterial Activity:

Several In vitro studies have found that B. bacteriovorus has a significant antibacterial activity.

In a study it was found that B. bacteriovorus reducing Klebsiella pneumonia populations by about 106 fold. But some of them get survival by developing phenotypic resistance strategies and regrow (Shatzkes K et al,2016). Interestingly, it was also found that a lytic bacteriophage associated with B.bacteriovorus has been found to enhance its efficacy, it leads to this complete eradication of prey bacteria in some cases (Hobley L et al,2020).

In another study, B. bacteriovorus showing antibacterial activity against biofilms farming oral Pathogens by penetrating and disrupting biofilms (Kadouri D et al,2005; Dashiff A et al,2011). Neverthless some residual prey population can also persist within biofilms due to the presence of substance like cyanide or indole produced by prey organisms (Mu W et al,2017).

In Vivo Antibacterial Activity :-

In vivo studies demonstrate that B.bacteriovorus also exhibits some notable antibacterial activity.

- In a rat lung infection model which is infected by Klebsiella pneumonia upon treating with B.bacteriovorus it seems to reduce intrapulmonary bacterial titers by 103 fold(Shatzkes K et al,2016).But after intravenous infection it does not decrease harmful bacterial loads in organs ,instead it improving the health of treated rats compared to the untreated rats (Shatzkes K et al,2017).

- In other studies showed Salmonella sp. infection in chicks are attenuated by B.bacteriovorus (Atterbury RJ et al,2011).This predatory bacteria can also cooperating with immune cells to care K.pneumoniae infections in Zebra fish larvae,and reducing mice infected with Yersinia pestis (Findlay JS et al,2019).

Surface Antigen profiling: A key to enhancing bacterial recognition and vaccine efficacy:

Researchers studied the outer membrane proteins (oMPs) of B.bacteriovorus and other related Bdellovibrio sp. like organisms (BALOs) to identify suitable targets for antibody development. They focus to find conserved surface proteins to develop antibodies applicable across various BALOs. A candidate of BALOs (primarily B.bacteriovorusHD100) antigens from homologs of known OMPs in E.coli were identified using uniport, emphasizing topology, localization and expression and completeness of genomic details. BALOs genomic data were analysed via ExPASy and BLASTp for similarity evalution and sequence alignment. Tow BALO OMPs were chosen for antibody development those are slc39 and TolC (Fig 2.). Were slc39 is a fall under solute carrier family and functions as an iron-regulated transporter (Uniprot C et al,2023). Also Tolc act as an outer membrane export factor that involved in efflux and it has the potential to act as an multidrug resistance factor (Uniport C et al,2023).

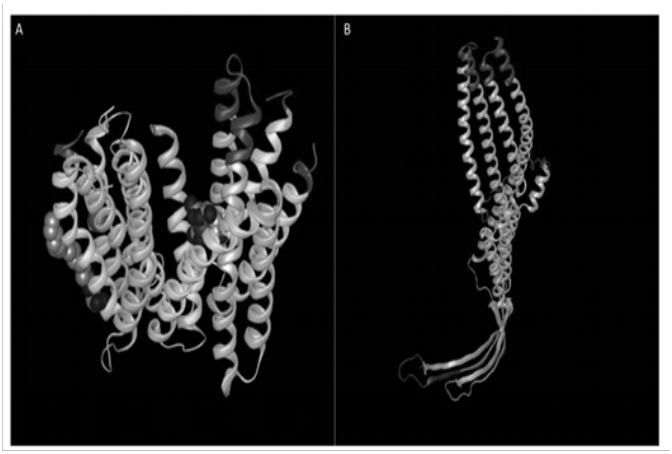

Fig 2.**Predictive structures of Slc39 (A) and TolC (B) B.bacteriovorus proteins generated by pacific Immunology.** Transmembrane domains are highlighted in yellow. Regions that would be most suitable for antigen development are highlighted in red (Reference:Greg Higgins,2024).

• Developing antigens and antibodies based on selected OMPs:

The selected protein sequences of Slc39 and TolC were forwarded to design antigen and antibody synthesis pourposes. Peptides from outside regions of transmembrane domains were selected and those two peptides were predicted as suitable antigens for each of Slc39 and TolC (TABLE).These peptides wrew attached to a keyhole Limpet Hemocyanin carrier and it was injected into rabbits, thus generating four antibodies with two biological replicates for each protein. For isolation pourposes final production bleeds were purified by affinity column chromatography (Greg Higgins,2024).

Verification of B.bacteriovorus OMP recognition:-

- **Preparation of coverslips:** Circular coverslips were sterilized by autoclaving and treated them with 70% ethanol for 15 minutes then dried them under UV light. Then these coverslips were coated with 200µl of 0.1% poly-L-lysine at room temperature. After that excess poly-L-lysine was removed and 3× with 1× PBS is used to wash coverslips. Further coated coverslips were dried under UV.

- **Application and fixation of cells:** 50µl of prepared B.bacteriovorus HD100 cells were applied to the coverslips and incubated them at room temperature for 1 hour. Then fixation was performed by using 200µl of 4% paraformaldehyde for 15 minutes at room temperature. Further coverslips were washed using 1× PBS.

- **Blocking and antibody staining:**500µl of a 10% normal goat serum is used as a blocking solution and coverslips were coated with this solution followed by overnight incubation at 4°C.Then blocking solution was removed and 50µl of the primary antibody were added to a triplicate wells followed by 1 hour of incubation. Washing step repeats again and further 50µl of secondary antibody followed by incubating them 1 hour in dark condition.

- **Counterstaining:** After washing a DAPI counterstain was added to the coverslips by applying 300µl of diluted DAPI followed by 5 minutes incubation a room temperature

in dark condition.

- **Mounting and Imaging:** After final washing coverslips were mounted on slides using Mowiol 4-88 and stored them at 4°c until imaging them using a Leica DM 500B epifluorescence microscope at 100× magnification . Velocity software used to analyze the images.

Safety studies to show that Bdellovibrio bacteriovorus does not affect animal health:-

- Ocular safety :- For the potential treatment of eye infections B.bacteriovorus applied on the ocular surface of live rabbits demonstrating itsnon-toxicity nature. Any clinical signs of inflammation or interference with corneal wound healing were not observed upon applying this predatory bacterium (Romanowski et al,2016).

- Respiratory and intravenous Administration: Upon administrating high dose of B.bacteriovorus (4×109 PFU intranasally, 1×108 PFU intravenously) were well-tolerated in mice and all mice were survived during 20 day observation period without any signs of distress.Their histological analysis revealed no adverse pathology in examined organs (Shatzke et al,2015).

- Oral administration in poultry: Oral dosing of Bdellovibrio bacteriovorus reduced salmonella sp. number in the ceca of birds it also preventing gut inflammation. It was observed that gut microbiota diversity was altered in non-infected chicks those were treated with B.bacteriovorus,but interestingly no adverse effect were observed in them (Atterbury et al ,2011).

- Lung infection in rats: Upon administration of B.bacteriovorus intranasally sublethal lung infection of Klebsiella pneumonia in rat reduced upto 3.0 log10 amount than the previous bacterial load (Shatzkes et al,2016).

- Zebra fish Embryo model: Lethal injection of Shigella flexneri into the hindbrain of zebra fish embryos were rescued by B.bacteriovorus injections into the same compartment. Here B.bacteriovorus predation nature is supported by the host immune cells but this predatory bacteria was ultimately

cleared by macrophages and neutrophils of the fish (Willis et al,2016).

Applications of Bdellovibrio sp.:Bdellovibrio sp. is a predatory bacterium,it has gained considerable interest in recent years due to its distinctive predatory behaviour and it has the potential to be used in multiple fields. As a natural predator of gram-negative bacteria, Bdellovibrio sp. presents a promising approach for managing bacterial populations across different settings. These bacteria have various applications in fields such as antibacterial development, environmental cleanup, biomedical research and agricultural, aquaculture enhancement. Those are as follows-

- Theraputic potential: From the various studies we have found that B.bacteriovorus can be used as a therapeutic or biological control agents in various pourposes those are as follows-
- It can reduce the Klebsiella pneumonia bacterial load within the lungs of rats.The B.bacteriovorus also did not affect negatively on host after their inoculation (Shatzkes K et al,2016).
- It also plays an important role to provide protection and increase life span in mice that were infected with lethal injections of Yersinia pestis which is responsible for the plague (Sutherland et al,2004).
- These bacteria reduce the infection of Salmonella enterica in chick ceca of a chicken (Atterbury et al,2011).
- Low toxicity: The LPS of a typical Bdellovibrio sp. has reduced toxicity compared to the other gram-negative bacteria, thus lowering the risks of inflammatory responses in human cells (Kessel M & Shilo et al,1976;Thomashow et al,1978).
- B.bacteriovorus act as a prey on gram-negative oncomicrobes those are found in human gut, and this property helps to be novel approachesto be used in therapies for CRC treatment (Greg Higgins,2024).

- Potential in combination therapies: It can used as antiseptics or β-lactam antibiotics because it shows limited resistance development and it works effectively in synergy (Thomashow et al,1978; Cotter et al,1992).

- Probiotic approaches:

  - **Presence in mammals:** From the recent studies we have found that Bdellovibrio sp. is present in mammalian guts, and it has the potential to act as a probiotic to combat pathogens in human microbiota (Lambert et al,2002).

  - **Selective targeting:**This bacterium specifically targeting gram-negative bacteria without harming human cells that making it a promising candidate for safe probiotic applications (Ruby et al,1992).

  - **Role in microbial ecology:** It has the ability to influence the microbial balance in ecosystems suggesting that it could positively impact in human gut health by reducing pathogenic bacteria (Fackrell et al,1973).

- Food Industry:

  - **Improvement of food safety in animals:** BALOs were tested in various animals to demonstrate significant reductions of Salmonella sp. and other pathogenic bacterial colonization to improve food safety (Atterbury et al,2011).

  - **Food preservation:**Bdellovibrio sp. has the ability to reduces food borne pathogens such as E.coli on chicken and canned meat, it minimizing the use of chemical preservative while improving food safety (Ottavinai et al, 2020) .

- Water treatment :-

  - **Potable water pre-treatment:** This is used in the pre-treatment step to reduce microbial load in waste water,thus limiting biofilm formation and enhancing membrane filter efficiency (Kim et al 2013) C.

  - **Rainwater purification:** Their predatory capability was successfully paired with solar-based methods to enhance the disinfection of harvested rain water (Waso et al , 2020).

- In biotechnological field :-
  - **Enzyme source**:Bdellovibrio sp. produces enzymes like proteases, DNases and RNases, which could serve as an antimicrobial agents or tools for antibody analysis and protein research (Bratanis et al , 2020) .
  - **Biofuel production:** This bacteria promotes microalgae growth by controlling bacterial contamination, aiding in eco-friendly biofuel production (Li et al ,2018).
  - **Recovery of Intracellular products:-** This predatory bacteria used as a biological agent to recovering bioplastics like polyhydroxyalkanoates by lysing gram-negative bacteria, thus in this way they reducing the need for chemical solvents (Martinez et al ,2016).
- In Agricultural field:-
  - **Prevention of spoilage:** It was found Bdellovibrio sp. is used to fight against Pseudomonas tolaasii a harmful bacteria that infect post-harvested mushrooms (Saxon et al, 2014).
  - **Prevention of soft rot in potatoes:** BALOs prevent bacterial soft rot in potato slices, but their protective effect become reducedif applied after the pathogen had already infected the crop. So we have to apply this as a pesticide before contamination (Youdeowei et al ,2020).
- In Aquaculture field:
  - **Enhancement of Marine Aquaculture species**:Bdellovibrio sp. improved growth , survival rates and overall health of species like black tiger shrimp (Penaeus monodon) and turbot fish (Scophthalmus maximus) by targeting harmful bacteria in rearing waters (Wen et al ,2010; Guo et al,2017).
  - **Curative effects against pathogens in fish:**Bdellovibrio sp. has the curative effects against fish pathogens thus improving channel catfish and turbot farming (Chen & cai 2011; Zeng et al,2017).
  - **Environmental benefits in Aquaculture:**Bdellovibrio

sp. reduces heterotropic bacterial populations and biofilm formation in aquaculture systems, thus promoting healthier environment to the aquatic organisms (Wen et al ,2010; Li et al ,2014).

Challenges for Bdellovibrio sp. survival: Bdellovibrio bacteriovorus a typical predatory bacteria facing various difficulty for their survival those are discussed below-

- **Discovery of Bdellophages:-** A tail less icosahedral single-stranded DNA bacteriophage was found that infecting B.bacteriovorus they known as "bdellophages". This viruses infect both host-dependent and host-independent variants of B.bacteriovorus (Hashimoto et al 1970; Althauser et al, 1972).

- Upon using B.bacteriovorus to treat gut related infections and disease require anaerobic environment but B.bacteriovorus is an obligatory aerobic microorganisms.

- **Threats from protists:-**B.bacteriovorus faces threats not only from bacteriophages but also from phagotropic protists that influence this bacterial community composition .Some ciliated protists varieties can also consume both living and dead B.bacteriovorus cells (Johnke et al, 2017).

- Bdellovibrio bacteriovorus have an unusual large genome (3.8 Mbp) were several genes support anaerobic respiration condition of this bacteria (Rendulic S et al,2004).

- **Impact of secondary metabolites:**Chromobacterium piscinae produce some secondary metabolites, such as violacein and cyanide which inhibit predation of B.bacteriovorus. This inhibition happen in a calcium/HEPES buffer but not in diluted nutrient broth, indicating medium dependent effects (Mun et al, 2017).

- It is difficult use this predatory bacteria to treat gut related diseases, but some studies mentioned that infection of Salmonella enterica in chick ceca is prevented by B.bacteriovorus supporting their possible anaerobic growth (Atterbury et al,2011).

Potential mechanisms by which pathogens avoid predation:

- **Outer membrane as a barriers to predation:** When B.bacteriovorus get survived in the extracellular environment they next faces the challenge to overcome prey's outer membrane. A wide range of gram-negative bacteria potentially prey to thispredatory bacteria but still variations in susceptibility to predation was observed even within the same species (Dashiff et al,2011). But variations in the outer membrane does not completely avoid predation by B.bacteriovorus (Varon M et al,1969). Further testing requires to predict their role in averting predation due to new outer membrane variants (fig 3a).

- **Role of capsules:** Bacterial capsule can prevent bacteriophage infections (Sutherland IW,1965). From the studies we have found that E.coli k29 capsule does not inhibit predatory attachment or penetration, because predator usually penetrates the capsule by flagellar motility (Koval SF et al,1997).(fig 3b)

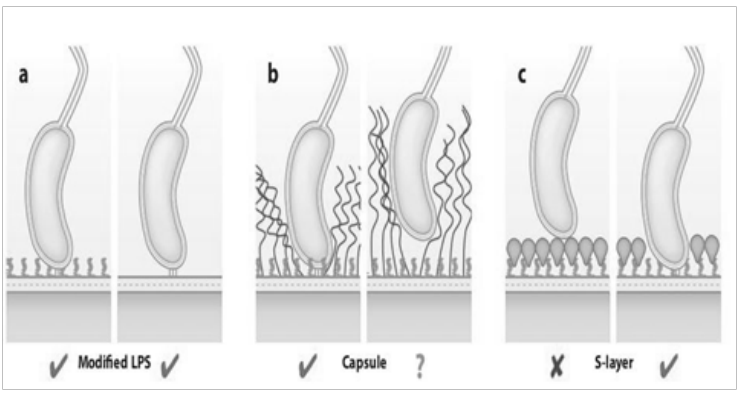

Fig .3 (a) Bdellovibrio bacteriovorus (yellow) attaches to a gram-negative bacterium with LPS outer membrane O antigen (left) or no antigen (right) (Varon M et al,1969). Other LPS modifications have yet to be studied. (b) B.bacteriovorus attaches to a gram-negative bacterium with a capsule. E.coli polysaccharide capsules are not a barrier of predation(left) (Koval SF et al,1991), but the effects of other biochemically

distinct prey capsules on predation have yet to be studied (right). (c) B.bacteriovorus attaches to a gram-negative bacterium with a surface layer (S-layer) only at point where the S-layer is incomplete (right), so predation is less efficient on S-layer-expressing prey (Koval SF et al,1991)

- **Role of S-layer in predation:** The S-layer is a common bacterial structure which is made up of crystalline protein subunits that may inhibit B.bacteriovorus attachment. B.bacteriovorus predation is protected by the S-layer of Aquaspirillum serpens, A.sinuosum and Aeromonas salmonicida bacteria (Koval SF et al,1991). partial expression of the S-layer can expose the outer membrane, making it susceptible to predation and highlighting how even small vulnerabilities in this defences can be exploited (fig 3c). Further investigation is needed to understand the role of the S-layer in reducing predation by medically significant pathogens.
- **LPS and O-antigen as barriers:** The LPS of gram-negative bacteria act as a potential barrier to the predator so they cannot access the outer membrane and periplasm. Itwas identified that 0-antigen a capsular component can also hindering predation. From the studies we have found that those Vibrio choleraisolates lacking 0-antigen were more susceptible to Bdellovibrio sp . than their wild type isolates (Seed et al,2012).

Conclusion: Bdellovibrio bacteriovorus is a predatory bacteria that holds a great promise to act as a biocontrol agent against various pathogenic bacteria and colorectal cancer (CRC) associated oncomicrobes including Fusobacterium nucleatum, enterotoxigenic Bacteroides Fragilis and pKs+ E.coli. It's specialized features such as high motility,chemotaxis and production of proteolytic enzymes enables them to efficiently target pathogenic bacteria thus paving the way towards living antibiotic .Various research demonstrates its safety in vivo an in vitro condition ,minimal inflammatory response and its ability to reduce bacterial populations.

However, some challenges remain such as reduced effectiveness in anaerobic environment,development of

bacterial resistance,presence of Bdellophage and presence of some predatory protists against them. Even B.bacteriovorus a innovative alternative to conventional antibiotics ,along with their potential applications in infection control, agriculture and biotechnology. Further research is necessary to enhancing its anaerobic efficiency, solve the resistance issue to totally use its potential as a therapeutic agent for harmful pathogens and CRC associated bacteria.

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ADVANCING VACCINE DEVELOPMENT AND DELIVERY: THE ROLE OF GENETIC ENGINEERING

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ABSTRACT

The advent of genetic engineering has revolutionized the field of vaccine development and delivery, offering innovative approaches to combat infectious diseases and emerging health threats. Traditional vaccine platforms, while effective, often face limitations in production speed, efficacy, and adaptability. Genetic engineering enables the design of precise, scalable, and highly targeted vaccines, leveraging techniques such as recombinant DNA technology, RNA-based platforms, and synthetic biology. This review highlights recent advancements in genetically engineered vaccines, including mRNA and DNA vaccines, viral vectors, and protein subunit vaccines, and explores their role in addressing global health challenges. Furthermore, it examines the innovations in delivery mechanisms, such as lipid nanoparticles and gene-editing tools, that enhance stability, immunogenicity, and accessibility. Emphasis is placed on the implications of these technologies for rapid pandemic responses, personalized medicine, and the potential to tackle diseases previously considered untreatable. Challenges in regulatory approval, scalability, and equitable distribution are also discussed, providing a comprehensive overview of the transformative

potential of genetic engineering in vaccine science.

Keywords:*Genetic engineering, infectious diseases, vaccine*

1. Introduction

Major concerns of the global public health system are the prevention and control of infectious diseases, which pose a serious threat to world health. Infectious illness prevention and control are achieved by a variety of tactics, including social distancing, monitoring, and immunization. Vaccination is the most economical and successful strategy for treating and preventing infectious illnesses among these strategies. (Rémy et al 2015).Throughout history, extensive vaccination campaigns have effectively eliminated the extremely virulent smallpox virus. The incidence and mortality rates of many serious infectious diseases, such as polio, rotavirus enteritis, hepatitis B, diphtheria, tetanus, pertussis, cervical cancer, mumps, and others, have also been considerably reduced as a result of vaccination. (Mühlemann et al 2020).The World Health Organization (WHO) reported that between 3.5 and 5 million lives can be saved annually if immunizations are widely used. There is no denying the impact of vaccines on public health, even though their accessibility and acceptance vary among countries and regions.(Sallam et al 2021). Emerging high-risk and highly variable viruses continue to pose serious dangers and provide major global health issues, as the unpredictable COVID-19 pandemic shows. (Lazarus et al 2022).

Advances in virology, immunology, molecular biology, and related fields have transformed vaccine research. These developments have led to the development of several molecular engineering tools, such as viral vectors, molecular adjuvants, gene editing tools, protein modification tools, and molecular delivery techniques (Baay et al. 2021).With the aid of these technologies, new vaccine technology platforms have been developed, current vaccine products have been improved and enhanced, and vaccine formulations have been safer and more effective. Furthermore, molecular engineering methods have made it possible to respond quickly to newly discovered high-risk infections, averting and managing

widespread epidemics. (Nguyen et al 2021).

This review aims to present a thorough analysis of the state of molecular engineering tool research and implementation, with an emphasis on how these tools have advanced different vaccine technology platforms. It will also cover possible future research directions for vaccine technology.

2. Advances in Genetically Engineered Vaccine Platforms

2.1. Viral vectors

The use of viral vectors for gene therapy has existed since the beginning of virology. However, the ongoing development of virology has made viral vectors crucial engineering tools that enable the development of a wide range of novel vector vaccines. (Travieso et al 2022). The viral vector can transfer the antigen to host cells by inserting target antigen sequences at the right locations, which initiates a thorough immune response.(Okuda et al 2014). Because of their safety, a broad range of host cells, low vector immunogenicity, and capacity to express exogenous genes for extended periods in vivo, replication-defective viruses like adenovirus, lentivirus, and poxvirus are particularly appealing for vaccine development among the different vector tools available. (Robert-Guroff et al 2007). Because of its quicker clinical conversion rate, adenovirus vector vaccines have been a popular option during the COVID-19 pandemic. Examples of these include Russia's Sputnik V and Johnson & Johnson's Ad26.COV.S, and AstraZeneca's ChAdOx1Ncov-19. (Sadoff et al 2021). Herpesviruses, retroviruses, and influenza viruses are examples of another kind of viral transmission agent that can replicate itself. These viruses are safe and effective, and recent vaccine research is concentrating on them. (Kamel et al 2019). Chen and associates used an influenza virus vector in 2022 to create a novel COVID-19 vaccine that, when inhaled, was successful in triggering mucosal immune responses, such as humoral and resident cell immunity, as well as mucosal IgA, which in turn prevented the virus from spreading. (Chen et al 2022).

Even though vaccine research has focused a lot of attention on designing vaccines using viral vectors, safety, and human

preexisting immunity are still issues. As an illustration, the first chimpanzee-based COVID-19 vaccine, which was authorized for use in several nations, has been halted because of the possibility of blood clots.(Voysey et al 2021).

2.1.1. Adenovirus Vectors

There are approximately 50 known serotypes of human adenovirus (Ad). Human adenovirus serotype 5 (Ad5) has been extensively studied as a gene delivery vector due to its ability to be produced at high titers easily. The genome of Ad5 consists of 36 kilobases (kb) of double-stranded, linear DNA. It is non-enveloped and features a capsid shell made up of hexon and penton structures. The Ad fibre protein plays a crucial role in determining the virus's tropism, as it facilitates the binding of the adenovirus to the coxsackievirus and adenovirus receptor (CAR).(Sumida et al 2005). The high transduction efficiency, high transgenic expression, and wide spectrum of viral tropism of recombinant Ad vectors make them popular. Both proliferating and non-dividing cells can become infected by them. Because the E1A and E1B viral gene regions are deleted, the majority of Ad vectors have replication defects. To make room for the transgene, the E3 genes are frequently removed as well. A common process for creating ad vectors consists of two parts. The Ad vector plasmid is first transfected into cell lines that complement E1 (the 293 cell lines are commonly utilized). Following infection, the Ad vector multiplies in the 293 cultured cells. Secondly, the vector is extracted from infected cells and ultracentrifuged to purify it.(Wohlfart et al 1988). The neutralizing reactions are dominated by antibodies directed against the hexon protein's hypervariable regions (HVRs). It has been researched to modify these HVRs and the fibre knob domain to circumvent pre-existing immunity.(Roberts et al 2006).Modifying the host immune response by lowering viral gene expression is an additional strategy. By decreasing the vector-derived immune response in infected cells, deletion of the E2 and E4 viral gene sections reduces toxicity. This vector is referred to as a second-generation advertising vector.(Gao et al 1996).Clinical trials have investigated vaccinations based on recombinant Ad vectors against solid tumors, influenza,

and HIV-1.(Chiappori et al 2013).

2.1.2. Retrovirus Vectors

The majority of retrovirus vectors are avian or murine, and they are usually replication-defective.Retroviral vector-based gene therapy has been used in clinical trials for individuals with malignant gliomas or X-linked severe combined immunodeficiency (SCID-X1).(Basile et al 2000). During preclinical testing, this safety concern also surfaced. When viral LTRs were incorporated into proto-oncogenes, cancer began. The risk of carcinogenesis may be decreased by non-integrating and self-inactivating (SIN) retroviral vectors because they preferentially incorporate close cellular gene promoters that control cell reproduction. (Maruggi et al 2009).During vector manufacturing, SIN vectors are rendered inactive and include partially erased LTRs. Because of this, SIN vectors have been suggested for SCID-X1 clinical studies. (Thornhill et al 2008).

2.1.3.Poxvirus Vectors

Highly attenuated vaccinia virus strains have been developed, including replication-competent strains like LC16m8 and replication-deficient strains such as NYVAC, ALVAC, TROVAC, and MVA.Numerous studies have shown that vaccines based on vaccinia virus vectors can induce a strong immune response due to the high transgenic expression of foreign antigens. (Martinez et al 2007). The vaccinia virus benefits from a scalable manufacturing process for the smallpox vaccine. (Belshe et al 2013). The MVA vector is usually produced in chick embryo fibroblast (CEF) cells for large-scale manufacturing. Specifically, the vaccinia virus is employed to transfect CEF cells with a plasmid containing the recombinant transgene. After transfection, centrifugation is used to purify the recombinant vectors that multiply within the CEF cells. (Mehtali et al 2010).

Enhancements to vaccinia virus vectors continue to be developed even today.(Davison et al 1990).

2.2. DNA Vaccine

DNA vaccines provide immunogenicity through the

activation of the adaptive immune response. When a plasmid is introduced close to a cell and then picked up (either passively or through facilitation), the target antigen can be produced by the cellular machinery that recognizes and expresses the plasmid DNA. (Hewitt et al 2003). The most popular method of antigen presentation in DNA vaccination is MHC Class I because plasmids are absorbed by the cell and the antigen to be presented is subsequently produced intracellularly by the transcription and translation of the supplied DNA. (Khan et al 2013).Intracellular peptides, which are endogenous proteins in this route and are most frequently seen on MHCI, are produced sequentially by DNA vaccines and activate CD8 T cells. Additionally, the translated proteins are exocytosed at the same time and then absorbed by additional antigen-presenting cells, which then drain to nearby lymph nodes. (Hobernik et al 2018).Macrophages, dendritic cells, and other monocytes are examples of antigen-presenting cells (APCs) that endocytose and/or phagocytose extracellular materials before processing and presenting them by MHCII, which preferentially activates CD4 T cells.Th1 and Th2 are two types of CD4 T helper cells that can either further activate CD8 T cells or encourage B cell differentiation to plasma cells to produce a humoral immune response, respectively.(Tussey et al 2000).Because of their basic structure, plasmids can include many CpG patterns, just like DNA. These CpG patterns can be optimally built into DNA vaccines. A CpG motif is a recognized adjuvant that stimulates plasmacytoid dendritic cells and TLR9-bearing B cells to generate powerful Th1-type immune responses. (Bode et al 2011).

Strong T cell-mediated immune responses, which are essential for both the removal of infected cells and the prevention of tumors, have been demonstrated to be induced by the DNA vaccine. (Chang et al 2021). Fascinatingly, the DNA vaccination delivery mechanism might guide the immune responses. While intramuscular (IM) delivery produces a stronger humoral, or Th2-mediated, immune response with an advantage in antigen expression, intradermal (ID) delivery may preferentially induce the Th1-immune response by recruiting more APCs and inducing a more polyfunctional cellular immune response.(Shedlock et al 2000).This indicates

that the proliferation of CD4 T helper cells toward Th1 or Th2 is somewhat directable.(Feltquate et al 1997).

However, it has been argued that the DNA vaccine's administration method—such as gene gun versus IM depot—is probably more directed. Adjuvants can also have specific stimulatory actions that guide CD4+ T cells toward either or both Th-1 and Th-2, thus the problem appears to be merely additional.(Olive et al 2010). Finally, plasmid DNA can enable tissue production of antigens for considerably longer periods than injected protein or subunit antigens, which have a very limited half-life. This could potentially provide superior immune system priming. (Petrovsky et al 2016).

DNA vaccines are presently being studied in immuno-oncology. DNA cancer vaccines target cancer cells to elicit both a broad and a customized immune response, in contrast to the conventional DNA vaccination that targets infectious organisms. The tolerogenic effect is the process by which cancer cells avoid detection by the immune system. The two most well-known mechanisms are PD-1 and CTLA-4.(Makarov et al 2014).

2.3. Protein Subunit Vaccines

Subunit vaccines derived from pieces of microbes can overcome these obstacles. Subunit vaccinations include only the pathogen's antigenic components necessary to elicit successful immune responses. Proteins, polysaccharides, and nucleic acids can all be employed as antigens (Bill et al. 2015). Immune responses are triggered by protein subunits that contain a particular virus product rather than the entire viral particle. SARS-CoV-2 includes both structural and non-structural proteins in addition to accessory proteins. The primary structural proteins of SARS-CoV-2 are the envelope (E), membrane (M), and S proteins. (Koupae et al 2021). The proteins present in the ribonucleoprotein core of the nucleocapsid (N) and the viral phospholipid bilayer play crucial roles in the virus's function. The S-proteins facilitate viral entry and adhesion to host cells by binding to ACE2 (angiotensin-converting enzyme-2). Protein S consists of two subunits: S1 and S2. The S2 subunit is responsible for

membrane fusion, essential for the virus to enter the host cell, while the S1 subunit serves as a C-terminal receptor-binding domain (RBD) that identifies the receptor. The M proteins are involved in the development of the virion envelope. Since antibodies bind to the S protein and neutralize the virus, the spike gene sequence or protein is a critical component of SARS-CoV-2 vaccines.(Sampson et al 2021).Due to its high antigenicity and capacity to trigger strong immune responses, the S protein of SARS-CoV-2 has been recognized as a leading candidate for vaccine development across various platforms. (Zepeda-Cervantes et al 2021) (Zheng et al 2020).

Some Recombinant Spike Protein Vaccines for COVID-19

2.3.1. COVAX-19 Vaccine

A South Australian biotech company called Vaxine Pty Ltd. has developed a COVID-19 vaccine candidate known as COVAX-19, or SpikoGen. This vaccine is based on recombinant spike proteins and combines a recombinant protein antigen with the Advax-SM adjuvant.Vaxine Pvt. Ltd. utilized computer models of the spike protein and the human ACE2 receptor to demonstrate that this vaccine not only reduces COVID-19 illness but also helps prevent virus shedding and transmission.(Vihol et al 2021).

2.3.2. Nanocovax Vaccine

Nanogen Pharmaceutical Biotechnology JSC makes a protein subunit vaccination called Nanocovax in Vietnam. The vaccine, which is based on the SARS-CoV-2 recombinant spike protein, uses aluminum hydroxide as an adjuvant. When tested in animal models, the vaccine has produced high amounts of anti-spike antibodies. Nanocovax has been shown to neutralize antibody titers against the Wuhan variety and anti-spike IgG antibodies. (Jacob et al 2020).

2.3.3. Nanoparticle Vaccine

Matrix-M1 adjuvant2 and a recombinant SARS-CoV-2 (rSARS-CoV-2) nanoparticle vaccine3 derived from the full-length, wild-type SARS-CoV-2 spike glycoprotein are both included in Novavax's NVX-CoV2373 protein subunit vaccine. Because it improves viral attachment to the host cell's

human angiotensin-converting enzyme 2 (hACE2) receptor, it targets the formation of antibodies and vaccines. The absence of cell-mediated immune responses, which define protein subunit vaccines, was addressed using the saponin-based matrix M1 adjuvant. (Bengtsson et al 2016).(Keech et al 2020).

2.4. mRNA vaccine

There are two RNA vaccine categories: self-amplifying and traditional mRNA-based immunizations.(Kramps et al 2017). Traditional mRNA vaccines solely encode the target antigen and are conceptually comparable to host cell mRNA molecules. SAM vaccines, on the other hand, encode a modified RNA viral genome that contains nonstructural proteins and an antigen cassette that replaces the genes encoding the structural proteins. Examples of these viruses are those produced from alphavirus single-stranded, positive sense (+)RNA molecules. (Geall et al 2012). Because of RNA template amplification in host cells, the resulting RNAs, known as replicons, express high amounts of the antigen gene. These replicons are unable to produce infectious virions and disseminate to nearby cells because they lack the genes for the viral structural proteins. The sequences of two open reading frames (ORFs) with untranslated regions (UTRs) at the 5C and 3′ ends make up the alphavirus-based replicon. A viral polyprotein that is translated from the genomic (+)RNA and proteolytically broken down into four nsPs is encoded by the ORF at the 5C-end.(Fros et al 2016).

Mice treated with liposome-delivered mRNA encoding the influenza nucleoprotein developed a response from antigen-specific cytotoxic T lymphocytes (CTLs). (Martinon et al 1993).

Advantage of mRNA vaccines

1. The first benefit of mRNA vaccines is how simple and quick they are to make. Delivering a transcript that encodes a target antigen or immunogen is the fundamental idea behind mRNA vaccines. The procedure is also simply scalable and cell-free, needing little platform modification throughout mRNA formulation and production. (Jackson et al 2020)

2. After transfection, an mRNA vaccine quickly expresses

the target protein (antigen) by translation from the mRNA. Since the cytoplasm, not the nucleus, is where the antigens are translated, mRNA vaccines are far more biosafe than DNA-based vaccinations since they have a lower chance of integrating into the genome. Furthermore, as mRNA is a temporary molecule that does not interact with the host genome and only contains a brief sequence that needs to be translated, it is a safer vector than DNA.(Jackson et al 2020)

3. While the host translation machinery translates mRNA vaccines and are therefore likely to yield an antigen that matches the protein's structure released from the viral genome, including the post-translational modifications, protein-based vaccinations are frequently made from bacteria. (Jackson et al 2020).

2.4.1. Production of mRNA Vaccines

In the production process, a DNA template containing the mRNA sequence is transcriptionally transcribed in vitro. A poly(A) sequence, a start codon, an open reading frame (ORF), a stop codon followed by a 3′ UTR, and a 5′ untranslated region (UTR) are all typical components of this mRNA. RNA for translation and stability as well as codon optimization have both been employed to optimize the ORF for expression. The mRNA is polyadenylated and capped to facilitate effective translation. The poly(A) sequence can be added either during or after transcription using poly(A) polymerase, and the cap can be added either way. Translation efficiency may be significantly impacted by the 5′ and 3′ UTR sequences. Using nucleoside triphosphates as the building blocks, a DNA-dependent RNA polymerase (often from phage T7) converts a linearized DNA template into mRNA to begin the process. The nucleosides could be either naturally occurring modified nucleosides or native nucleosides. (Liu et al 2019) (Pardi et al 2018) (Thess et al 2015).

The removal of nucleotides, enzymes, and the DNA template is crucial for mRNA purification. As recently summarized, various manufacturers have optimized their specific constructs in terms of the sequence, the nucleosides used (i.e., how modified, if they are modified), and for the

lipid nanoparticle formulations to achieve the appropriate immunogenicity, potency, and thermostability. (Zhang et al 2019).

3. Innovations in Delivery Mechanisms

3.1. Lipid nanoparticles (LNPs)

The pharmaceutical industry has seen lipid nanoparticles (LNPs) as attractive delivery systems for a range of medicinal medicines. Other industries, including medical imaging, nutrition, cosmetics, agriculture, and other cutting-edge domains like nanoreactors, have also begun to use LNPs. LNPs are currently in the news as an essential part of the COVID-19 mRNA vaccines, helping to preserve and deliver mRNA to cells.

Reaching the full potential of RNA-based vaccines can be facilitated by using LNPs as delivery methods since they shield the big encapsulated nucleic acid molecule from nuclease destruction.(Reichmuth et al 2016)

Different applications of RNA-based vaccines against infectious diseases and cancer have resulted from the diverse functions that RNA plays in both preventive and therapeutic applications. Numerous studies that have examined LNPs as delivery methods for conventional and self-amplifying mRNA against a range of infectious illnesses have demonstrated strong and quick immune activation in various animal species. (Lutz et al 2017) (Bahl et al 2017) (Pardi et al 2018)

For example; the **mRNA-1273** mRNA vaccine was given against COVID-19 via this delivery system in phase three manufacture by Moderna Therapeutics/National Institute of Allergy and Infectious Diseases (NIAID). (Baden et al 2020)

3.2. Electroporation

Electroporation (EP) is a process that employs short electric pulses to temporarily and reversibly permeabilize the cell membrane. This temporary state allows for the enter of large molecules, such as nucleic acids, into cells, either in vivo (within a living organism) or in vitro (in a controlled environment). Over the past decade, this method has advanced from a

purely experimental technique to one that is actively used in various clinical trials aimed at transferring medications and nucleic acids to specific target organs. (Sardesai et al 2011).

In the investigations by Petkov et al. and Lambert et al., BALB/c and CB6F1 mice were immunized intramuscularly with DNA vaccines against influenza and HIV-1, respectively, using a **BEX CUY21EDITII** electroporator. (Petkov et al 2022). (Lambert et al 2016).

4. Gene editing tool: CRISPR/Cas9 for vaccine development

CRISPR/Cas9-based gene-editing technology has been extensively used in vaccine research due to its high efficiency, specificity, versatility, flexibility, simplicity, and low cost when compared to other viral genome editing techniques. This has proven to be an effective and potent strategy for the future development of genetically engineered vaccines, in addition to studies on gene function, gene therapy, and virus-host interaction. (Okoli et al 2018)

Guide RNA (gRNA) and the Cas9 nuclease, which breaks DNA, make up the CRISPR/Cas9 functional system. The Cas9 nuclease is directed by the gRNA to bind to the specific sequence 5′-NGG-3′ upstream of the Protospacer adjacent motif (PAM) and then initiate the Cas9 nucleases' double-strand break (DBS) activity. Variants of the Cas nuclease enzyme cause variations in the PAM sequence. In the functioning mechanism, the Cas9 creates the double-strand break (DSB) at three base pairs upstream of PAM, and the gRNAs use sequence complementarity to determine the specificity of the target DNA. The DBS initiates two cellular DNA repair mechanisms: homology-directed repair (HDR), which results in gene knock-in (KI), and non-homolog end joining (NHEJ), which results in gene disruption or knock-out (KO). By using donor DNA as a template to rectify gene mutations, the HDR pathway activates and repairs the DSB when a foreign sequence (donor DNA template) is introduced into the first cell stage using Cas9 and gRNA.(Asmamaw et al 2021).NHEJ, a strong, error-prone, common, and quick DNA repair process, often carries out the repair of DSB when it happens in cell DNA. Throughout the cell cycle, this route

remains constant. HDR is another parallel route that is only active during the cell cycle's S and G2 stages. The "core complexes" that identify the damaged DNA regions and their "unique processing factors" that repair the DSB involve both the NHEJ and HDR processes.(Symington et al 2011).

The human immunodeficiency virus (HIV) was prevented from replicating in the host cells by editing and modifying its DNA using the CRISPR/Cas9 system. The lengthy terminal repeats of HIV-1 act as a promoter to propel viral genome integration and replication into host genomes. One treatment approach to lessen HIV replication in host cells is to edit the LTR sections.The U6 promoter was used to guide the design of the gRNA expression vector. Two target locations, T5 and T6, were chosen to be edited in the HIV-1 virus's LTR sections. In addition to eliminating and preventing the expression of latently integrated HIV-1 provirus from the host genomes, this approach dramatically decreased HIV-1 expression and replication in the host cell lines. To target the replicative factors of HIV-1 in latently infected Jurkat C11 cell lines, the researchers employed modified Cas nuclease, Staphylococcus aureus (SaCas9), with eight tested gRNAs. This resulted in a significant decrease in HIV replication and production in the host cell lines without any off-target effects. (Ebina et al 2013).

5. Applications in Global Health

The scientific community around the world is showing renewed interest in vaccine research and development. This is primarily due to four factors: (1) the absence of effective treatment for numerous debilitating infections; (2) the rise of bacteria that are resistant to multiple drugs; (3) the necessity of enhancing the safety of the more conventional licensed vaccines; and (4) the enormous potential for novel vaccine research and design with the convergence of omics sciences, including immunomics, proteomics, genomics, and vaccines. (Bagnoli et al 2011).

The vaccine industry continued its standard cycle of discovery, development, and distribution until the end of 2019. Then, a sudden and unheard-of global public health emergency struck, upending lives, economies, and society

as a whole. In Wuhan, China, SARS-CoV-2, or severe acute respiratory syndrome coronavirus 2, first appeared. A variety of COVID-19 clinical outcomes can arise from exposure to SARS-CoV-2, ranging from asymptomatic infection to severe acute respiratory distress and death. (Zhou et al 2020).

It is extremely difficult to develop vaccines for diseases that are common in low-resource environments because they require extensive biologics manufacture, immunologic characterisation, and the management of organisms with complex life cycles. In the impacted areas, these initiatives are hindered by inadequate clinical trial infrastructure and a lack of finance. However, the chances of success have increased due to developments in immuno-profiling, antigen and antibody screening, and manufacturing methods. Within the next ten years, promising vaccine candidates for HIV, RSV, TB, malaria, and Shigella may become widely used. Furthermore, monoclonal antibodies and strong, reasonably priced long-acting medications may soon be able to treat these illnesses in low-income nations.Using modular biomanufacturing to facilitate regional production and cut costs, as well as embracing people-centered care techniques, are future challenges. To improve vaccination efficacy, more study on pathogen life cycles, antigen selection, and immunopathogenesis is still necessary. The diseases that plague low-resource areas can be eradicated and made extinct with the help of modern instruments and technologies. (Heaton et al 2020).

5. Challenges and Future Directions

The rapid pace of genetic engineering necessitates robust regulatory frameworks to ensure vaccine safety and efficacy. Ethical concerns, particularly regarding gene-editing technologies, must also be addressed. Regulatory agencies must adapt to evaluate the unique aspects of genetically engineered vaccines, ensuring they meet rigorous safety and efficacy standards without unnecessary delays. Manufacturing genetically engineered vaccines at scale remains a challenge, particularly in low-resource settings. Infrastructure for large-scale production and the availability of raw materials, such as lipid nanoparticles, are often limited. Strategies

to ensure equitable access, including technology transfer, global collaboration, and investment in local manufacturing capabilities, are critical to address these challenges and prevent disparities in vaccine distribution. Public skepticism toward genetically engineered vaccines highlights the need for transparent communication and education. Misinformation and distrust can undermine vaccination efforts, necessitating community engagement and proactive efforts to inform the public about the safety and benefits of these vaccines.

Future research should focus on improving the stability, storage, and delivery of mRNA and DNA vaccines, such as developing thermostable formulations that eliminate cold-chain requirements. Advances in delivery technologies, including self-amplifying RNA systems and next-generation nanoparticle carriers, could further enhance vaccine efficacy. Additionally, exploring the potential of artificial intelligence and bioinformatics to optimize vaccine design and predict immune responses will enable more precise and effective vaccine platforms.

6. Conclusion

Genetic engineering has revolutionized vaccine science, offering unprecedented speed, precision, and adaptability in combating infectious diseases and emerging health threats. Innovations in mRNA, DNA, and viral vector vaccines, as well as novel delivery systems, have proven transformative, particularly during the COVID-19 pandemic. These advancements promise to address neglected diseases, enhance personalized medicine, and improve global health outcomes. However, challenges such as scalability, equitable distribution, and public acceptance remain critical. Continued research, collaboration, and investment are essential to maximize the potential of genetic engineering and ensure vaccines are accessible to all, paving the way for a healthier and more prepared future.

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EFFECTS OF INTOXICATING SUBSTANCES ON GUT AND ORAL CAVITY

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ABSTRACT

The Human body harbors trillions of microorganisms, known as natural microflora or commensal microbes. They present all over body, forming complex ecosystem. Dysbiosis condition among themselves create unhealthy condition as all of them have significant function to play. However, the consumption of intoxicating substances such as betel nut, gutka, cigarette, alcohol can disrupt their balance of the microbiome, creating favorable condition to grow the opportunistic microbes and pathogen and leading to various diseases. This review aims to summarize the effect on natural microflora of the most exposed part of the body to these intoxicants particularly oral cavity and gut, their mode of action of causing diseases and altering normal microflora. We highlighted the risk of diseases, those are directly linked by having these intoxicants such oral and gastrointestinal cancer, cardiovascular diseases and other disorders. The relationship between intoxicating substances and the human microbiome provided valuable insights on prevention and treatment of related diseases.

Keywords: *Gut, intoxicating substances, microflora, oral cavity*

Introduction

Human body is the home to trillions of microorganisms including bacteria, fungi and viruses. Among them bacteria are more abundant. These microorganisms form normal flora of the body,providing nutrients and stable environment tohost and form symbiotic relationship withthem.They also play different roles in our body. Balance between the commensal microbes and the host is essential for the normal function of the body. Anincrease or decrease in their number can cause disturbance in body. Human mouth and gut are more frequently exposed with microbes through foods, air, water. Consuming of Intoxicating Substances like Betel nut, Gutka,Cigarette, alcohol causes disbalance between in commensal microbes of gut and oral cavity, facilitating the growth of opportunistic microbes and pathogens and ultimately leading to various diseases. (Reyaz Ahmad Khan, et al.,2023). Betul nut or areca nut is a seed of areca palm, composed of tannins, arecoline arecaidine,free fatty acids, polysaccharides etc. For preparing betel quid or betel paanbetel nut is main ingredients. A toxic mixture of areca nut, tobacco, slaked lime, catechu is known as gutka, also define as smokeless tobacco. it is placed between cheek and gum for chewing and Often get with various flavors to enhances the ability of mouth fresheners (Bharat Sankhla, et al.,2018). When fined cut tobacco rolled in thin paper with different flavors or without flavors known as cigarette. Composed of approximately 600 ingredients, among of them at least 69 are carcinogen. Nicotine, tar, tobacco, arsenic, benzene, acrolein, carbon monoxide, hydrogen cyanide these are the few components of the cigarette those lead to make it carcinogenic(Helen Keane, et al.,2020; Yoon-Seok Seo, et al.,2023).Alcohol is another intoxicant, composed with only carbon, hydrogen and oxygen. Accumulationof alcohol metabolites lead to cause cancer like diseases (Alessia Stornetta, et al.,2017). All of theseintoxicants ultimately cause cancer by disrupting the normal function of the body's normal microbiome. On the basic of environmental factors, personal's food habit, hygiene human oral microbiome can be two type core or pioneer microbes and variable microbes. Bacteria can colonize in the hard tissue of teeth or soft tissue

of oral mucosa of oral cavity. Microbes get access of nutrients from teeth, tongue, gums, cheeks, tonsils, gingival sulcus etc. Recent study showed thatnewborns acquire microflora during delivery, feeding and while exposing environmental factors. Fusobacterium nucleatum was the most common phyla found in the oral cavity, besides that more 11 phyla (Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes, Chlamydiae,Chloroflexi, Spirochaetes, Synergistetes, Saccharibacteria (TM7) and Gracilibacteria) were observed. Among them 54% are officially named, 14% are unnamed but cultivated and 32% are known but uncultivated. All of these phyla have distinct characteristic so all of them contain with specific microorganism. After birthStreptococcus salivarius, Lactobacillus, Actinomyces,and Veillonella like aerobic bacteria mainly can grow as the pioneer microorganism in the oral cavity (Priya Nimish Deo, et al.,2019). In the gut ecosystem, 10- 100 trillions of microbes were developed after birth and influenced by diet and host genotype. Factors like age, diet, antibiotics and consumption of intoxicating substances can affect the microbial stability of the gut. Helicobacter pylori, a spiral shaped member of gram negative Epsiloproteobacteria inhabits in the acidic pH of mucus layer of the stomach. Whose presence make drastic changes in the stomach microbiota. Hpylori hasthe positive and negative correlation with Spirochaetae and Bacteroides, Choloroflexi, and other phyla respectively (Gabriele Capurso, et al.,2017). Acid resistance bacteria like Lactobacillus sp, Veillonella sp and Clostridium sp are dominants in stomach microbiota. Whereas, species like Escherichia coli, peptococcus sp, Klebsiella sp, Bacteriodes sp show decline growth in acidic environment. Corynebacterium sp, Proteus sp, Enterobacter sp, Propniobacterium sp were found as constant bacteria in average concentration (Bruno Zilberstain, et al.,2007). N-nitroso compounds (NOCs) are the key component of the cigarette smoke and gutka. Which are formed by the reaction of nitrate or nitrite with any amino group of the body. NOCs are Predominantly found in meat, smoked food, salted fish. The main sources of nitrate and nitrite are vegetables like beetroot, spinach, and cabbages, drinking water (in most countries)and animal food like processed and smoked foods respectively. These N-nitroso

compound(N-nitrosidimethylamine) acts as alkylatingagent, react with the DNA and cause mutation (Monireh Sadat Seyyedsalehi et al.,2023). Besides NOCs,complex mixture of nicotine, aldehyde, acrolein, heavy metals are found in the several intoxicating substances including cigarette smoke and ultimately cause dysbiosis.Restricted the crucial functions include intestinal development, barrier integrity, metabolism, immunity, inflammation and neurological signalling regulation performed by gut microbiome. Leading to cause diseases like irritable bowel syndrome, cardiovascular disease, obesity, rheumatoid arthritis, systemic lupus erythematous, central nervous systemic disease and ultimately cancer (Xiaohua Gui et al.,2021). Not only in the gut, oral microbe like Firmicutes, Actinobacteria, Bacteroidetes also get effected and get disrupted their normal function such as, food digestion, maintenance of oral pH, immune system modulation, defensive mechanism etc.Some pathogenic microbes such as; Streptococcus pyogenes, Staphylococcus aureus, Bacillus species, Escherichia coli, Pseudomonas aeruginosa, Candida species, Entamoeba gingivalis and Porphyromonas gingivalis may be present in the oral cavity due to poor oral hygiene. These microbes can cause diseases and disorders such as; gingivitis, pharyngitis, stomatitis, tonsillitis, sinusitis, dental decay, oral thrush, halitosis, periodontitis, oral cancer and so on (Ofonime M. Ogba, et al.,2017; Sandhya Singh Kushwaha, et al.,2021). Disbalanced microflora in gut or oral cavity is the main reason of getting susceptible to various diseases caused by intoxicants. Change the concentration of inhabits microbes facilitate the opportunistic organisms and pathogens to grow. Once their concentrations get increased, cause distress. Several studies showed direct correlation with the microbes and the disease like increased concentration of Helicobacter pylori causes gastric cancer, Fusobacterium nucleatum andPorphyromonas gingivalis cause oral cancer. Factors like life style, geographical status, genetical history may affecttheecosystem of gut and oral cavity. Maintaining oral hygiene, having proper diet, or taking probiotics like Lactobacillus acidophilus, Bacillus subtilis, Bifidobacterium bifidumcan restore the microflora by stimulating immune system, inducing apoptosis, reducing cancer related

symptoms (Stanislav Sitkin, et al.,2022).

Oral Microflora

Microbes are present every part of the body, affect every aspect of human life. Oral cavity, one of the densely populates sites of the human body, often referred as the entrance or gate of the gut or digestive tract. From the oral cavity foods and liquid reach to the stomach through esophagus. Variety of sites are available where microbes can colonize and each site have specific environment that allow specific microbes to grow, not all the microbes can grow all the parts of oral cavity (Neetu Sharma, et al.,2018; Nobuhiro Takahashi, et al.,2025; M Killan, et al.,2016).Phylum Firmicutes (gram positives with low GC content) such as species of Streptococcus, Gemella, Eubacterium, Selenomonas, Veillonella, and related ones, the Actinobacteria (gram positives with high GC content) such as species of Actinomyces, Atopobium, Rothia, and related ones, the Proteobacteria (gram negative) species of Neisseria, Eikenella, Campylobacter, and related ones, the Bacteroidetes (Gram negative bacteria) species of Porphyromonas, Prevotella, Capnocytophaga, and related ones, the Fusobacteria (obligate anaerobe, nonsporforming, gram negative species of Fusobacterium and Leptotrichia, and the TM7 phylum are more abundant in the oral cavity(Jørn A. Aas, et al.,2005). TheSubgingival dental plaque, Supragingival dental plaque, Gums, Cheek, Tonsil,Throat, Saliva, Tongue soft tissues oforal cavity are populated by various microbes, providing different growth environment(Lu Gao, et al., 2018). In the Supragingival sites saccharolytic microorganism like Streptococcus sp, Actinomyces sp are predominant, take nutrients from continuously supplied saliva, fermenting carbohydrates produce lactic acid and creating acidic environment. The subgingival sites contain gingival crevicular fluid(GCF) which derived from the blood plasma so nutritionally rich in amino acids and proteins. Asaccharolytic bacteria such Fusobacteria sp, Prevotella sp metabolize nitrogenous compound and creating neutral pH.Haemophilus sp abundant in cheeks. Sulphur compounds are being producing by asaccharolytic microorganisms in the tongue coating, causing oral malodour. Additionally oral

microflora also can form biofilm where 500 microbial species communicate with each other's, act cooperatively and form community (Nobuhiro Takahashi, et al.,2025; Lu Gao, et al.,2018). Principle bacterial genera found in the healthy oral cavity are –

- Gram positive(cocci) – *Abiotrophia sp., Peptostreptococcus sp., Streptococcus sp., Stomatococcus sp.*
- Gram positive(rods)- *Actinomyces sp., Bifidobacterium sp., Corynebacterium sp., Eubacterium sp., Lactobacillus sp., Propionibacterium sp., Pseudoramibacter sp., Rothia sp.*
- Gram negative(cocci) -*Moraxella, Neisseria, Veillonella*
- Gram negative(rods) - *campylobacter, Capnocytophaga, Desulfobacter, Desulfovibrio, Eikenella, Fusobacterium, Hemophilus, Leptotrichia, Prevotella, Selemonas, Simonsiella, Treponema, Wolinella(Priya Nimish Deo et al.,2019).*

Gut Microflora

Human intestine poses diverse community, where 500-1000 bacterial species, 1800 genera found. Its 150 times higher than the human genome so now it also known as a discrete "organ" within human body. Bacterial inoculation had developed after post-partum and stabilizes near adolescence and stay intact till adulthood. 90% human intestine are dominated by the Firmicutes, Bacteroicidetes phyla and followed by Proteobacteria, Fusobacteria, Verrucomibrobia (JohnTsiaoussis,et al.,2019). Some factors effect the gut microbiome like, in vaginal delivery neonate are more exposed to the mother vaginal microbes and dominated by Lactobacillus, Prevotella, and sneathia genera. While in c-Section delivery newborn poses less diverse gut microbiome with more skin -Dwelling microbes like, Staphylococcus, Corynebacterium, Propioniobacterium etc. Additionally, breast milk contains own bacteria such Bifidobacterium sp. E. coli, Clostridium difficile, Bacteroides fragilis and lactobacilli, Enterobacterisceae spare dominant in formula fed infants(Zahraa Al Bander, et al.,2020;Elizabeth Thursby, et al.,2017). In Infants of rural Africa dominate by the Actinobacteria, Bacteroidetes phyla due to having starch,

fibre and plant polysaccharide in diet(Carlotta De Filippo, et al.,2010). Helicobacter pylori have a complex relationship with gut microbiome.It protects against allergic, auto immune disorders and inflammatory bowel disease. More over help to maintain balance and diversity of GI. Eradication of H pylorialtered the bacterial metabolism,associated with the presence of Rhodococcus, Lactobacillus, and Sphingomonasand changing the gastric microbiota by increasing proteobacteria, Enterobacteriaceae, Enterococcus sp and decreasing Actinobacteria sp(Elizabeth Thursby, et al.,2017).Lactobacilli spare the members of lactic acid bacteria play vital role in the gut eco system,Produce lactic acid as the main product of the carbohydrate metabolism. There are 17 species of lactobacillus associated with the human gut. Not only the gut, also abundant in fermented food, plants etc. Used as probiotic for their potential health benefits (Jens Walter, et al.,2008).

Intoxicating Substances and their mode of action

A mixture of powdered tobacco, Betel nut or areca nut and slaked lime are formed a form of smokeless tobacco, gutka. Other component of gutka include perfumery compounds such as sandalwood and musk ketones and commercially available in tins and sachets. consumption of gutka is increasing day by day due to lack of awareness of its negative impact on health, easy access and less expensive. It is prior placed between teeth chewed then held against buccal mucosafor long duration chewed and sucked. Arcea nut also can be used as Betel quid or paan, a blend of areca nut, slaked lime, artificial sweeteners and sometimes tobacco wrapped in Piper betel leaf. Zarda and Khaini are others form of smokeless tobacco(Bharat Sankhla, et al.,2018; Fawad Javed, et al.,2009). Habitable gutka use has been directly associated with the several oral mucosal disorders including oral submucosa fibrosis (ORS), oral cancer and periodontal disease. Investigation showed that patient with ORS (excess accumulation of collagen fibre in the oral mucosa) intolerance to spicy food, rigidity lips, tongue and palate and limited mouth opening and tongue movement ability.Fine grains of areca nut cause mechanical injury to oral tissue and fascinating the binding of ground

tobacco to the injured mucosa, leading to membrane damage and morphological changes. More over it increase the arterial blood pressure,heart rate. Arcea nut, tobacco and slaked lime, all of them have the different pathophysiological event that ultimately cause mucosal disorders. Acrolein is the one of the key alkaloids (basic nitrogen containing compounds) that can bind to the DNA by covalently, transferring the alkyl group to the DNA, or binding to major or minor groupof specific DNA sequence led to cause breakage of DNA strand and DNA damage,arrest the cell cycle at G2/M stage, suppresses the p53. Indirectly by generating reactive oxidative species or inhibiting DNA repair enzyme (such as O6-methylguanine-DNA methyltransferase (MGMT) cause DNA damage (Fawad Javed, et al.,2009). Combination of Tobacco and areca nut with slaked lime in the gutka increase the concentration of ROS in saliva and produce superoxide anion and hydrogen peroxide, high ROS level cause direct tissue damage. Tobacco specific Nitrosamines present in the saliva of the chewers which converts procarcinogen to the carcinogen, metabolites of the tobacco specific Nitrosamines, that react with nucleophilic sites of on DNA bases causing damage. Polycyclic aromatic hydrocarbons (PAHs) is a tobacco mutagen that activated by the microsomal cytochrome P450, an enzyme that play a significant role in the metabolism of tobacco carcinogen(Bharat Sankhla, et al.,2018). Amphipathic nature of the ethanol makes it rapid absorbed through passive diffusion by stomach and liver. Hydrophilic properties of ethanol allow it to distribute throughout the body's water compartments by forming quick hydrogen bond with water. First metabolite of ethanol is acetaldehyde, a group 1 carcinogen to human, which is formed by alcohol dehydrogenase (ADH) in liver. Another two enzyme, those are involved in ethanol metabolismare aldehyde dehydrogenase (ALDH) and cytochrome P450. Aldehyde dehydrogenase form acetate from acetaldehyde, which denote as less harmful than acetaldehyde. Where as cytochrome P450 oxidizes ethanol to acetaldehyde. Specific mutation in the ALDH2 gene leads to deficient enzyme activity causing disbalance condition of ADH and ALDH result in accumulation of acetaldehyde. Acetaldehyde is an electrophilic molecule, which can react with the nucleophilic

centers of cellular constituents. By the imine formation between carbonyl carbon of acetaldehyde and amino group of DNA nucleotides acetaldehyde-DNA adduct is formed. N2-ethyldeoxyguanosine, one of the major DNA adduct and potent mutagen. Acetaldehyde can alter DNA methylation pattern, Histone modification, protein misfolding (Alessia Stornetta, et al.,2018; Mikko T. Nieminen, et al.,2018; Tingting Yan, et al.,2020). By binding to the mitochondrial electron transport chain (specifically complex I and III) acetaldehyde form superoxide anion (O2-) which further react with other molecule and form additional reactive e oxygen species (H2O2,OH-). Additionally, ROS also can be formed as byproduct of the acetaldehyde metabolism. While metabolizing of ethanol by cytochrome P450 ROS generate (Masato Tamura, et al.,2013).ROS causes oxidative damage to protein, nucleic acid, lipid and by forming toxic lipid radicalsROS initiates lipid peroxidation (Hsu-Sheng Yu, et al.,2010). Alcohol consumption has been linked to increase risk of oral cavity cancer, oropharynx cancer, hypopharynx cancer, colon cancer, liver cancer, rectum cancer, female breast cancer (Alessia Stornetta, et al.,2018). Smoking is a significant risk factor for numerous diseases including cardiovascular diseases, respiratory conditions and various form of diseases. Cigarette smoke is a mixture 7000 chemicals including gases like carbon monoxide, carbon dioxide, nitrogen oxides, particular matter like tar, nicotine polycyclic aromatic hydrocarbons(PAHs), organic compound like acetaldehyde, acrolein, toluene, phenol, cresol, metal ions like As, Ba, Cu, Cs, Cd, Mn, Na, Zn Fe, K, Li etc. (Xiaohua Gui, et al.,2021). Nicotine is primarily absorbed by lung but also can be absorbed by skin, GI tract. It increases heartrates, blood pressure and generates ROS. It can easily pass blood brain barrier and influences function of nervous system. Nicotine binds to the nicotine acetylcholine receptors (nAChRs), located on the surface of the neurons, muscles cells and tissue. When nicotine binds to the receptors leading to an influx of positively charged ions such calcium, sodium into the cells. These cations further allowing calcium entry and release neurotransmitters including dopamine, acetylcholine. (Neal L Benowitz, et al.,2010; Liang Chi, et al.,2017). Heavy metals

of the cigarette could cause dysbiosis in intestinal ecosystem like lead and arsenic exposure increase the abundancy of Barnesiella and firmicutes and decreases firmicutes and Bacteroidetes respectively. PAHs in the cigarette smoke cause DNA damage, mutation that led to activation of oncogenes and inactivation of tumor suppressor genes, by forming covalent bond with DNA bases it also can form DNA adduct these are metabolized by the cytochrome P 450 enzymes, which convert them into reactive oxygen species. More over cigarette smoke itself contain with radicals and ROS which further contribute to produce oxidative stress. It also can disrupt the electron transfer chain of mitochondria causing mitochondrial damage (Xiaohua Gui, et al.,2021., William A Pryor, et al., 1976).

Conclusion

In conclusion, dysbiosis or alteration of the functions of commensal microbes in any parts of body remarks great changes.Prolong Ignorance of changes cause harmful disease. All of the microbes have specific and significant role in our body.All of the intoxicatinghas some similar composition in it like aldehyde, nicotine, heavy metals etc. Their ultimate mode of action is similar in the body. Generation of free radicals or reactive oxidative species is main cause of various disease by forming DNA adduct or mutation. Genetic evolution or genetic history also play a crucial role of having disease in offspring.Future investigation is going on long term effect of intoxicant use on physical and metal health, effect of geographical differences, effect of e- cigarette and vaping product use, role of specific microflora preventing harmful effect of intoxicants. To avoid theaddiction of intoxicating conveying the awareness about the risks and consequences of intoxicant use can be effective way. By organising health fairs, public service announcements, public lectures, workshops or providing counselling to the addicted people or establishing supportive group awareness can be speared. Self-realising is very crucial to total get rid from the addiction.

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DEFENDER OF THE GENOME: UNRAVELLING THE MECHANISM OF TUMOR SUPPRESSOR GENES (tsgs)

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ABSTRACT

So, it's almost to our knowledge that tumor suppressor genes (TSGs) which makes the cell division slow and functions the cell that when they will die. They are regulating processes like DNA damage repair, checking the cell cycle and genomics stability etc. Inactivation or mutations of these genes play a critical role in cancer growth because they result in the loss of regulatory mechanisms, allowing for unregulated cell division and genetic damage accumulation. Suppose a mutation inactivates a tumor suppressor gene, the protein it encodes is either not generated or does not function effectively, which can result in uncontrolled cell division. If we talk about the complex mechanisms of TSGs which are evaluated in this study, recognition is given to crucial members such as TP53, RB1, BRCA1/BRCA2 and PTEN. In this review, there's a more about the various classes of TSGs into caretakers, gatekeepers, and landscapers by illuminating their functions into various cellular pathways. On top of that, new and modified medication options for TSG pathways, including as gene therapy and synthetic lethality are in the process of establishment. Therefore, we get a better understanding

of TSGs mechanism and mutational changes which assists the progress for better cancer diagnostics, prognosis, and precision therapy.

Keywords: *Cancer, gene therapy, genomics, tumor suppressor genes, synthetic lethality*

1. Introduction

We all know that cancer is increasing day by day. In this area the tumor suppressor genes as genome's guardian by controlling the oncogenic transformations. Genes accountable for these were found and classified as "oncogenes." Although "antioncogenes" have been introduced, there has been a lack of definitive evidence for years. In the year 1969, Knudson gave a prediction that existence of tumor suppressor genes (TSGs) based on the development of inherited and sporadic retinoblastomas (Knudson et al.,1993). In 1986, the retinoblastoma (RB1) gene was effectively cloned, supporting his postulated "2-hit" hypothesis for carcinogenesis (Knudson et al., 1971). Inactivation of tumor suppressor genes usually results in a phenotype when both copies are lost. In cancer, inactivating one copy of a TSG causes loss of the remaining copy, followed by emergence. One cannot overstate the importance of TSGs for maintaining cellular integrity. Most relevantly we see the reaction to unusual growth signals, genotoxic stress and other carcinogenic impulse, acting as genomic soldiers. Understanding the molecular mechanisms of TSGs, classifying them, and taking into account their importance for cancer treatment are the main goals of this review. By comprehending these pathways, scientists and medical professionals can create customized treatments to lessen the effects of TSG inactivation in cancer. Kinzler and Vogelstein classified TSGs into two categories: "gatekeeper" genes and "caretaker" genes (Kinzler et al., 1997). Caretaker genes maintain genomic integrity, whereas gatekeeper genes control how cells grow or divide. Understanding the distinctions between these two gene types is critical for developing treatment approaches. Nowadays, practically all molecular targeted therapies block oncogenes such as kinases Kinase inhibitors are among the most effective cancer treatments ever developed (Kinzler et al., 2004). Through

this review let's emphasize and explore the molecular mechanisms, classification, and therapeutic implications which are related to tumor suppressor genes.

2. Mechanisms of Tumor Suppressor Genes (TSGs)

2.1 Regulation of Cell cycle

Tumor suppressor genes such as RB1 and CDKN2A are very much crucial for regulating the G1/S checkpoint. The retinoblastoma protein (pRB), which is encoded by the RB1, allows to bind and inhibits E2F transcription factors, therefore it prevents transcription of genes which is required for S-phase entry (Weinberg et al., 1995). When the loss occurs in RB1 function is because of different mutations that aggregate in the preceding cell proliferation (Knudson et al.,1971). One more key regulator, is CDKN2A, which encodes p16INK4a and this protein also inhibits the cyclin-dependent kinase 4/6 (CDK4/6), averting the phosphorylation of pRB and securing that cells do not prematurely advance throughout the G1 phase. Deprivation of CDKN2A function, frequently through deletion or promoter methylation, is a hallmark of many cancers(Sherr & McCormick et al., 2002).

2.2 DNA Damage Response (DDR) and Repair

One of crucial gene TP53, it encodes for p53, which is a central regulator for DDR. If we talk about DNA damage, then p53 accumulates and operates the transcription of targeted genes, including GADD45 for repair, CDKN1A (p21) for cell cycle arrest, and pro-apoptotic genes like BAX (Levine, 1997; Vousden & Prives, 2009). In over a half of the human population with cancer, mutations are found in TP53, impair the DDR and allows the cells to heap up genetic abnormalities (Kandoth et al., 2013).

2.3 Apoptosis

When the process of cellular damage becomes irreparable then TSGs open a door for promoting apoptosis. We can also see that PTEN antagonizes the PI3K/AKT pathway in a subtle way, by vanquishing anti-apoptotic signals and which promotes cell death (Cantley & Neel et al., 1999). After, PTEN decreases, it gives rise to increased survival

signaling which contributes to the tumorigenesis (Salmena et al., 2008). Additionally, there are BAX and BCL2-associated pathways that are regulated through p53 to initiate various mitochondrial-mediated apoptosis (Chipuk et al., 2004).

3.Classification of Tumor Suppressor Genes

So, the tumor suppressor genes (TSGs) play an important and very crucial role in generating cellular processes and preventing excessive cell development, which is one of the characteristics of cancer. Hence, these genes are divided into three categories: caretakers, gatekeepers, and landscapers, they have their unique roles in maintaining the genomic stability and altering the tumor microenvironment. They also help in regulating cell proliferation.

3.1 Caretaker

Here, the tumor suppressor genes help to polish up cohesion of genome by finding and reconstructing the DNA damage. Then it ensures the chromosomal stability, and prevents mutation, which can lead to oncogenesis. Their primary function is to act as "genomic defenders," safeguarding the fidelity of genetic material.

If we look upon the significance there are genes like BRCA1 and BRCA2. These genes are very crucial and essential for the homologous recombination repair pathway, which is exact mechanism for rebuilding of DNA double-strand breaks. When BRCA1 or BRCA2 function is disapproved, cells proceed to error-prone ways such as non-homologous end joining (NHEJ), which increases genomic instability and the probability of mutations. The occur of germline mutations in BRCA1 or BRCA2 likely increases the risk of hereditary breast and ovarian cancer (Venkitaraman et al., 2002). Lack of these genes also causes exposure to DNA-damaging drugs, which is the base for medicines like PARP inhibitors.

3.2 Gatekeeper

In this, the tumor suppressor genes are straight away involved in supporting the cell growth and apoptosis. These genes act as "brakes" on cell proliferation, not allowing cells from dividing fastly or surviving irreversible harm. Let's

give a look towards PTEN that how it works. It is a negative selector of the PI3K/AKT pathway, PTEN conquers cell for survival and proliferation. Its reduction aims to unchecked activation of this pathway, assisting to tumor growth. Now here comes RB1. The RB1 gene is well known for encoding the retinoblastoma protein (Rb), as it regulates the G1/S checkpoint of the cell cycle. Therefore, hen RB1 is mutated, cells bypass through this checkpoint and enters the S phase without proper growth control (Lane et al., 1992).

3.3 Landscaper

Here the tumor suppressor genes affect the tumor microenvironment by ensuring correct tissue architecture and extracellular matrix integrity. While they do not directly govern cell proliferation, their absence produces a favorable environment for tumor development.

If we see the loss of APC that leads to aberrant activation of Wnt signaling, promoting the heaping of β-catenin in the nucleus and driving unidentified cell division. APC mutations are a hallmark of familial adenomatous polyposis (FAP) and sporadic colorectal cancers (Polakis et al., 2000).

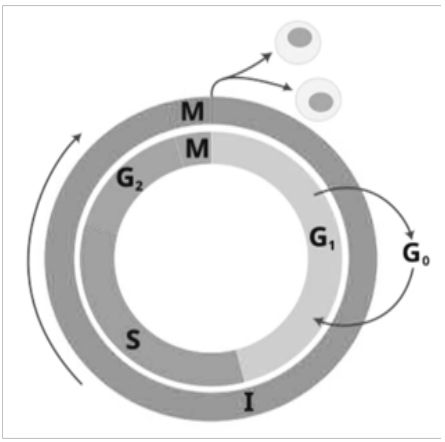

Figure:1

4. Different functions and mutational patterns in Tumor Suppressor Genes (TSGs)

As we know, so much about the mechanism of Tumor suppressor genes (TSGs). It plays a crucial role in preventing tumorigenesis by regulating cell cycle arrest, maintaining apoptosis, and promoting genomic integrity.

4.1 Genetic Mutations

Tumor suppressor genes (TSGs) genetic mutations include point mutations, nonsense mutations, and frameshift mutations, all of which sometimes result in the growth of shortened or non-functional proteins. The missense mutations in TP53, one of the most essentially researched TSGs, they mostly alter the DNA-binding domain etc. These mutations interrupt the transcriptional control of downstream genes involved in apoptosis, DNA repair, and cell cycle arrest (Soussi et al., 2007). There are few other TSGs, such as RB1 (retinoblastoma protein), are frequently deactivated by nonsense or frameshift mutations, resulting in impaired cell cycle control (Knudson et al., 1971).

4.2 Epigenetic Silencing

The transcriptional silencing in TSGs is due to epigenetic changes, methylation of DNA and alteration of histone. As CDKN2A promotes hypermethylation, which encodes p16^INK4a^, is a widespread route of inactivation in hostility such glioblastoma, melanoma, and large cell of lung cancer. When the silencing occurs, it distrupts the RB pathway, permitting for unlimited cellular generation(Herman et al., 1995). Now let's look into epigenetic suppression of the BRCA1 through promoter methylation, which has been seen in breast and ovarian cancer by various, that hinders the homologous repair and become the cause to genomic instability (Esteller et al., 2000). Afterwards these epigenetic changes are unique and reversible, making them main targets for diagnosis like DNA methyltransferase and histone deacetylase inhibitors.

4.3 Loss of Heterozygosity (LOH)

So, loss of heterozygosity is a commonly caused in cancer, which results the lack of the second allele of a tumor suppressor

genes through different actions like deletion of chromosomes, mitotic recombination, or uniparental distomy. In TP53 loss of heterozygosity is a well-documented event in cases such as lung and breast cancer, it assists co-occurring of mutations in the left over allele (Malkin et al., 1990). On the other hand, loss of heterozygosity in the RB1 locus is seen in retinoblastoma and osteosarcoma, which highlights the role of carcinogenesis (Harbour et al., 1988). After performing studies, it's noticed that LOH is a critical "second hit" in the Knudson two-hit hypothesis, which states that both alleles must be inactivated for tumor growth (Knudson et al., 1971).

5. Therapeutic Process

There are various therapies introduced. By the help of tumor suppressor gene modifications has been noticed in cancer treatment. All this type of therapy includes techniques like gene therapy, targeted therapies and also immunotherapy.

5.1. Targeted therapies

The good effects created by tumor suppressor genes deficits caused in cancer cells is used in therapies. A very well-known example is synthetic lethality, where the cancer cells with few mutations are specifically destroyed by targeting compensating processes. For example, BRCA1/2-mutant cancers, they have deficits in homologous recombination repair, so they are in extremely exposure to PARP inhibitors. These inhibitors hampers the base excision repair mechanism, resulting in DNA damage reconstruction and cell death (Farmer et al., 2005). These modified and precised medicine techniques have indicates great success in treating BRCA-mutant breast and ovarian cancers.

5.2. Gene Therapy

Gene therapy is one of the best ways for curing cancer and also it is better than chemotherapy and radiotherapy. Its working is, it mainly aims to reimpose the working of inactivated Tumor suppressor genes and then activates them. It is also a practicable treatment approach and reaching to many peoples. Gene therapy is considered of the safest method. Now let's take example of adenoviral vectors

with a functional TP53 gene, they have showed promisable results in preclinical and early clinical trials for diagnosis of non-small cell lung cancer (Martins et al., 2006). While they have promising results,challenges still remain, including as productive delivery to tumor cells and prevailing over immune responses. New modifications in delivery technologies, such as nanoparticle-based vectors, are also looked over to intensify therapeutic efficacy and safety (Kaiser et al., 2018).

5.3. Immunotherapy

Immunotherapy is made to strengthen our immune system so that it's fight cancer more effectively. It engages the immune system to fight cancer and overcome it, so that it can help to reduce the effect forloss of tumor suppressor genes. Mutations that occurin TSGs, likely TP53, subsequently leads to the generation of neoantigens, which are main peptides seen on the tumor cell surface. T-cells can target these neoantigens, following in immune-mediated cancer cell death as it destroys them. Immune checkpoint inhibitors, likely PD-1/PD-L1 blockers, have been productive in exploiting these neoantigens to boost and magnify anticancer immunity. Immunotherapy is essential treatment option in TSG-mutated cancers because it promotes the immune system's ability to acknowledges and attack cancer cells and destroys then.

6. Conclusion

So, this is the mechanism of the tumor suppressor genes. When the inactivation, caused due tomutations, different epigenetic changes, or loss of heterozygosity, they result in the lack of these protective mechanisms and enhances tumor growth. It is a helpful process. As we got to know about the regulation of cell cycle, the DNA damage response and apoptosis etc. Tumor suppressor genes such as TP53, RB1, PTEN, and BRCA1/2 indicates the diversity of function and critical functions these genes play in cell control. By understanding the molecular mechanisms of TSGs has provided insights into cancer biology while also opening up new treatment approaches. Synthetic lethality, targeted gene therapy, and immunotherapy use the vulnerabilities generated by TSG loss to selectively eradicate cancer cells while preserving healthy

tissues. Despite these gains, obstacles remain, particularly in restoring TSG function and overcoming resistance to treatments.So, we should concentrate on creating novel therapeutic techniques, leveraging developments in gene editing, and investigating the tumor microenvironment's role in TSG regulation. A better understanding of TSGs will improve our ability to create precise, effective medicines, eventually benefiting cancer patients.

7. Future Prospective

The study of mechanism of tumor suppressor genes (TSGs) tells us about the future-comings of cancer research, diagnostics, and therapeutics. It focuses on the advanced genomics and personalized Medicine. Many advancementsare seen in genomics, gene editing, and computational biology are likely to revolutionize our understanding of TSGs functions and their role in cancer prevention and progression. Gene-editing tools like CRISPR-Cas9 offer immense potential for restoring TSG function in cancer cells. TSGs such as APC and PTEN are known to influence the tumor microenvironment. Future research may focus on manipulating the microenvironment to inhibit tumor growth and metastasis.TSGs and their regulatory pathways offer potential as biomarkers for early cancer detection and prognosis. Several areas of focus are emerging as key directions for future exploration.

8. Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this paper.

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MOLECULAR MECHANISM OF LUNG CANCER: DYSREGULATION OF PROTO-ONCOGENE AND TUMOR SUPPRESSOR GENE

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ABSTRACT

Lung cancer remains a critical public health challenge, representing the leading cause of cancer-related mortality worldwide. Lungcanceraccounts for approximately 20%of all cancer deaths, translating to around 1.6 million fatalities annually. The two primary classifications of lung cancer are small-cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC), with NSCLC comprising approximately 85% of cases. Key driver mutations in genes such as EGFR, KRAS, MET, HER2, BRAF, ROS1 and NTRK have been identified as pivotal in the development of NSCLC. In addition to genetic alterations, various signaling pathways such as PI3K/AKT/mTOR and MAPK contribute significantly to tumor grow than dsurvival mechanisms in lungsqua mous cellcar cinoma.Through altering chromatin structures and the specific expression of genes, epigenetic modifications in lung cancerplay a significantrole incelltrans for mation. These in clued DNA methy lation, histone and chromatin protein modification, and micro-RNA, all of which suppress tumor suppressor genes while promotingthe expression of on cogenes.Understanding the molecular mechanisms

behind lung cancer presents signify cant opportunities for enhancingearly detection and develop ping targeted therapies.

Keywords: *Epigenetic changes, Lungcancer, Proto-oncogene, Tumor suppress or gene*

Introduction:

Lung cancer is a complex disease characterized by uncontrolled cell growth in lung tissues, primarily driven by genetic and environmental factors. With an estimated death toll of over 160,000 in 2006, lung cancer is the most common cause of cancer-related fatalities in the US for both sexes. Surgical resection, platinum-based doublet chemotherapy, and radiation therapy, either separate lyorin combination, are currently considered conventional treat ments. Sadly, the disease is rarely cured by these treatments, and the 5-year survival rate is still only 15% overall (Jemal A et al. 2006).

The main causative agent for lung cancer is tobacco smoking. There are over 60 carcinogens in tobacco smoke, and over 20 of them are closely linked to the development of lung cancer. The most well-known of these substances are the tobacco-specific nitrosamine 4- (methylnitrsosamino)-1-(3-pyridyl)-1-butanone and the polycyclic aromatic hydrocarbons, both of which cause genetic alterations by forming DNA a dducts (Hecht SS et al. 1999). According to the epidemiology studies, smokers have a 14-fold increased risk of lung cancer compared ton on smokers, only 11%of heavy smokers will ever get the disease (Amos CIetal. 1999).

The molecular mechanisms underlying lung cancer involve a series of alterations in cellular processes, including mutations, changes in gene expression, and epigenetic modifications that

Canlead to tum origenes is.Key genetic mutation soft enimplicate dinlung can cerinclude those in the epidermal growth factor receptor (EGFR), KRAS, and anaplastic lymphoma kinase (ALK) genes. These mutations can activate signaling path ways that promote cell proliferation and survival, contributing to the aggressive nature of lung cancer. In addition to genetic mutations, thetumor microenviron

ment, which includes surrounding cells, blood vessels, and immune elements, play sasignifican trole in lung cancer progression. The interaction between cancer cells and their micro environment can lead to enhanced tumor growth, metastasis, and resistance to therapies.

Base donitshis to logical type, lung cancer is categorized. Small cell lung cancer (SCLC) makes up 15–20% of lung cancer patients, whereas non-small cell lung cancer (NSCLC) makes up the remaining 80–85% (Pikor LA et al. 2013). It is believed that neuroendocrine cells are the source of SCLC, which mainly arises from the central airways. Adenocarcinoma (LUAD), squamous cell carcinoma (LUSC), and large cell carcinoma (LCC) are the three main histological subtypes that make up non-small cell lung cancer (NSCLC). The most prevalent is LUAD, which make supabout 40% of all lung cases. LUAD typicall yarises from glandular epithelium, from bronchioal veolar stem cells, club (Clara) cells or type II pneumocytes in the lung periphery. LUAD is also the predominant subtype that arises in patients who have never smoked (Chen Z et al. 2014). About 20% of all instances of lung cancer are LUSC, which mostly occurs in the central airways and segmental bronchi and is closely correlated with a history of smoking. LCC is categorized as a tumor that lacks the common characteristics of SCLC, LUAD, or LUSC and can develop anywhere in the lung (Pikor LA et al. 2013).

Currently, it is essential to carry out a suitable his to logical cate gorization, choose eachpatient for a treatment plan based on a thorough examination of the molecular tumor characteristics, and assess predictive factors (Travis WD et al. 2013). Under standing the intricate molecular mechanis msoflung cancer is also very crucial for develop in gtargeted the rapies an dimproving patient outcomes. Insights into these mechanisms pave the way for innovative treatment strategies, including the use of targeted therapies and immune otherapies that can specifically address the unique molecular profiles of lung cancer.

ABNORMAL ITIESINPROTO-ONCOGENES MEDIATED PATH WAYS EGFR

Family- Epidermal growth factor receptor (EGFR) is atrans membranety rosinek inase receptor that plays a central role in regulating cell division and death. It belongs to the HER family of receptors, which includes EGFR (HER1/ ErbB1), ERBB2 (HER2/neu), ERBB3 (HER3), and ERBB4 (HER4). The epidermal growth factor receptor is thear chetypal member of this family. A transmembrane segment, an intracellular TK domain, and an extracellular ligand-binding domain make up an ERBB receptor. A regulatory C-terminal section comes after the intracellular TK domain. The TK domain contains the highest sequence homologies (59–81%identity) of thefourgenes (SharmaSVet al.2007).When ERBBreceptors attach to ligands, they either homodimerize or heterodimerize, which triggers intrinsic kinase activities that startintr a cellularsign altrans duction cascades, such as the MAPk in a sepath way. In about 30% of NSCLCs, particularly adenocarcinomas, ERBB2 (HER2/neu) is strongly expressed (WeinerDBetal1990).According toTrans fection studies inimmor talized human bronchial

Epith elial cells, ERBB2 over expression play sarole in tum origenicity (NoguchiMetal. 1993). Mutations in the genes involved in the EGFR signaling pathway have been discovered in NSCLC (and extremely rarely in other tumors). EGFR and KRAS mutations have also been found in approximately 10–30% of NSCLCs, depending on the region (Shigematsu H et al. 2006). The most common RAS gene activation in lung cancer is KRAS, which is typically caused by mutations at codon 12 but sometimes occasionally at codons 13 and 61. The downstream KRAS gene codes for a tiny guanosine-5′-triphosphate-binding protein. It is the most common oncogene known to be active in human malignancies, and missense mutations in many of these cancers usually activate it. About 20% of non-small cell lung cancers have KRASmutations, particularlyin ADC and smokers. There is mutual exclusion between EGFR and KRAS mutations, according to a number of studies that examined the presence of both mutations in the same tumors. Both KRAS and BRAF are members of the EGFR family

signaling cascade because they link to each other. However, compared to KRAS mutations, BRAF mutations are seen in lung cancer far less frequently (0–3%) (Dhomen N et al. 2007).

P13k-Inlungcarcinogenesis, the PI3K/AKT/m TOR path way is dysre gulated, and it has been linked to advanced illness (stage III) and high-grade tumors (G3–G4) (Scrima M et al. 2012). PI3K signaling inhibitors have therefore been proposed as possible treatment agents for NSCLC. Deregulation of this pathway can be caused by a number of processes, such as PIK3CA amplification, activation of tyrosine kina serecep tors up stream of PI3K, mutations in KRAS, PI3K, or AKT, or loss of negative regulation by the tumor suppressor gene PTEN. According to reports, 3–10% of SQCLC and 0–2.7% of ADK have PI3KCA activating mutations in exons 9 (E545K) and 20 (H1047R), which affect the protein's helix binding domain and catalytic subunit, respectively (Stjernstrom A et al. 2014). Interestingly, EGFR, KRAS, and ALK mutations may coincide with PIK3CA mutations in ADK (Chaft JE et al. 2012). The loss of PTEN function may be caused by mutations, deletions or transcriptional repression viapro moterhyper methy lation. PTEN loss and PTEN mutations are reported in8– 59% and 3–10% of SQCLC, and 4–46% and 2–5% of ADK, respectively (Scrima M et al. 2012).

MYC family- Nuclear proto-oncogene products like MYC are eventually activated by RAS signaling, and upon heterodimerization, these products transcriptionally activate downstream genes that promote cell growth. MYC, MYCN, andMYCL (which was first identified from a lung cancer49) are members of the Myc family. While the others often exclusively affect SCLC, MYC is the most commonly activated and affects both SCLC and NSCLC. Overexpression of proteins is the result of either transcriptional dysregulation or gene amplification (~20–115 copies per cell). About 15% to 30% of SCLC and less often NSCLC have amplified MYC genes. Specific to neuroen docrine lung tumors, such as large cell neuroen docrine carcinoma (LCNEC) and small cell lung cancer (SCLC), L- and N-MYC am plification occurs. A high degree of tran scription causes MYC protein over expression more often than amplification (Gazzeri S et al. 2004).

ABNORMAL ITIES IN TUMOURS UP PRESS ORGENE PATH WAYS

Thep 53 path way-In the face of car cinogens, UV radiation, and γ-induced DNA damage, p53 preserves genomic integrity. p53, a sequence-specific transcription factor that controls down stream genes like p21, MDM2, GADD45, and BAX, rapidly in creasesin responseto

DNA damage or hypoxia. This helps to control the G1/S cell cycle transition, G2/M DNA damage checkpoint, and apoptosis. The accumulation of several mutations and the eventual developmentofacancercellaremade possible by p53 dys function, whichpermits genetically damaged cells to survive inappropriately. P 53 is essential for lung cancer; 75% or more of SCLC sand roughly 50% of NSCLC shave mutation alin activation of there maining allele, and the chromo some 17p13 locus is commonly hemizygously deleted. The G-T conversions anticipated of tobacco smoke carcinogens are mostly responsible for the 42, 52, 53 p53 alterations in lung cancers that correlate with cigarette smoking (Greenblatt MS et al 1994). Research has indicated that 40% to 70% of SCLCs and 40% to 60% of NSCLCs (squamous cell carcinomas higher than adenocarcinomas) exhibit aberrant p53 expression by immunohisto chemistry (Brambilla E et al.1996).

P 16-cyclin D1-CDK4-RB path way-

The retino blastoma, Rb gene- The Rb gene, which activates the cyclin-dependent kinase (CDK) inhibitor p21, is the downstream effector of p53-mediated G1 arrest and was the first tum or suppress orgenetobe identified. The G1check point function of Rbis close lyre lated to its phosphorylation status; in contrast, hypophosphory lated Rb binds the transcription factor E2F1 to sustain a cell cycle arrest in G0–G1. Rb phosphorylation is another method of Rb deactivation. When CDK4-6, cyclinD, and CDK2-cyclin Ephosphory late Rb, the transcription factor E2F1 is released, causing the G1–S transition. Therefore, the G1 checkpoint control is disrupted by both the often-observed hyper phosphorylation of Rb in NSCLC and the absence of Rb protein, which is the most common escape strategy from the G1 checkpoint in SCLC.

Less than 15% of NSCLC and more than 70% of high-grade neuroendocrine LCNEC and SCLC tumors, respectively, had loss of Rb protein (Ding L et al. 2008).

Cyclin D1 and CDK4- Cyclin D1 stimulates CDK4 to phosphorylate RB, which in turn suppresses its activity. There fore, another mechanism for interfering with the pl6-cyclin D1- CDK4-RB path way besides RB mutation is cyclin D1 over expression. Between 25% and 47% of initial NSCLC shave over expressed cyclin D1, which has occasionally been linked to a poor prognos, is (Betticher DC et al. 1996). Whena cyclin D1 antisense construct is transfected into lung cancer cell lines, it destabilizes RB and slows development by preventing any tumor- related cyclin D1 synthesis (Driscoll B et al 1996).

p16- Many lung tumors have chromosome 9p allele deletion (Merlo A et al. 1994). In lung cancer, p16 (p16 INK4 CDKN2), which is located at 9 p21, frequently experien cesmutation and allele loss. Disrupting the pl6-cyclin D1-CDK4-RB cell cycle control pathway can also be accomplished via p16 activation, since p16 controls RB function by suppressing CDK4 and CDK6 kinase activity. NSCLC frequently exhibits p16 abnormal ities; although SCLC rarely does (RB mutations typically affect the pathway). 10–40% of NSCLCs have homozygous deletion or point mutations. Loss of pl6 expression from the remaining allele may also result from hyper methy lationin the 5´Cp Gisland of the maintained allele (Otterson GAetal.1995).

EPIGENETICC HANGESIN LUNG CANCER

Epigenetic changes in lung cancer refer to alterations in gene expression that do not involve changes in the under lying DNA sequence.These changes can affect various cellular processes, including cellgrowth, differentiation, and apoptosis and are known to play a keyrole in the Development and progression of lung cancer. Some of the common epigenetic changes observe dinlung cancer include DNA methy lation, His tone modifications, Non-coding RNAs, Chromatin remodelling etc.

DNA Methylation - The most researched epigenetic regulation mechanism is DNA methylation. Various DNA methyl transferases (DNMTs) finish the methylation of the CpG island, which may result in gene silence. A methyl group is transferred from S-adenosyl-L- methionine to the CpG islands 5′-cytosine carbon by three active DNMTs (DNMT1, DNMT3a, and DNMT3b) (Forde PM et al. 1994). The pathophysiology of lung cancer is linked to DNMT over expression. Lung cancer patients may have elevated DNMT levels due to over expression of transcriptional activators, loss of micro RNAs that inhibit DNMTs, or compromised hsp 90 proteasomal DNMT break down. Smokers with lung cancer had higher levels of DNMT1 expression, most likely as a result of tobacco-specific nitrosamines that inhibit DNMT1 ubiquitination and degradation (Damiani LA et al. 2008). Additionally smoking-induced chronic inflammation and increased reactive oxygen species generation, leading toincreased DNA methy lation (O'HaganHMetal.2011). Two cigarette carcinogens, methy lnitrosurea (MNU) and benzo (a) pyrenediolepoxide 1 (BPDE), were administered to immortalized normal human bronchial epithelial cells (HBECs) for a duration of 12 weeks. Following exposure to these carcinogens, cell transformation was avoided by stable knock down of DNMT1, but not DNMT3 (Merlo A et al. 1995).

Histone modifications- The chromosomal building blocks known as nucleosomes are made up of two molecules of each of the main histones H2A, H2B, H3, and H4. About 1.8 times, DNA encircles the octameric core of the nucleosome (Luger K et al. 1997). In contrast to healthy lung cells, lung cancer cells showed abnormal patterns of histone H4 modification, including loss of H4K20 trimethylation, hypoacetylation of H4K12/H4K16, and hyperacetylation of H4K5/H4K8. Their results point to H4K20me3 as a possible biomarker for the early identification and treatment of lung cancer and show significant role forhistone H4 alterations (Van Den Broeck A et al.2008). Furthermore, the patterns of HAT and HDAC expression in tumor samples differ from those in normal samples, which may have therapeutic ramifications for prognostication, early tumor diagnosis, and the direction of epigenetic-targeted treatments (Ozdağ H et al. 2006).

The histone changes may also be influenced by respiratory epithelia exposed to cigarette smoke. Tobacco smoke contains nickelcations,which cause his tone deacety lation an denhance histone H3K9 dimethylation as well as smoking carcinogenic components such as nickel, chromate, and arsenite also cause H3K4 methylation (Zhou X et al. 2009).

MicroRNA- Through direct base-pairing interactions, miRNAs, a type of tiny (~22 nucleotides) nonprotein-encoding RNA molecules, modify the activity of particular messenger RNA targets to control gene expression. Over one-third of coding mRNAs are regulated by more than 1,000 miRs, each of which has the ability to control hundreds of target RNAs (81).There fore, methy lation-induced silencing of miR scan significantly alter the initiation and spread of tumors (Cowland JB et al. 2007).

Conclusion and Future implications

The molecularme chanism sunderlying lung cancer continues to be anarea of intense research, particularly given the disease's status as a leading cause of cancer-related mortality in the Western world. Recent advances have illuminated various pathways involved in lung carcinogenesis, revealing how genetic and environmental factors contribute to tumor heterogeneity and resistance to conventional ther apies. This under standing is crucialas it lays the foundation for developing more effective treatment strategies tailored to individual patient profiles. The traditional one-size-fits-all approach based on disease stage is gradually giving way to precision medicine that considers the unique molecular characteristics of each tumor. As researchers delve deeper into the molecular underpinnings of lung cancer, several key implications for future clinicalpracticee merge. First, there is potential forearly detection through identifying specific biomarkers associated with the onset of lung cancer. These biomarkers could facilitate the development of sensitive screening tools capable of detecting tumors atearliers tages when they are more amenable to treatment. Such advancements could significantly improve survival rates and quality of life for patients diagnosed with this aggressive form of cancer. Moreover, advancements in molecular classification

are paving the way for targeted therapies that exploit specific vulnerabilities within different subtypes of lung cancer. By integrating genomic data with treatment protocols, clinicians can develop personalized the rapeutic regimens that enhance efficacy while minimizing adverse effects (Massion & Carbone, 2023). This shift toward individualized therapy represents a transfor mative approach in oncology that holds promise not just for lung cancer but also for other malignancies. Under standing the molecular mechanisms behind lung cancer presents significant opportunities for enhancing early detection and developing targeted therapies. As research progresses, it will be essential to translate these findings into clinical applications effectively. Future studies should focus on refining biomarker identification processes and optimizing therapeutic strategies based on tumor genomics. Ultimately, such endeavors will not only improve patient outcomes but may also provide insights applicable across various forms of cancer (Massion & Carbone et al. 2023).

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THE INFLUENCE OF PROTO-ONCOGENES ON TYPICAL PATTERNS OF GROWTH & DEVELOPMENT

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ABSTRACT

Proto-oncogenes, also known as cellular oncogenes, plays a vital role in regulating normal cell growth. Their malfunction can lead to cancer, highlighting their significance. Most oncogenes encode intracellular proteins involved in signal transduction pathways and belong to closely related gene families. Families like erbB, ros, and fms encode membrane receptors with tyrosine kinase activity. Src family oncogenes, also tyrosine kinases, act as kinase subunits for independent receptors. Intracellular signaling occurs through phosphorylation of substrates such as phosphatidylinositol pathway kinases and cytoplasmic kinases. Ras oncogenes, conserved GTP-binding proteins, modulate signaling from membrane kinases. Oncogenes coding for nuclear DNA-binding proteins, phosphorylated by kinases like raf, regulate gene expression through dimerization. DNA-binding proteins, such as helix-loop-helix proteins (myc, MyoD, myogenin) and leucine zipper proteins (fos, jun), influence cell growth. Antioncogenes, like Rb and p53, counteract growth by inhibiting it.

Keywords: *GTP-binding proteins, Helix-loop-helix proteins, Intracellular signaling, leucine zipper proteins, Tyrosine kinase*

activity

INTRODUCTION

Oncogenes code for proteins that play a vital role in cell growth regulation and are involved in the onset of cancer. Proto-oncogenes, or cellular oncogenes, ensure proper regulation of cell division and differentiation during normal growth. Alterations like mutations, translocations, or viral effects can lead to their dysfunction, resulting in abnormal cell growth-a hallmark of cancer.

HISTORICAL CONTEXT OF THE RETROVIRUS GENE

Retroviruses, which have RNA instead of DNA as their genetic material, were pivotal in the discovery of oncogenes. Upon infection, retroviruses replicate by converting their RNA into viral DNA (V-DNA) using a reverse transcription process. This V-DNA, flanked by long terminal repeats (LTRs), integrates randomly into the host genome as a "provirus." The host then transcribes the viral DNA, producing viral RNA and proteins as though they were part of its genome. Occasionally, viral RNA may incorporate host DNA sequences, producing hybrid RNA and fusion proteins. This phenomenon, known as transduction, allows retroviruses to acquire and transmit host genetic material, some of which may have oncogenic potential. The discovery of cellular homologs to viral oncogenes revealed that normal regulatory genes, when disrupted, could drive cancer, thus offering a strategy to study cellular proliferation and differentiation.

TERMINOLOGY

Proto-oncogenes, also known as normal cellular oncogenes, are denoted as c-"onc." However, transforming oncogenes are not labeled as c-"onc," even if they originate from mutations, deletions, or other changes in cellular genes. When the function of a gene product is unknown, nomenclature often reflects the source (e.g., c- for cellular, v- for viral) and the viral homolog's identity (e.g., v-src from the Rous sarcoma virus). Typically, the gene is better understood than the protein product, as researchers often focus on oncogene sequence homology to uncover regulatory mechanisms.

Proteins encoded by oncogenes are named after the oncogene or their molecular weight (e.g., Myc protein or pp60c-src). Similar names, like ras and ros, do not imply related genes.

CELLULAR CONTROL & THE PROCESS OF SIGNAL TRANSDUCTION

Cellular oncogenes typically encode proteins involved in intracellular signal transduction. These oncogene products facilitate the reception of extracellular signals, transmission across the cytoplasm, and modulation of gene expression in the nucleus to elicit a cellular response. While the understanding of these regulatory pathways is growing, significant uncertainties remain.

Oncogene families represent distinct components of signal transduction pathways, enabling tissue-specific responses to stimuli through differential gene family expression. This discussion explores these families at various signal transduction stages.

Signal transduction, often reliant on phosphorylation cascades, enables external stimuli to cross membranes, reach the nucleus, and influence gene expression. Receptors with tyrosine kinase activity or associated kinases initiate these cascades. Phosphorylated cytoplasmic kinases, including serine kinases, activate DNA-binding proteins.

The phosphatidylinositol (PI) pathway, involving lipid phosphorylation, offers an additional mechanism for membrane kinases to activate cytoplasmic kinases. Adrenergic pathways can regulate kinase activity through protein kinase C or protein kinase A. Similarly, oncogenes resembling G proteins, components of adrenergic pathways, contribute to transduction, albeit differently.

Steroid and thyroid hormones bypass typical pathways, directly entering cells due to their lipophilic nature and binding nuclear receptors that regulate gene expression. Cross-talk between kinase pathways and hormone-receptor interactions influences gene regulation through phosphorylation.

Protein-protein interactions, including transient complexes and dimers, are pivotal in signal transduction. These

interactions ultimately regulate DNA-binding protein activity in the nucleus, facilitating changes in gene expression.

ONCOGENE FAMILIES

TYROSINE KINASE MEMBRANE RECEPTORS

Certain oncogenes encode families of membrane receptors with tyrosine kinase activity that respond to peptide hormones or growth factors. These include receptors similar to the insulin receptor, platelet-derived growth factor (PDGF) receptor, epidermal growth factor (EGF) receptor, and fibroblast growth factor (FGF) receptor. These receptors share structural features, including glycosylated extracellular ligand-binding domains, a membrane-spanning domain, and a cytoplasmic tyrosine kinase domain. Ligand binding induces structural changes that activate tyrosine kinase activity, leading to signal transduction via tyrosine phosphorylation of intracellular substrates. Autophosphorylation of the receptor itself often further modifies receptor function.

The Insulin receptor-like family includes the insulin receptor, insulin-like growth factor (IGF) receptor, and oncogenes such as ros, met, and trk. These receptors consist of four subunits: two extracellular subunits for ligand binding and two transmembrane subunits with tyrosine kinase activity. The extracellular ?-subunit has a cysteine-rich ligand-binding region, while the ?-subunit's intracellular domain contains the tyrosine kinase and autophosphorylation sites. Autophosphorylation enhances kinase activity, removing insulin dependence, while phosphorylation by protein kinase C on serine and threonine residues reduces insulin binding and tyrosine kinase activity.

Another oncogene family encodes receptors similar to the EGF receptor, such as neu, erbB2, and erbB3. The EGF receptor is composed of a single polypeptide chain with cysteine-rich extracellular ligand-binding domains. Tyrosine phosphorylation of the cytoplasmic domain activates the kinase, similar to autophosphorylation in insulin receptors. Serine and threonine phosphorylation can inhibit EGF receptor kinase activity.

Additional families include PDGF and CSF receptors, as well as receptors for FGF and flg oncogenes. These receptors have extracellular domains resembling immunoglobulins, with the PDGF and FGF receptor families featuring interrupted tyrosine kinase domains. The interruptions are variable but do not impact kinase activity directly, though autophosphorylation in these regions may play a role in substrate interaction.

All tyrosine kinase membrane receptors form functional dimers. The insulin receptor exists as ?-? dimers, while EGF receptors form dimers upon ligand binding, facilitating autophosphorylation and receptor activation. Some ligands, like PDGF, form dimers (e.g., PDGF-A and PDGF-B), which bind to specific receptor isoforms with varying affinities. This dimer assembly allows cells to modulate their response to signals efficiently by controlling a few genes.

NON-RECEPTOR TYROSINE KINASES

Non-receptor tyrosine kinases, such as those from the src family (src, fgr, fin, lyn, lck, and yes), are membrane-associated proteins that share similarities with receptor tyrosine kinases, although they do not have intrinsic receptor activity. These kinases likely function as kinase subunits for receptors without their own kinase activity.

The lck oncogene serves as a representative model for src family tyrosine kinases. In T lymphocytes, the CD4 surface protein interacts with MHC II antigens, and when it binds with p56lck (the product of lck), the complex activates the kinase activity of p56lck, leading to the phosphorylation of the T cell receptor subunit. This activation only occurs when p56lck is bound to CD4, not when CD4 is uncomplexed.

The src family proteins, like pp60src and p56lck, lack a transmembrane domain but are membrane-associated via myristic acid at their amino terminus. They have both serine and tyrosine phosphorylation sites, similar to receptor kinases. Tyrosine phosphorylation at two distinct sites regulates the activity of src proteins-one stimulates and the other inhibits kinase activity. These phosphorylation events are mainly intermolecular, involving src proteins or other kinases. The inhibition of pp60src and p56lck by tyrosine phosphorylation

near the catalytic domain is not fully understood, but the phosphatase CD45 plays a key role in regulating the activity of these kinases by dephosphorylating specific sites.

Additionally, src proteins undergo serine phosphorylation at various sites, with minimal effect from protein kinase A and protein kinase C. However, cyclin-dependent kinase (p34cdc2) increases pp60src's tyrosine kinase activity through serine phosphorylation during mitosis. This phosphorylation may regulate the interaction between src proteins and receptor subunits.

The src family proteins also contain two regions, SH2 and SH3, which are crucial for their function and share similarities with other oncogene products and regulatory proteins. For example, the abl protein, a tyrosine kinase, contains SH2 and SH3 domains and can localize to the cytoplasm or nuclear membrane. These domains are also present in the regulatory regions of other proteins, such as ras GAP and phospholipase C. In src proteins, the SH2 domain activates kinase activity, while mutations in this region reduce activity. Conversely, mutations in the SH3 domain lead to oncogenic transformation, indicating its role in negatively regulating kinase activity. The presence of SH2 and SH3 domains in various proteins suggests a shared regulatory mechanism, potentially involving interactions between different proteins.

SUBSTRATE OF TYROSINE KINASE

Tyrosine phosphorylation, once considered a rare event, is now understood to occur in various proteins, even though it represents less than 0.1% of total phosphoproteins. While many proteins can be phosphorylated at low levels in vitro under non-physiological conditions, up to 50 proteins are phosphorylated in response to oncogenes that encode for tyrosine kinases. The challenge in identifying key tyrosine kinase substrates arises due to the overwhelming background of serine and threonine phosphorylation (90% and 10%, respectively) and the diversity of phospho-tyrosine proteins. This complexity is further compounded by the fact that proteins like the EGF receptor and erbB2, though structurally similar and both part of the same oncogene family, activate

distinct mitogenic pathways.

Membrane-associated tyrosine kinases phosphorylate several types of proteins. Common mechanisms include autophosphorylation by the kinase itself or intermolecular phosphorylation by another membrane-bound kinase. Membrane-bound proteins and cytoplasmic proteins such as serine kinases and metabolic enzymes may also be regulated by tyrosine phosphorylation. Phosphatases that dephosphorylate serine or tyrosine residues can be phosphorylated to reverse kinase activation. Since both receptor and non-receptor kinases are membrane-associated, membrane proteins are likely candidates for phosphorylation.

Enzymes in the phosphatidylinositol (PI) pathway are also substrates for tyrosine kinases, although the exact effects of their phosphorylation remain unclear. These include PI kinase, which produces phosphatidylinositol phosphate, and phospholipase C, which generates diacylglycerol and inositol triphosphate. Both enzymes are phosphorylated and form complexes with activated membrane receptors, such as the PDGF and EGF receptors. Activation of PI kinase or phospholipase C results in protein kinase C activation, a serine/threonine kinase that might inhibit receptor tyrosine kinase activity.

The protein pp85, a substrate for src or PDGF receptor-mediated tyrosine phosphorylation, has been linked to PI kinase activity in src-transformed cells. This protein exists in various forms in different tissues, and differential phosphorylation may lead to distinct activities. This could explain unexpected findings, such as pp85 generating novel PI phosphates not recognized by phospholipase.

Several cytoplasmic serine/threonine kinases are also phosphory lated by tyrosine kinases, including microtubule-associated protein kinase (MAP kinase) and casein kinase II. MAP kinase, activated by insulin-mediated phosphorylation, may be identical to pp42, a protein responsive to multiple growth factors. Casein kinase II, involved in various cell functions, is activated by insulin, IGF-I, and EGF and plays a role in Alzheimer's disease. It can phosphorylate various

cytoplasmic and nuclear enzymes, including the myc protein and the insulin receptor tyrosine kinase.

The raf protein, a serine kinase encoded by the raf oncogene, is phosphorylated by tyrosine kinases such as PDGF, EGF, or FGF receptors. Raf can form a complex with membrane receptor tyrosine kinases, PI kinase, and phospholipase C. This large complex may function as a multifunctional signal transfer particle, with various members binding at multiple sites, as deletions in the PDGF receptor gene can block PI kinase binding without affecting raf or phospholipase C binding.

NUCLEOTIDE BINDING PROTEINS OF GUANINE

Guanine nucleotide-binding proteins, such as those in the ras family, play a significant role in cellular processes and are associated with the cell membrane. These proteins are not kinases but are involved in the activation of the ras oncogene product through the ras GTPase activating protein (GAP). The ras family includes several genes, such as ha-ras, ki-ras, N-ras, ral, and others, with high homology between them. Although their exact function is still unclear, ras proteins likely participate in cellular signal transduction between membrane-bound oncogenes like src or fms and cytoplasmic kinases like raf, possibly through activation of protein kinase C independently of the PI pathway.

Ras proteins are attached to the cell membrane via a carboxyl terminal palmitic acid, and this attachment site is crucial for their oncogenic activation. For example, exchanging palmitic acid for myristic acid at the amino terminal can activate ras oncogenically. These genes are highly conserved across species, with mammalian and yeast ras genes being functionally interchangeable. Ras overexpression leads to enhanced cell growth and lower serum requirements, resembling the behavior of transformed cells, though without full transformation. Ras overexpression also results in the production of PDGF and TGF-?, while reducing responsiveness to external PDGF. Such abnormalities are often seen in transformed cells, indicating that ras plays a role in transformation processes.

Ras proteins function through guanine nucleotide binding, much like G-proteins in adrenergic signaling. The active form, P21ras, binds GTP, while the inactive form binds GDP. The GTPase activity of P21ras is low but is activated by the GTPase Activating Protein (ras-GAP). Unlike adrenergic G-proteins, which release GDP in response to hormones, p21ras releases GDP with the help of a releasing factor (rus-GRF). The ras activation-deactivation cycle involves GTP, GAP, and GRF proteins. Although GAP is present in higher concentrations than P21ras, the regulatory role of GAP is still not fully understood, and it may be regulated by ras rather than the other way around.

A portion of GAP is phosphorylated, possibly indicating a regulatory function. GAP can be phosphorylated by src or by PDGF or EGF receptors in response to growth factors. Phosphorylated GAP can bind to the PDGF receptor and other proteins like PI kinase, phospholipase C, and raf. However, GAP binding is not essential for the interaction of these proteins, suggesting that GAP may influence their function. Additionally, GAP can form complexes with other tyrosine-phosphorylated proteins, pointing to complex interactions among membrane-bound oncogenes. Another ras family member, rap-I, can block v-ras oncogenic transformation, similar to how inhibitory and stimulatory G-proteins function. Rap protein has its own GTPase activity and is not activated by rap-GTPase activating protein

CYTOPLASMIC KINASES

Cytoplasmic kinases are important in the signal transduction pathway, linking the membrane to the nucleus. Several oncogenes encode cytoplasmic serine kinases, with raf (also known as mil). The mos oncogene, a cytoplasmic kinase, is expressed exclusively in germ cells and plays a role in oocyte meiosis. The abl oncogene, a cytoplasmic tyrosine kinase, may also associate with the nuclear membrane. Raf kinase is activated by PDGF through tyrosine phosphorylation and can also form a complex with the PDGF receptor. Interestingly, raf can also be activated by insulin (within 2 to 6 minutes) through serine phosphorylation, suggesting the involvement of an intermediate kinase like MAP or casein kinase. Although

raf substrates are not well-defined, cytoplasmic kinases may target nuclear oncogenes like myc, fos, ski, and myb for serine phosphorylation.

In addition to their role in activating signaling pathways, cytoplasmic kinases may also help deactivate receptors. Many membrane-bound tyrosine kinases interact with the PI pathway, leading to the activation of protein kinase C (PKC). Both PKC and protein kinase A can phosphorylate serine residues on receptors, causing their inactivation. Receptor deactivation can occur through various mechanisms, including decreased ligand binding affinity, inhibition of tyrosine kinase activity, or enhanced internalization of the receptors.

NUCLEAR BINDING PROTEINS

Nuclear binding proteins encoded by oncogenes plays a crucial role in the final stage of signal transduction, as they bind to DNA. These proteins are often serine phosphoproteins, and their phosphorylation carried out by cytoplasmic kinases like raf or nuclear kinases like abl. Oncogenes that produce DNA-binding proteins belong to various families with distinct mechanisms but share some key characteristics. Many of these genes, known as "immediate early genes," are involved in regulating the expression of other genes. These genes are usually expressed at low levels in resting cells but are rapidly activated by growth factors or other mitotic signals. Their expression does not require protein synthesis, suggesting that the necessary regulatory pathways are already in place, allowing the cell to respond to stimuli and alter gene expression without needing new proteins to be made.

The Induction of these "immediate early genes" can occur within 1 to 2 hours after a stimulus, and their response is brief. The mRNA for these genes has a short half-life, typically around 15 to 30 minutes, with the level of mRNA being regulated by changes in transcription or reduced stability, often due to AT-rich 3′ ends. The protein products from these genes also have short half-lives, ranging from 15 to 90 minutes

A useful way to categorize DNA-binding proteins is based on their binding mechanism. These categories include helix-

loop-helix (HLH) proteins, leucine zipper proteins, and zinc finger proteins. A key regulatory feature within these classes is the ability to form homodimers or heterodimers. Heterodimers regulate target gene expression differently from homodimers, and various heterodimers exhibit distinct activities. The formation of symmetrical dimers-almost but not entirely symmetrical in the case of heterodimers-aligns with the frequent presence of palindromic consensus binding sequences for these DNA-binding proteins.

HELIX-LOOP-HELIX PROTEINS

Helix-Loop-Helix (HLH) proteins, such as myc, myf, MyoD, myogenin, CEBP, and E12, belong to a class of oncogenes. These proteins have two regions that form amphipathic helices, separated by a loop. These regions are important for dimer formation, which influences DNA binding specificity, though the regions themselves do not directly bind DNA. The DNA binding region is highly basic and essential for the protein's function, and in some cases, proteins lacking this region are inhibitory. For example, a dimer missing a basic DNA-binding region (like ID:myoD) would not be active because the DNA-binding region would be asymmetrical.

Some HLH proteins, such as MyoD and myogenin, are crucial in muscle development, while others, like CEBP, are expressed in tissues related to lipid or cholesterol metabolism. Myc, one of the most studied HLH proteins, is highly expressed in proliferating cells and overexpression leads to hyperplasia. Although myc expression decreases during differentiation, this decrease is not required for differentiation, as cells can still differentiate with overexpressed myc. The relationship between HLH gene expression and differentiation is complex, likely due to dimer formation. For example, MyoD, although highly expressed in myoblasts, does not initiate muscle protein synthesis until it forms a homodimer. This is facilitated by the ID protein, which lacks the basic DNA binding region and forms a heterodimer with MyoD, allowing for the homodimer to form once ID levels decrease during differentiation. Alternatively, other HLH heterodimers, like MyoD-E12, can enhance DNA binding and promote muscle protein synthesis.

LEUCINE ZIPPER PROTEINS

Leucine zipper proteins, including fos and various jun proteins, are part of a family that forms dimers through interactions in a region with high homology, known as the leucine zipper. This region contains four to five leucine heptad repeats. The DNA binding domain is highly basic, similar to that in HLH proteins. Although these leucine zipper proteins share structural similarities, their effects on DNA binding can vary. They may be expressed in response to signals from protein kinase C or protein kinase A. Expression is also regulated by the proteins themselves, though the nature of this regulation differs. For instance, fos protein inhibits its own expression, while jun protein promotes its own. Both fos and jun are phosphoproteins, but the specific role of phosphorylation in their regulation is not fully understood.

Proteins encoded by the fos and jun oncogenes share significant homology with the AP-1 transcription factor and may bind to AP-1 or related proteins. AP-1 is a key member of a large family of transcription regulators. Fos and jun proteins can activate genes with AP-1 binding sites, and the fos gene regulates its own expression through AP-1 sites. Additionally, fos and jun proteins activate fat-specific element 2 (fse2), which is involved in glycerol phosphate dehydrogenase expression during early adipocyte differentiation. The impact of fos protein binding to AP-1 sites varies depending on the dimer formed. A jun:fos dimer activates transcription at AP-1 sites, while a jun:fos dimer represses it. This illustrates how different heterodimers can have opposing effects, particularly since jun and junB levels can fluctuate significantly. For example, in BC3H1 muscle cells treated with TGF-?, jun protein levels increase 2.5 times within 15 minutes, but junB levels rise about 20-fold, favoring the formation of the repressive junB:fos complex despite an increase in jun protein.

ZINC FINGER PROTEIN: HORMONE RECEPTORS FOR STEROIDS AND THYROID

Steroid and thyroid hormone receptors belong to a group of DNA-binding proteins that form dimers. This family includes receptors like the glucocorticoid, mineralocorticoid,

progesterone, estrogen, androgen, vitamin D3, and thyroid hormone receptors, among others. These proteins have separate regions for DNA binding, hormone binding, and a domain that regulates their activity. Unlike HLH or leucine zipper proteins, the zinc-containing domain in these receptors is responsible for DNA binding, not dimer formation. The binding consensus sequence of these proteins is longer than that of HLH or leucine zipper proteins, and different members of the family have varied effects when binding to this sequence. The v-erb-A oncogene, a fusion protein from a virus, is a well-known example of an oncogenic member of this family, as it shares DNA binding and hormone binding domains with thyroid hormone receptors.

These receptors are regulated by phosphorylation, similar to HLH and leucine zipper proteins. Phosphorylation, potentially mediated by nuclear serine kinases like the abl protein, usually enhances hormone binding and is induced by hormones, though this has not been fully confirmed. Hormone binding also triggers dimer formation, suggesting that phosphorylation and dimerization may be linked. Multiple phosphorylation sites can lead to different receptor forms with varying binding affinities, which may prevent functional dimer formation. The physiological significance of this regulation is clearer for steroid and thyroid hormone receptors, as they are involved in well-understood endocrine pathways. For instance, insulin or EGF can increase estradiol-induced progesterone receptor induction, possibly through phosphorylation of the estrogen receptor. This process typically occurs during the estrous cycle but is inhibited during starvation, when insulin is low, and estrous cycling is inappropriate.

Several oncogenes are involved in the response to steroid or thyroid hormone receptors, explaining the role of steroids in tumor promotion. For example, glucocorticoids affect Ras and abl, while estrogen increases the expression of proteins like fos, myc, H-ras, and erb B. On the other hand, thyroid hormones reduce the levels of fos, myc, and ras in cardiac muscle.

Myb : DNA-BINDING PROTEIN

The myb oncogene product is mainly expressed in hematopoietic cells and functions as a DNA-binding protein, with no evidence of dimer formation. Phosphorylation directly regulates its DNA-binding ability. Specifically, a serine phosphorylation, mediated by casein kinase II, inhibits DNA binding in a reversible manner. Casein kinase II plays critical roles in both the cytoplasm and nucleus and is inhibited by phosphorylation. This suggests that when a growth factor activates a receptor tyrosine kinase, it phosphorylates and inhibits casein kinase II. As a result, reduced casein kinase II activity leads to dephosphorylation of myb protein by nuclear phosphatases, enabling it to bind DNA and promote transcription for protein synthesis from a target gene.

ANTI-ONCOGENES : TUMOUR SUPPRESSING GENES

Tumor suppressor genes, also known as anti-oncogenes, have the opposite function of oncogenes by inhibiting growth rather than promoting it. While oncogenes stimulate cell growth and must be carefully regulated for normal cell function, mutations or deletions in tumor suppressor genes can lead to uncontrolled cell growth, a hallmark of cancer. For instance, the retinoblastoma gene (Rb) and p53 gene both serve to inhibit growth, and mutations or deletions in these genes can result in cancerous transformation. The Rb gene is crucial for preventing growth in certain cancers like retinoblastoma, and p53 is involved in regulating cell cycles, with mutations found in a significant number of colon cancers.

Both Rb and p53 are proteins that bind to DNA and can act as transcriptional inhibitors to suppress growth-related genes. They interact with transforming viral proteins, indicating a shared regulatory mechanism. Rb can be phosphorylated, but only its dephosphorylated form can bind to viral proteins like large T antigen, which prevents cell growth. Phosphorylation of Rb in normal cells removes this inhibition and promotes cell cycle progression, supporting the idea of phosphorylation playing a key role in regulating growth signals, especially as cells transition from the G1 to S phase.

CONCLUSION

The review paper offers a detailed overview of the role oncogenes that plays in cellular regulation and signal transduction. It highlights how oncogenes are involved in essential cellular processes like cell growth and differentiation, and how their malfunction can contribute to cancer. The review also underscores the complexity of oncogene activity, with various oncogene families playing roles at different stages of signal transduction.

The paper discusses several families of oncogenes, such as tyrosine kinase membrane receptors, non-receptor tyrosine kinases, guanine nucleotide-binding proteins, cytoplasmic kinases, and nuclear binding proteins. These families are integral to signal transduction, and their disruption can lead to oncogenic transformation.

A central theme of the paper is the significance of protein-protein interactions in oncogene function. It highlights how processes like dimerization, phosphorylation, and other post-translational modifications regulate oncogene activity. The review also points out the intricate nature of oncogene regulation, with numerous feedback loops and regulatory pathways involved.

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UNRAVELLING THE MOLECULAR BASIS OF LUNG CANCER: PATHWAYS TO PROGRESS

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ABSTRACT

Lung cancer remains one of the primary causes of cancer-related deaths worldwide. The disease's intricacy stems from its heterogeneity, which includes numerous genetic, epigenetic, and molecular mechanisms. This review delves into the critical molecular mechanisms driving lung cancer progression, such as genetic mutations, epigenetic changes, signal transduction pathways, and immune evasion strategies. Understanding these pathways sheds light on targeted medicines and personalized medicine methods for lung cancer treatment. Key oncogenic drivers, including KRAS, EGFR, and TP53 mutations, as well as ALK and ROS1 rearrangements, are highlighted for their role in cell cycle dysregulation, apoptosis evasion, and increased metastatic potential. Epigenetic modifications, such as DNA methylation, histone acetylation, and non-coding RNAs, contribute to gene expression changes in lung cancer cells. Dysregulated signaling pathways, including PI3K/AKT/mTOR, RAS/RAF/MEK/ERK, and Notch, are investigated regarding tumor development, angiogenesis, and therapeutic resistance. Furthermore, the review emphasizes the heterogeneity of lung cancer and the interaction of the tumor microenvironment,

immune evasion, and therapy resistance. Recent advances in targeted therapeutics and immunotherapies are briefly discussed, focusing on the implications for personalized medicine. The goal of the review is to offer a thorough grasp of the biology of lung cancer, opening the door for innovative treatment approaches that will enhance patient outcomes.

Keywords: *Immune evasion, Lung cancer, Oncogenic drivers, Personalized medicine, Targeted therapies, Tumor microenvironment*

INTRODUCTION:

Lung cancer is the leading cause of cancer-related fatalities worldwide, accounting for around 1.6 million deaths annually. Approximately 85 % of NSCLC refers to a range of histological subtypes, with the most frequent being lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). Tobacco use is the leading cause of lung cancer, accounting for about 80% of cases in the US and other nations where smoking is prevalent. Smoking is linked to all main histological subtypes of NSCLC, including small cell lung cancer (SCLC), however, LUSC and SCLC have a larger connection than LUAD, which is the most prevalent histology in nonsmokers.

Non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) are the two main forms of lung cancer, which cause more than 2 million new cases each year. Because of aggressive disease progression and delayed detection, survival rates are still low despite advancements in treatment. Numerous pathways essential to the pathophysiology of lung cancer have been revealed by molecular research, providing possible targets for treatment.

Genetic Mutations in Lung Cancer:

1) Oncogenes and Tumor Suppressors

a) KRAS mutations: KRAS is frequently mutated in NSCLC, leading to uncontrolled cell proliferation via the MAPK and PI3K-AKT pathways.

b) EGFR mutations: Mutations in EGFR contribute to constitutive activation of tyrosine kinase signaling, promoting tumor growth and survival.

c) TP53 mutations: Loss of TP53 function disrupts cell cycle control and apoptosis, enhancing tumor aggressiveness.

2. Fusion Proteins

a) ALK and ROS1 fusions: These gene rearrangements are frequent in a subgroup of non-small cell lung cancer (NSCLC) and endorse oncogenesis by activating constitutive kinase activity.

3) Epigenetic Alterations

a) DNA Methylation

Tumor suppressor genes like CDKN2A are silenced by hypermethylation, which promotes unregulated cell proliferation.

b) Histone Modification

Changes in histone acetylation and methylation affect gene expression and chromatin structure. For instance, a poor prognosis is linked to histone deacetylase (HDACs) overexpression.

c)Non-coding RNAs

Lung cancer is regulated by long non-coding RNAs (lncRNAs) and microRNAs (miRNAs). For example, miR-21 targets tumor suppressors such as PTEN to promote invasion and proliferation.

4. Signal Transduction Pathways

a) MAPK/ERK Pathway

Mutations in KRAS and EGFR cause aberrant signaling in this system, which encourages the growth and survival of tumor cells.

b) PI3K-AKT-mTOR Pathway

Angiogenesis, metabolism, and cell growth are all supported when mutations or amplifications activate this pathway

c) Wnt/β-Catenin Pathway

Dysregulated Wnt signaling promotes invasion, metastasis,

and the characteristics of cancer stem cells.

5.Immune Evasion Mechanisms

a).Immune Checkpoint Dysregulation

Lung malignant cells use immunological checkpoints, such as PD-1/PD-L1, to elude immune detection.

b)Tumor Microenvironment

The tumor microenvironment (TME) facilitates immune evasion by attracting immunosuppressive cells such as regulatory T-cells (Tregs) and myeloid-derived suppressor cells (MDSCs).

6. Therapeutic Implications

a) Targeted Therapies

Treatment approaches for certain subtypes of lung cancer have changed as a result of tyrosine kinase inhibitors (TKIs) that target EGFR, ALK, and ROS1.

b)Immunotherapy

Nivolumab and pembrolizumab are examples of immune checkpoint inhibitors that have demonstrated potential in boosting immune responses against lung cancer.

c).Epigenetic Drugs

To reverse the epigenetic silencing of tumor suppressor genes, researchers are investigating the use of HDAC inhibitors and DNA methyltransferase inhibitors.

ROLE OF TUMOR SUPPRESSOR GENE

In human lung tumors, allele loss in the short arm of chromosome 3 at 3p21.3.1 is the first premalignant chromosomal abnormality. This loss of heterozygosity happens in almost all cases of small-cell lung cancer (95%) Additionally, some smokers and ex-smokers have normal bronchial epithelium.2–4 According to homozygous deletion research about three lung cancers, the smallest deletion area specific to the 370 kb span of established SCLC cell lines (GLC20, NCI-H740, and NCI H1450) contains 19 genes

(RBM6, RBM5, SEMA3F, GNAT1, NAT1/SLC38A3/G17, GNAI2, SEMA3B, IFRD2, HYAL3, FUS2/NAT6, HYAL1, HYAL2, FUS1/TUSC2, RASSF1/123F2, BLU/ZMYND10, NPRL2/TUSC4, 101F6/ TSP10/CYB561D2, PL6/TMEM115, and CACNA2D2), and 3 open reading frames encoding hypothetical proteins (LOC100129060, LOC100287609, and C3orf45/FLJ38608).Five

Regarding regulating processes like cell differentiation, proliferation, signal transduction, and apoptosis, most of the 19 genes exhibit variable levels of tumor suppressor activity. It has been proposed that these genes work together as a sizable, cohesive, biologically functionally diverse tumor suppressor unit.3

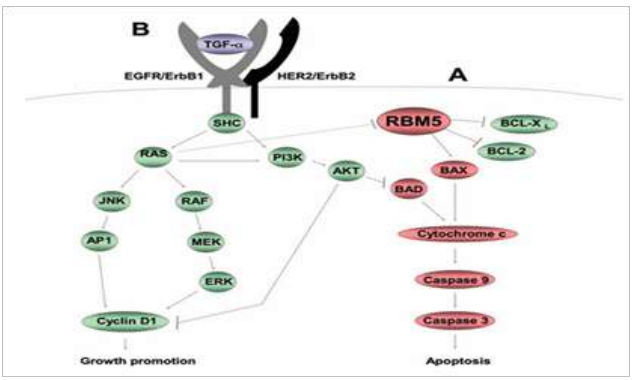

FIGURE 1. The relationship between transforming growth factor (TGF)-and RBM5 signaling and the modulation of apoptosis. AProapoptotic activity associated with RBM5 overexpression is related tothe down-regulation of BCL-XL and up-regulation of BAX expression, and antisense RBM5 overexpression is related to the up-regulation of BCL-2 expression. Overexpressed RBM5 increases cytochrome c release from the mitochondria and activation of caspases 9 and 3. B, activating mutations in epidermal growth factor receptor (EGFR), HER2, or KRAS result in increased growth and decreased apoptosis. For instance, RBM5 expression is down-regulated by the RAS activating mutant RAS(G12V), which would inhibit apoptosis. Likewise, increased EGFR and

HER2 activities can lead to phosphorylation—and subsequent inhibition of—the proapoptotic activity associated with the BCL2-associated agonist of cell death.

ROLE OF ONCOGENE

Therapeutic Targeting of Oncogenes

Small Molecule Inhibitors; Erlotinib, afatinib, and Osimertinib are first, second, and third-generation EGFR TKIs. ALK inhibitors include brigatinib and crizotinib.

Antibody-Based Therapies

Monoclonal antibodies that target HER2 for uncommon HER2 mutations.

Combination Therapies

TKIs and immune checkpoint inhibitors work in concert

Overcoming Drug Resistance

Next-generation inhibitors that target resistant mutations are being developed (for example, EGFR C797S).

FUTURE PROSPECTS

Advancements in the understanding of molecular mechanisms underlying lung cancer provide an optimistic outlook for precision medicine. Future research should integrate multi-omics approaches, such as genomics, proteomics, and metabolomics, to uncover novel biomarkers and therapeutic targets. Artificial intelligence and machine learning hold the potential to revolutionize diagnostic accuracy and treatment personalization. Additionally, developing combination therapies addressing resistance mechanisms and targeting tumor microenvironments will enhance treatment efficacy. Expanding immunotherapy options and exploring epigenetic modulation as therapeutic avenues are promising strategies. Collaborative efforts in clinical trials will be crucial to accelerating the transition of these discoveries into clinical practice.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ILLUMINATING THE ER-DIABETES RELATIONSHIP: THE HIDDEN CATALYST IN DIABETES DEVELOPMENT AND PROGRESSION

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ABSTRACT

Endoplasmic reticulum (ER) stress has emerged as a critical factor in the development and progression of diabetes, encompassing both type 1 diabetes (T1D) and type 2 diabetes (T2D). The ER plays a pivotal role in protein folding, lipid synthesis, and calcium homeostasis. However, when the ER is subjected to stress, it activates the unfolded protein response (UPR) to restore homeostasis. Prolonged ER stress and chronic UPR activation have been implicated in pancreatic β-cell dysfunction, insulin resistance, and inflammation, all of which are hallmarks of diabetes.Recent studies have highlighted the complex interplay between ER stress, oxidative stress, and inflammatory pathways in the pathogenesis of T1D and T2D. Furthermore, research has shown that targeting ER stress and the UPR may offer novel therapeutic strategies for the treatment and prevention of diabetes. This review aims to provide a comprehensive overview of the current understanding of ER stress in diabetes, including its molecular mechanisms, cellular consequences, and potential therapeutic interventions. By synthesizing findings from recent studies, this review seeks to contribute

to the development of innovative treatments for diabetes and to shed light on the intricate relationships between ER stress, β-cell function, and glucose metabolism.

Keywords: *Diabetes, Endoplasmic reticulum stress, Insulin resistance, Pancreatic β-cell dysfunction, Unfolded protein response*

INTRODUCTION

Diabetes is a severe metabolic condition that causes excessive blood glucose levels in millions of people on a global scale, divided into two categories namely, Type 1 Diabetes (T1D) and Type 2 Diabetes (T2D). The former causes total deficiency of insulin production for the fact of being an autoimmune disorder where the immune system accidentally targets and disables the pancreatic beta cells to produce insulin (Atkinson & Eisenbarth, 2001). The latter is a multifactorial disease, showing insulin resistance, declined insulin secretion, and elevated glucose production in the liver (DeFronzo & Ferrannini, 1991). Now the connection between ER stress and diabetes is well complicated. Within eukaryotic cells, the endoplasmic reticulum (ER) is a vitalorganellesynthesizing proteins, metabolizing lipids and storing calcium. It is a network of membranous tubules and sacs called cisternae, extending from the nuclear envelope to the cell membrane. The endoplasmic reticulumis present in two distinct forms, namely the Rough Endoplasmic Reticulum (RER) where the roughness is due to the presence of ribosomes, henceperforming in protein synthesis; and the other form is the Smooth Endoplasmic Reticulum (SER) which is involved in lipid synthesis and detoxification. The ER is sensitive to various stressors, such as glucose deprivation, oxidative stress, and environmental toxins, which can disrupt its normal functioning. This disruption leads to the accumulation of misfolded proteins in the ER lumen, triggering a stress response known as ER stress (Ron & Walter, 2007).Therefore, the ER stress is caused when the organelle cannot do a proper protein folding which results in the accumulation of misfolded or unfolded proteins. Unfolded protein response (UPR) is a cellular stress responsetriggered to restore normal functioning by making a temporary stop of protein translation,degrading misfolded proteins, and ultimately activating signalling

pathways that lead to apoptosis if the stress is not resolved (Schroder & Kaufman, 2005).Implications of the ER stress in various diseases, including neurodegenerative disorders, cancer, and metabolic disorders made it a vital area of study (Hetz, 2012). In the context of diabetes, ER stress has been shown to play a critical role in both T1D and T2D. In T1D, ER stress has been linked to the apoptosis of pancreatic beta cells, contributing to the development of the disease (Oyadomari et al., 2002). In T2D, ER stress has been implicated in insulin resistance, impaired insulin secretion, and pancreatic beta cell dysfunction (Ozcan et al., 2004).This review paper aims to examine the role of ER stress in the pathogenesis of both type 1 and type 2 diabetes, explore the molecular mechanisms underlying the effect of ER stress on diabetes, discuss the potential therapeutic implications of targeting ER stress pathways for diabetes treatmentand also highlight areas for future research.

MOLECULAR MECHANISM OF ER STRESS

The stress is occurred when there is a disruption in the ER's homeostasis due to which the ER lumen gets accumulated with misfolded or unfolded proteins.

- A number of factors are there triggering this condition in the body. For example, the endoplasmic reticulum's protein folding machinery gets overwhelmed when there is an excessive protein production, often due to increased cellular demands or genetic mutations. If the genes whose role is to encode proteins gets mutated, then obviously misfolding of that protein will show up in the ER. Oxidative stress can successfully cause a stress in the ER for the fact that unnecessary amount of Reactive Oxygen Species (ROS) causes protein damage and further misfolding. There are other vital factors causing up the ER stress like lack of sufficient nutrients in the body can limit the availability of substrates required for protein synthesis and folding, moreover viral infections too can interfere with protein synthesis and processing. Disruptions in calcium homeostasis within the ER can affect protein folding and trigger ER stress. (Hetz et al., 2011)

- Unfolded Protein Response (UPR): When there are any misfolding or misfolding of proteins happening in the ER lumen, the UPR comes up as a quality control mechanism. is a highly conserved cellular signaling pathway which aims to restore proper functioning of the endoplasmic reticulum, thereby helps regaining the organelle's homeostasis. Three most important activity is performed by this response mechanism. Firstly, it prevents further accumulation of misfolded proteins by reducing or making a temporary halt in the protein production. Secondly, it upregulates the expression of chaperones and other proteins which are involved in protein folding to make the ER more capable in doing its job. Last but not the least, is that the misfolded proteins need to be degraded else it would be problematic for the ER to do its job under such accumulations, such degradation is executed by this response via mechanisms like ER-associated degradation (ERAD).

Key Features of the UPR:The most important character of this response mechanism which makes it a successful quality control is its high sensitivity, which has the ability to detect even subtle changes in the protein-folding environment within the ER. The UPR is particularly activated in response to unfolded or misfolded proteins, not other forms of cellular stress, which makes it highly specific in nature.The UPR involves a complex network of signaling pathways, including three major transmembrane sensors: PERK, IRE1, and ATF6. It performs a homeostatic regulation to ensure that it is activated only when necessary and that it does not lead to excessive cellular stress or apoptosis.

Activation of the UPR: The UPR gets activated when the ER stress sensor, BiP (Binding Immunoglobulin Protein), is dissociated from the ER transmembrane sensors, PERK (PKR-like ER kinase), ATF6 (Activating Transcription Factor 6), and IRE1 (Inositol-Requiring Enzyme 1). This dissociation occurs when the ER is subjected to stress, such as increased protein synthesis or secretion demands, impaired protein folding or degradation, ER calcium depletion, oxidative stress, viral infections etc.

1) UPR transmembrane proteins: There are three main proteins or sensors monitoring the protein-folding status within the ER lumen. They are Protein Kinase RNA-like ER Kinase (PERK), Inositol-Requiring Enzyme 1 (IRE1), Activating Transcription Factor 6 (ATF6).When ER stress occurs, these sensors are activated, leading to a cascade of signalling events that aim to restore ER function.

2) UPR Signaling Pathways:

The PERK Pathway of UPR goes through four sequential stages. At first, it is activated by the dissociation of BiP, leading to its autophosphorylation and activation. The activated PERK phosphorylates and activates the eukaryotic translation initiation factor 2 alpha (eIF2α). Now on one hand the phosphorylated eIF2α reduces protein synthesis by inhibiting the initiation of translation, and on the other hand it also induces the expression of ATF4, a transcription factor that regulates the expression of genes involved in amino acid metabolism and oxidative stress response. (Harding et al. 1999)

The second one, which is the ATF6 Pathway starts with the activation of ATF6 by the dissociation of BiP, leading to its translocation to the Golgi apparatus. ATF6 is proteolytically processed by the site-1 protease (S1P) and site-2 protease (S2P), releasing a cytosolic fragment. The cytosolic fragment now translocates to the nucleus, where it activates the expression of genes involved in protein folding, quality control, and ERAD. (Yoshida et al. 2001)

Third one is the IRE1 Pathway. IRE1 is a transmembrane kinase and endoribonuclease that dimerizes and auto-phosphorylates in response to ER stress. The activated IRE1 splices the mRNA of X-box binding protein 1 (XBP1), converting it into a potent transcription factor. Then XBP1 upregulates genes involved in ER protein folding, degradation, and lipid biosynthesis. IRE1 also activates the Regulated IRE1-Dependent Decay (RIDD) pathway, which targets mRNAs encoding ER-resident proteins for degradation. (Iwawaki et al. 2003)

3) UPR Outputs: There are two main categories in which the UPR outputs can be classified. First one is the

adaptive outputs where the UPR can restore ER homeostasis by reducing protein synthesis, increasing protein folding capacity, and enhancing protein degradation. The second one is the apoptotic outputs which shows that prolonged or severe ER stress can lead to the activation of apoptotic pathways, resulting in cell death.

ER STRESS ANDDIABETES (TYPE 1)

T1D is an autoimmune disease which is represents in demolition of the beta cells of the pancreas, resulting in lack of insulin. ER stress triggers in producing pro-inflammatory cytokines, such as IL-1β (interleukin-1 beta) and TNF-α (tumour necrosis factor-alpha), which exacerbate beta cell destruction. These cytokines activate various signaling pathways, including the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway, which promotes the expression of pro-inflammatory genes (Cnop, M., et al. 2005). ER stress impairs insulin biosynthesis by reducing the expression of key enzymes involved in insulin production. Proinsulin convertase 2 is responsible for converting proinsulin to insulin. Now the ER stress reduces its expression, leading to impaired insulin production. ER stress also reduces the expression of key transcription factors, like PDX1 (pancreatic and duodenal homeobox 1), which regulates insulin gene transcription.

Key Molecular Players: The keys players of ER stress-induced beta cell destruction in Type 1 Diabetes (T1D) can be broadly categorized into three groups: sensors, transducers, and effectors.

- Sensors: They are thefirst line of defense against the ER stress. Basically, they are proteins that detect the stress condition and trigger the unfolded protein response. These proteins are embedded in the ER membrane and are responsible for monitoring the protein-folding environment within the ER. When ER stress occurs, sensors detect changes in the ER environment and activate downstream signaling pathways to restore ER homeostasis. There are three main types of sensors involved in ER stress detection: PERK (PKR-like ER kinase), IRE1 (Inositol-requiring

enzyme 1), ATF6 (Activating transcription factor 6).The PERK is a transmembrane protein which is activated by the binding of misfolded proteins to its ER luminal domain. It phosphorylates eIF2α, leading to the attenuation of protein synthesis. Such attenuation of protein synthesis helps lower the protein-folding load on the ER and restore ER homeostasis. The IRE1 is a transmembrane protein that activates the IRE1-XBP1 pathway, which results splicing XBP1 mRNA withsubsequently activating UPR target genes which helpsrestore ER homeostasis by increasing the expression of ER chaperones and other UPR target genes. The ATF6 is a transmembrane protein activating the ATF6 pathway, leading to the transcription of UPR target genes.

- Transducers: They are the signal relay stations of the UPR, which basically are the proteins that transmit the ER stress signal from the sensors to the effectors. These proteins play a vital role in amplifying and processing the ER stress signal, allowing the cell to respond appropriately to the stress. The three main transducers involved in the UPR are: eIF2α (Eukaryotic translation initiation factor 2 alpha), XBP1 (X-box binding protein 1), and CHOP (C/EBP Homologous Protein).eIF2α is a key regulator of protein synthesis which is phosphorylated by PERK in response to ER stress, hereby inhibiting global protein synthesis which will ultimately help to alleviate ER stress by reducing the protein-folding load on the ER. XBP1 is a transcription factor which gets spliced by IRE1 in response to ER stress, resulting in the activation of its transcriptional activity, thereby uplifting the expression of ER chaperones and other UPR target genes, hence helping to restore ER homeostasis.CHOP is a transcription factor which gets activated by ATF6 and PERK. It increases the expression of pro-apoptotic genes that results in the contributionof beta cell apoptosis in response to chronic ER stress.

- Effectors: They are the executioners of the UPR. They are also proteins that are responsible for implementing the decisions made by the UPR, such as restoring ER homeostasis, initiating apoptosis, or activating autophagy. Different types of effectors involved in the UPR are: Bcl-2 family proteins, JNK (c-Jun N-terminal kinase), Caspase, Autophagy-related

proteins.The Bcl-2 family proteins are the key regulators of apoptosis, which gets split into two distinct subgroups- one is the anti-apoptotic (e.g., Bcl-2; Bcl-xL), other one is the pro-apoptotic (e.g., Bax, Bak). They regulate the release of cytochrome c from the mitochondria, which triggers the activation of caspases and apoptosis thereby helping to eliminate damaged or dysfunctional cells, maintaining tissue homeostasis. JNK is a protein kinase which phosphorylates and activates pro-apoptotic proteins such as Bax and Bak, and regulates the expression of autophagy-related genes. Caspases are a family of proteases whose function is to cleave and activate pro-apoptotic proteins, leading to the execution of apoptosis and thus maintaining tissue homeostasis. And lastly, Autophagy-related proteins regulate the formation of autophagosomes, which engulf and degrade damaged or dysfunctional cellular components.

ER STRESS AND DIABETES (TYPE 2)

In the context of Type 2 diabetes, ER stress contributes to insulin resistance, a hallmark of the disease. The UPR activates various signaling pathways, including the IRE1α-XBP1 pathway, which can lead to insulin resistance (Ozcan et al. 2004). Specifically, the activation of IRE1α can phosphorylate and inhibit the insulin receptor substrate 1 (IRS1), impairing insulin signaling (Nakatani et al. 2005). ER stress also plays a critical role in pancreatic beta-cell dysfunction, a key feature of Type 2 diabetes. Chronic ER stress can lead to beta-cell apoptosis, reducing insulin secretion and contributing to hyperglycemia. The UPR can also impair beta-cell function by reducing the expression of genes involved in insulin biosynthesis and secretion.

Key Molecular Players: The variety of keys players of ER stress in Type 2 Diabetes (T2D) are as follows.

Insulin Receptor Substrate 1 (IRS-1), the key substrate of the insulin receptor tyrosine kinase, plays a crucial role in insulin signaling by transmitting signals from the insulin receptor to downstream effectors. Reduced expression and/or function of it contribute to insulin resistance in T2D. IRS-1 serine phosphorylation, which inhibits its function, is increased in

insulin-resistant conditions.

Phosphatidylinositol 3-Kinase (PI3K) is a lipid kinase that plays a central role in insulin signaling. It catalyses the production of phosphatidylinositol 3,4,5-trisphosphate (PIP3), which activates downstream effectors. Impaired PI3K activation contributes to insulin resistance in T2D. Reduced PI3K activity leads to decreased glucose uptake in peripheral tissues.

Protein Kinase B (Akt) is a serine/threonine kinase playing a key role in insulin signaling. It is activated by PI3K-generated PIP3 and regulates various cellular processes, including glucose metabolism. Under any impairment, it contributes to insulin resistance in T2D. Reduction in its activity leads to decreased glucose uptake in peripheral tissues and impaired insulin suppression of glucose production.

Glucagon-Like Peptide-1 (GLP-1) is an incretin hormone produced by intestinal L cells, enhancing insulin secretion in response to glucose and has beneficial effects on pancreatic β-cell function and survival. Impaired GLP-1 secretion and/or action contribute to β-cell dysfunction in T2D. GLP-1 receptor agonists are used therapeutically to enhance insulin secretion and improve glycemic control.

Hepatocyte Nuclear Factor 1α (HNF1α) is a transcription factor that regulates the expression of genes involved in glucose and lipid metabolism. It plays a crucial role in maintaining pancreatic β-cell function and glucose homeostasis. Variants in the HNF1α gene are associated with an increased risk of T2D while impaired HNF1α function contributes to β-cell dysfunction and impaired insulin secretion.

Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) is a nuclear receptor that regulates glucose and lipid metabolism. It is activated by fatty acids and plays a crucial role in adipocyte differentiation and glucose homeostasis. Its activation improves insulin sensitivity and glucose uptake in peripheral tissues and also leads to increased expression of genes involved in glucose and lipid metabolism, such as GLUT4, adiponectin, and lipoprotein lipase. The PPARγ agonists, such as thiazolidinediones (TZDs), are used

therapeutically to improve insulin sensitivity. However, TZDs can have adverse effects, such as weight gain and fluid retention.

AMP-Activated Protein Kinase (AMPK) is a kinase that regulates glucose and lipid metabolism. It gets activated by increased AMP:ATP ratios, which occur during energy-depleted states, such as exercise or calorie restriction. Its activation improves insulin sensitivity and glucose uptake in peripheral tissues and inhibits glucose production in the liver and improves fatty acid oxidation.

Sirtuin 1 (SIRT1) is a deacetylase that regulates glucose and lipid metabolism. It is activated by calorie restriction and plays a crucial role in maintaining metabolic homeostasis. Its activation leads to increased expression of genes involved in glucose and lipid metabolism, such as PGC-1α, and inhibits the expression of genes involved in inflammation and oxidative stress.

Nuclear Factor Kappa B (NF-κB) is a transcription factor that regulates inflammatory responses. It is activated by various stimuli, including cytokines, oxidative stress, and bacterial infections. When it gets activated, itresults in the elevation of expression of pro-inflammatory genes, like TNF-α, IL-1β, and IL-6, impairingnot only insulin signaling but also glucose metabolism.

ER STRESS AND GLUCOSE HOMEOSTASIS: A COMPLEX RELATIONSHIP

Endoplasmic reticulum (ER) stress and glucose homeostasis are intricately linked through multiple mechanisms. ER stress can disrupt glucose homeostasis by impairing insulin signaling, activating gluconeogenic pathways, and suppressing glycogen synthesis. Conversely, glucose metabolism can also influence ER stress.

Impairing Insulin Signaling- ER stress can activate JNK, which phosphorylates and inhibits IRS-1, leading to impaired insulin signaling (Ozcan, U., et al. 2004). It can also reduce Akt activation, which is necessary for insulin signaling (Hirosumi, J., et al. 2002).

Activating Gluconeogenic Pathways- ER stress can induce the expression of gluconeogenic genes. Phosphoenolpyruvate carboxykinase (PEPCK) and Glucose-6-phosphatase (G6Pase) are two vital enzymes whose expressions are induced by ER stress.

Suppressing Glycogen Synthesis- GSK3β activates, which further phosphorylates and inhibits glycogen synthase.

Influence of Glucose Metabolism on ER Stress- High glucose levels can induce ER stress by increasing the demand for protein folding and lipid synthesis thereby increasing the chances of misfolding. Also, glucose deprivation can induce ER stress by reducing the energy available for protein folding and lipid synthesis.

GENETIC SYNDROMES LINKED TO ER STRESS AND DIABETES

Wolfram Syndrome is a very infrequent autosomal recessive condition,which causes mutations in the WFS1 gene, whose function is to encode a transmembrane protein involved in ER calcium homeostasis. The WFS1 protein plays a crucial role in maintaining ER calcium homeostasis, and mutations in this gene lead to ER calcium depletion, triggering ER stress and pancreatic beta-cell dysfunction. It typically develops in childhood or adolescence. It shows progressive degeneration of the optic nerve, leading to blindness. It also causes sensorineural hearing loss, an impaired vasopressin secretion, leading to polyuria and polydipsia. Ataxia, seizures, and cognitive impairment are some other serious neurological abnormalities here.

Wolcott-Rallison Syndrome is a very infrequent autosomal recessive disorder, which causes mutations in the EIF2AK3 gene, whose function is to encode the PERK protein. The PERK protein plays a crucial role in the UPR, and mutations in this gene lead to impaired UPR signaling, exacerbating ER stress and pancreatic beta-cell dysfunction. It typically develops in infancy or early childhood. It results in impaired growth and development. It shows epilepsy, developmental delay, and cognitive impairment as different serious neurological abnormalities. It also causes the liver enzymes to be elevated

and also impairs kidney function.

Akita Mice (Izumi et al. 2003)- is a mouse model of diabetes caused by a mutation in the Ins2 gene, which encodes proinsulin. The mutation leads to the production of misfolded proinsulin, which accumulates in the ER and triggers ER stress. The Akita mouse model highlights the importance of proper protein folding in the ER and the consequences of ER stress on pancreatic beta-cell function. It typically develops in young adulthood. This model shows impaired insulin secretion and beta-cell apoptosis.

Permanent Neonatal Diabetes Mellitus (PNDM) is a rare form of diabetes caused by mutations in the KCNJ11 or ABCC8 genes, which encode subunits of the ATP-sensitive potassium channel. These mutations lead to ER stress and pancreatic beta-cell dysfunction. The KCNJ11 and ABCC8 genes play a crucial role in regulating pancreatic beta-cell function, and mutations in these genes lead to ER stress and impaired insulin secretion (De León, D. D., & Pinney, S. E. 2008). It is generally observed to develop within the first 6 months of life.

THERAPEUTIC APPROACHES TARGETING ER STRESS

Targeting ER stress has emerged as a potential therapeutic strategy for managing diabetes and offer promising avenues for disease management and prevention.

Chemical chaperones are small molecules that assist in the proper folding of proteins within the endoplasmic reticulum (ER). They help alleviate ER stress by stabilizing protein conformation, improving the ER's folding capacity, and facilitating the trafficking of proteins. This is particularly important in conditions like diabetes, where ER stress plays a significant role in disease progression. Chemical chaperones work through several mechanisms to reduce ER stress. They help in the proper folding of newly synthesized proteins, preventing the accumulation of misfolded proteins that can trigger ER stress. By reducing the accumulation of misfolded proteins, they can inhibit the activation of the UPR, which is a cellular response to ER stress. Chemical chaperones also have anti-apoptotic properties that protect cells from ER stress-induced apoptosis, which is particularly important

in preserving pancreatic β-cell function in diabetes. The therapeutic potential of chemical chaperones in diabetes is significant. By reducing ER stress, chemical chaperones can improve insulin sensitivity and glucose homeostasis. They help protect pancreatic β-cells from ER stress-induced apoptosis, preserving their function and insulin secretion. ER stress is associated with inflammation, and chemical chaperones can help reduce inflammatory responses, further improving metabolic health. 4-Phenylbutyric Acid (4-PBA) acts as a chemical chaperone by stabilizing protein folding and reducing the accumulation of misfolded proteins in the ER. It also inhibits the activation of the unfolded protein response (UPR), which is triggered by ER stress. Studies have shown that 4-PBA can reduce ER stress markers and improve insulin sensitivity in diabetic models. It has been found to decrease fasting blood sugar levels and improve insulin resistance (Amany Abdel-Ghaffar, et al. 2019) (Jeon JH, et al. 2022). Tauroursodeoxycholic Acid (TUDCA) is a bile acid derivative that helps in protein folding and reduces ER stress. It also has anti-apoptotic properties, which protect cells from ER stress-induced apoptosis. It has been shown to alleviate ER stress and improve glucose homeostasis in diabetic models. It has been found to reduce ER stress markers and improve insulin sensitivity (Amany Abdel-Ghaffar, et al. 2019) (Jeon JH, et al. 2022). Trimethylamine N-oxide (TMAO) is another chemical chaperone that assists in protein folding and reduces ER stress. However, it is less effective compared to 4-PBA and TUDCA. It has shown some potential in reducing ER stress and improving metabolic parameters, but its effects are not as pronounced as those of 4-PBA and TUDCA (Amany Abdel-Ghaffar, et al. 2019).

Small molecule inhibitors are those who target these UPR pathways and help mitigate ER stress and its associated cellular damage (Li A, et al 2020). By stabilizing protein folding and reducing the accumulation of misfolded proteins, these inhibitors help maintain ER homeostasis. And in cases where ER stress is severe, small molecule inhibitors can promote the apoptosis of stressed cells, thereby preventing the spread of damage. GSK2606414 and GSK2656157 are the inhibitors that target the PERK pathway by inhibiting the interaction

of PERK with ATP, thereby blocking the signaling cascade (Khanna, M., et al 2021). They have shown promise in reducing ER stress in experimental models. NCI 12487 is the small molecule inhibitor has been studied for its effectiveness in colorectal cancer models and may have potential applications in diabetes (Rozpedek-Kaminska W, et al 2022). It selectively targets cancer cells with induced ER stress conditions, promoting apoptosis and reducing cell viability. 4μ8C is the inhibitor which targets the IRE1 pathway by blocking its endoribonuclease activity, which is crucial for the splicing of XBP1 mRNA. By inhibiting this pathway, 4μ8C can reduce ER stress and its downstream effects. STF-083010 is another IRE1 inhibitor, STF-083010, also blocks the endoribonuclease activity of IRE1, thereby reducing ER stress and improving cellular homeostasis. Ceapin-A7 is a small molecule inhibitor that targets the ATF6 pathway by preventing the activation of ATF6, a transcription factor involved in the UPR. By inhibiting ATF6 activation, Ceapin-A7 can reduce ER stress and its associated cellular damage.

Gene therapy involves the use of genes to treat or prevent diseases. Gene therapy approaches targeting ER stress have shown promise in reducing ER stress and improving glucose metabolism in diabetes. X-box binding protein 1 (XBP1) is a transcription factor which is involved in the UPR pathway. Overexpression of XBP1 has been shown to reduce ER stress and improve glucose metabolism in mouse models of diabetes. Glucose-regulated protein 78 (GRP78) is a molecular chaperone involved in protein folding. Overexpression of GRP78 has been shown to reduce ER stress and improve glucose metabolism in mouse models of diabetes. Knockdown of pro-apoptotic genes such as CHOP (C/EBP homologous protein) can reduce cell death and improve cellular function (Oyadomari S, et al. 2002).

Anti-inflammatory agents are substances that reduce inflammation and oxidative stress in the body. Inflammation and oxidative stress are closely linked processes that contribute to the development and progression of various diseases, including diabetes. By targeting these processes, anti-inflammatory agents can help mitigate the harmful effects

of ER stress and improve overall metabolic health (Yu, X., Pu, H., & Voss, M. 2024), (Blagov AV, Summerhill VI, et al 2024), (Al-Khayri JM, et al 2022). Anti-inflammatory agents reduce the production of pro-inflammatory mediators by inhibiting enzymes like COX and lipoxygenase. decrease the production of inflammatory cytokines, such as interleukin-1 (IL-1), tumour necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6). Antioxidants neutralize ROS, reducing oxidative damage to cells and tissues. Some anti-inflammatory agents modulate the expression of genes involved in inflammation and oxidative stress, leading to a reduction in these processes. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) like aspirin, ibuprofen, and naproxen work by inhibiting the activity of cyclooxygenase (COX) enzymes, which are involved in the production of pro-inflammatory prostaglandins. By reducing the levels of these inflammatory mediators, NSAIDs help alleviate inflammation and oxidative stress. Corticosteroids are potent anti-inflammatory agents that work by suppressing the immune response and reducing the production of inflammatory cytokines. They also have antioxidant properties that help reduce oxidative stress. Prednisone, dexamethasone, and hydrocortisone are commonly used corticosteroids. Flavonoids are natural compounds found in fruits, vegetables, and other plant-based foods. They possess anti-inflammatory and antioxidant properties by modulating the expression and activity of various inflammatory enzymes and cytokines. Flavonoids also inhibit the production of reactive oxygen species (ROS), thereby reducing oxidative stress. Quercetin, genistein, apigenin, kaempferol, and epigallocatechin gallate (EGCG) are well-known flavonoids with anti-inflammatory effects. Omega-3 fatty acids, found in fish oil and certain plant oils, have anti-inflammatory properties by inhibiting the production of pro-inflammatory eicosanoids and cytokines. They also enhance the production of anti-inflammatory mediators, such as resolvins and protectins. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the primary omega-3 fatty acids with anti-inflammatory effects. Vitamins C and E are potent antioxidants that neutralize free radicals and reduce oxidative stress. By decreasing oxidative damage, these vitamins help alleviate inflammation and improve

cellular function. Ascorbic acid (vitamin C) and tocopherol (vitamin E) are commonly used antioxidant vitamins. Curcumin is a polyphenol with anti-inflammatory properties that has been shown to reduce inflammation and improve glucose metabolism in mouse models of diabetes.

Lifestyle interventions also play a very crucial role in managing and reducing ER stress, which is a key factor in the development and progression of diabetes. These interventions focus on improving overall metabolic health through regular physical activity and a balanced diet, thereby alleviating ER stress and its associated complications. Regular physical activity has been shown to have numerous benefits for metabolic health, including reducing ER stress. Exercise enhances the ability of cells to respond to insulin, which helps lower blood glucose levels. This is particularly important for individuals with type 2 diabetes. Physical activity reduces the levels of pro-inflammatory cytokines, which are often elevated in conditions of ER stress and diabetes. Exercise stimulates the production of new mitochondria and improves mitochondrial function, which can help reduce oxidative stress and improve cellular energy metabolism. Regular exercise helps in maintaining a healthy weight or achieving weight loss, which is associated with reduced ER stress and improved metabolic health. Exercise promotes autophagy, the process by which cells remove damaged components, including misfolded proteins that contribute to ER stress. There are varieties of effective exercises those help reduce ER stress like walking, jogging, cycling, and swimming improve cardiovascular health and overall metabolism. Weight lifting and resistance exercises enhance muscle mass and improve insulin sensitivity. Yoga and Pilates can improve overall physical fitness and reduce stress levels. Beside exercises, a balanced diet rich in nutrients can significantly impact ER stress and metabolic health. Antioxidant-rich foods help reduce oxidative stress, a major contributor to ER stress. Examples include fruits (berries, citrus fruits), vegetables (leafy greens, bell peppers), nuts, and seeds. Incorporating anti-inflammatory foods into the diet can help lower inflammation levels. These include fatty fish (salmon, mackerel), olive oil, nuts (almonds, walnuts), and spices

(turmeric, ginger). A diet rich in fibre can improve glycemic control and reduce insulin resistance. Whole grains, legumes, fruits, and vegetables are excellent sources of dietary fibre. Including sources of healthy fats, such as avocados, olive oil, and nuts, can improve lipid profiles and reduce inflammation. Ensuring a balanced intake of carbohydrates, proteins, and fats is essential for stable blood sugar levels and overall metabolic health. Reducing the consumption of processed and high-sugar foods can help prevent spikes in blood sugar and insulin levels, thereby reducing ER stress. There are even some specific dietary patterns which can be followed. Mediterranean Diet emphasizes fruits, vegetables, whole grains, nuts, seeds, and healthy fats (mainly from olive oil), with moderate consumption of fish and poultry. It is known for its anti-inflammatory and antioxidant properties. Dash Diet approaches to stop hypertension (DASH) diet focuses on reducing sodium intake and increasing the consumption of fruits, vegetables, whole grains, and lean proteins. It helps manage blood pressure and improve metabolic health. Low-Carbohydrate Diets limit the intake of carbohydrates and emphasize proteins and fats, can improve insulin sensitivity and promote weight loss.

FUTURE PROSPECTIVES

Ongoing research in the field of endoplasmic reticulum (ER) stress and diabetes is promising and aims to uncover novel therapeutic strategies. Several studies are currently exploring the potential of targeting ER stress pathways to prevent and treat diabetes(Yuan, S., et al. 2024).

- Biomarker Identification: Recent studies have identified several ER stress-related biomarker genes in Type 2 Diabetes (T2D). For instance, genes such as RTN1, CLGN, PCSK1, IAPP, ILF2, IMPA1, CCDC47, and PTGES3 have been implicated in protein folding, membrane composition, and metabolism regulation. These biomarkers could be used for early diagnosis and personalized treatment strategies (Yao, L., et al. 2024).
- Small Molecule Drugs: Research is underway to develop small molecule drugs targeting ER stress-related

genes. Drugs like D002994 and D001564 have shown potential in targeting these genes and alleviating ER stress in diabetic models (Yao, L., et al. 2024).

- Genetic and Epigenetic Therapies: Advances in genetic and epigenetic therapies are being explored to modulate ER stress responses (Yuan, S., et al. 2024). For example, miRNAs such as miR-197-5p, miR-320c, miR-1296-3P, and miR-6133 have been found to be dysregulated in diabetic samples, suggesting their potential as therapeutic targets (Yao, L., et al. 2024).

- Therapeutic Strategies for Diabetic Complications: ER stress is also being investigated as a therapeutic target for diabetic complications such as nephropathy. Maintaining ER homeostasis through the unfolded protein response (UPR) and ER-phagy is being considered as a potential strategy to mitigate diabetic nephropathy(Yang, M., et al. 2023). Autophagy is a cellular process that helps clear damaged proteins and organelles. Research is exploring ways to enhance autophagy to alleviate ER stress and improve cellular function in diabetes (Klionsky DJ, et al. 2016).

- Advanced Gene Editing Technologies: CRISPR-Cas9 technology offers the potential to precisely edit genes involved in ER stress pathways. This could be used to correct genetic mutations that contribute to diabetes or to enhance cellular defenses against ER stress (Hsu PD, et al. 2014).

- Stem Cell-Based Therapies: It show promise for regenerating damaged pancreatic beta cells and other tissues affected by diabetes. Research is investigating the role of ER stress in stem cell differentiation and function.

- Artificial Intelligence (AI) and Machine Learning:AI and machine learning are being used to accelerate the discovery and development of new drugs targeting ER stress pathways. These technologies can analyze vast amounts of data to identify promising therapeutic candidates.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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PESTICIDE RESIDUE ANALYSIS IN MARKETED GREEN CHILI'S: A COMPARATIVE STUDY WITH ORGANICALLY GROWN GREEN CHILIES

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ABSTRACT

The presence of pesticide residues in vegetables raises significant public health concerns, especially with rising demand for food safety. This study focuses on analysing pesticide residues in marketed green chilies, with a comparison to organically grown varieties purchased from various e-commerce platforms. We employed the QuEChERS extraction method to isolate pesticide residues, ensuring a reliable and efficient sample preparation process. For precise detection and quantification, we used advanced Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) and Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS) techniques, which allowed for a comprehensive analysis of both polar and nonpolar pesticide residues. Our findings reveal a notable presence of pesticide residues in the conventionally grown green chilies available in local markets. Surprisingly, some pesticide residues were also detected in samples marketed as organically grown chilies, raising concerns about the authenticity and purity of organic labelling standards. The data suggest potential contamination or inadequate control measures in the organic supply chain, especially when products are sourced from e-commerce platforms. This comparative analysis underscores the need for stringent regulatory standards and improved monitoring mechanisms for both conventional and organic produce. Our study contributes valuable insights to consumers, regulatory

bodies, and policymakers, highlighting the importance of transparent food safety practices in reducing potential pesticide exposure from both conventional and organically marketed green chilies.

Keywords: *Chromatography, GC-MS/MS, Green chili, LC-MS/MS, Pesticides, QuEChERS, Spectrometry, Vegetables.*

Introduction

Green chili (Capsicum annuum) is a crop of economic and nutritional significance. Its cultivation supports the livelihoods of millions of farmers worldwide, particularly in tropical and subtropical regions (Olatunji et al., 2018). Green chili is valued not only for its distinctive taste but also for its health benefits. One of the main suppliers of micronutrients, such as vitamins and minerals, which are required for human development and growth, are vegetables that are freshly picked (Godswill et al., 2020). We can get the Recommended Dietary Intake (RDI) of these vital nutrients by consist of vegetables in our daily meals (Olson et al., 1987). A major earnings product that is widely cultivated across India is chillis. According to the 2nd advance estimates, India's chili production estimate was 20.60 lakh tons for 2022-23 as against 18.36 lakh tons in 2021-22. Along with contributing to its gastronomic value, chili offers significant potential for therapeutic uses due to its abundance of beneficial phytochemicals, such as vitamins, minerals, flavonoids, carotenoids, and capsaicin (Duranova et al., 2022). These compounds also give chili analgesic, antioxidant, antibacterial, anti-cancer, and anti-obesity properties (Batiha et al., 2020).

Pesticides are chemical substances used to prevent, destroy, or control pests that harm crops (Yadav et al., 2017). They play a crucial role in modern agriculture by reducing losses caused by insects, fungi, and weeds, thereby increasing productivity (Manosathiyadevan et al., 2017). However, the indiscriminate use of pesticides raises concerns about food safety and environmental sustainability (Abrol et al., 2014). Pesticide residues can persist on fruits and vegetables, entering the human body through consumption and causing various health problems (Syed et al., 2014), ranging from acute

poisoning to chronic illnesses like cancer and neurological disorders.

Chili is often cultivated with neonicotinoid pesticides, carbamate insecticides, and organophosphorus herbicides (GURU et al., 2018). In 2020, India used over 61,000 tons of pesticides for agricultural purposes. This is relatively low compared to other countries, such as China, Argentina, and Brazil (Sharma et al., 2022). While pesticide is used correctly, they provide enough defense against herbicides, diseases, and insects. On the other hand, improper application could leave residues on food and agricultural commodities. For both humans and animals that consume these products, such residue may have significant chronic and long-term adverse effects (Boobis et al., 2017). Highly hazardous pesticides (HHPs) may cause harmful impacts on the liver, kidneys, blood, lungs, immune system, and digestive system after short-term exposure (Cestonaro et al., 2022). Pesticides containing dithiocarbamates and its metabolites (ethylene thiourea, carbon disulfide) may trigger malignancy, Parkinson's disease, hypersensitivity, allergic dermatitis, brain damage, reduced brain enzyme activity, and lymphocyte sensitivity (Cocco et al., 2022). HPPs promote cancer, especially in young children who continue to grow, and pregnant or nursing women, infants and children, individuals with weak immune systems, and people who suffer from malnutrition can suffer more severe health effects from these insecticides (Food and Agriculture Organization (FAO) & World Health Organization (WHO), 2016).

Most of the consumer exposure to pesticides comes through residues on food items that are consumed. High-risk pesticides are banned, and Maximum Residue Limits (MRL) have been determined for low-risk pesticide to protect people from the harmful effects of these (Carrère et al., 2018).

1. Materials & Methods

1.1 Location and Sampling

In this study, pesticide residues in green chili samples collected from local markets of Kolkata, India (Table 1) and the Organically grown green chili samples were purchased

from various E-commers platforms. Almost 500g of green chili samples was labelled, put into different bags, and transferred in packages to the Regional Food Laboratory (National Food Laboratory), where pesticide analyses were performed out the following day.

Table 1.Collected marketed chili samples and their location

Sample No.	Market Locations of collected Chili samples
JKP_ 1	Behala, south kolkata
JKP_2	Kalighat, south kolkata
JKP_3	New Town, North kolkata
JKP_4	Thakurpukur, south kolkata
JKP_5	Shyambazar, Kolkata
JKP_6	Garia, south kolkata
JKP_7	Bansdroni, kolkata
JKP_8	Baranagar, North kolkata

The samples of organically grown chilies **JKP_9, JKP_10, JKP_11,** and **JKP_12** were purchased from multiple E-commerce platforms.

1.2 Reagent and Chemicals

All pesticides standards would be obtained from authentic and trusted sources like Agilent, Merck, that can provide a certificate of analysis as proof of traceability. Methanol- LCMS grade, Water- Milli Q, Acetonitrile- LCMS grade, Anhydro sodium Sulfate- AR grade, Anhydro sodium Sulfate- AR grade, Acetic acid, glacial- LCMS grade, Ammonium-formate LCMS grade, Primary secondary amine (PSA) & Graphitized Carbon Blank (GCB) from United Chemical Technologies or Equivalent, Agilent Sample QuEChERS EN extraction kit.

1.3 Preparation of Mobile Phase

Prepare 5m Mammonium formate inwater by dissolving 0.317 gammonium formate in 1000m LHPLC water. Adjust with 0.1% formic acid (PhaseA). Prepare 5mM ammonium formate in methanol by dissolving 0.317 g ammonium formate in 1000 mL methanol. Adjust with 0.1% formic acid (Phase B).

1.4 Preparation of Sample

To make sure that no contamination happened throughout processing, collect and examine examples free of pesticides. Before reporting, reduce any found pesticide levels from the values obtained. To ensure the accuracy of the method of extraction, use a blank sample that has been treated with using known pesticide amounts. For batch recovery identification, prepare a single QC sample for each batch. About 250gm green chili sample homogenized with blender. Weigh 10.00 ± 0.10 g of homogenized sample into a 50 mL polypropylene centrifuge tube. Incubate for ten minutes at 4°C after introducing 10 mL of acidified acetonitrile (0.1% acetic acid) and vortex for 1 minute. Upon pouring the QuEChERS EN extraction salt (6.5 g Magnesium Sulfate + 1 g Sodium Acetate) and vortexing for one minute, refrigerate for ten minutes at 4°C (Lawa et al., 2018). Centrifuge at approximately 6500 rpm for 10 minutes. Transfer 2 mL of extract to a clean tube containing 50 mg PSA and 150 mg $MgSO_4$ (Ramalepe et al., 2022). Vortex for 30 seconds and centrifuge at 10,000 rpm for 10 minutes at 4°C. For LC-MS/MS, filter the supernatant through a 0.2 µm PTFE syringe filter and for GC-MS/MS, filter 1000 µL extract through a 0.2 µm PTFE syringe filter. Inject 10µLfromtheextractintotheLC-MS/MSusingSOPforthe respectiveLC-MS/MS system.

1.5 PreparationofStandards

Apply pesticide standards from trusted suppliers with appropriate credentials, (such as Sigma-Aldrich, Agilent, and Merck.). Dilute about 10 mg of pesticide in 10 mL of diluent (ethyl acetate for GC-MS/MS; acetonitrile for LC-MS/MS) for Stock Solutions. Keep refrigerate at -20 °C. A reference standard mixture with a minimum of five different concentrations (such as 0.002–0.1 µg/mL) that is appropriate

with the mobile phase needs to be prepared. To make sure that the instrument is acceptable, inject a mid-range standard every day. Each day, new standards need to be introduced.

2.5.1 Matrix-MatchedStandardsforLC-MS/MS

Spikepesticidestandardsintoresidue-free sample extracts to match the calibration range, e.g., 0.001–0.02 µg/mL.

2.5.2 ExternalCalibrationStandards for GC-MS/MS

Prepareaworkingrange(e.g., 0.005– 0.1µg/mL)inethylacetate. Useatleastfivelevelsofconcentrationandpreparefreshly before use.

2.5.3 Matrix-Matched Standards for GC-MS/MS

Spike standards post-extraction into residue-free sample extracts to create calibration standards (e.g., 0.005–0.1 µg/mL).

1.6 Chromatographic Condition

Samples were analysed using LC–MS/MS and GC–MS/MS devices. LC–MS/MS analyses were performed using liquid chromatography (PerkinElmer LX-50 UHPLC) coupled with mass spectrometry. The column used was Kinetex C18 (2.6 µm, 100 Å, 150 × 4.6 mm). The mobile phase flow rate was 0.5 mL/min (Table 2). The column temperature was maintained at 45°C, and the injection volume was 10 µL. The ionization mode was ESI (positive) with a drying gas flow of 120. The HSID temperature was 300°C, and the nebulizer gas ranged from 1–300. The electro spray voltage (V1 positive) was set at 5500 V, and the source temperature was 320°C.

GC–MS/MS analyses were performed using gas chromatography (Agilent 7000D GC/MS Triple Quadrupole) coupled with mass spectrometry (7000D GC/MS Triple Quadrupole). The autosampler used was the Agilent 7693A. The column used was a DB-5MS capillary column (30 m × 0.25 mm × 0.25 µm). The injection type was MMI-SSPL with an injection volume of 2 µL. The injection temperature was maintained at 280°C (Table 3), and the column flow rate was set at 1.2 mL/min. The ion source and transfer line temperatures were both maintained at 280°C.The LC-MS/MS and GC-MS/

MS instruments have been employed for analysing a number pesticide forms to determine their LOQ Value (Table 4).

Table 2.The Gradient Mobile phase flow program

Time	Flow	%A	%B	Shape
0	0.5	80	20	Linear
Time	**Flow**	**%A**	**%B**	**Shape**
0.5	0.5	80	20	Linear
1	0.5	50	50	Linear
4	0.5	40	60	Linear
15	0.5	10	90	Linear
17	0.5	2	98	Linear
20	0.5	80	20	Linear
22	0.5	80	20	Linear

Table 3. Oven temperature program

Rate(°C/min)	Target(°C)	Hold time(min)	Runtime(min)
Initial	**90**	**1**	**1**
35	**130**	**0**	**2.14**
10	**240**	**1**	**14.14**
15	**300**	**3**	**21.14**
Totalruntime	**21.14**		

Table 4.The LOQ readings of the analytes undergoing analysis

LOQ (mg/Kg)	Analytes
5	**Carbendazim**
0.1	**Acetamiprid**

0.01	**Methomyl, Dinotefuran, Carbofuran, Paclobutrazole,Tridemorph, Phosphamidon, Imazamox, Buprofezin, Fenazaquin, Fenamidone, ButachlorHexaconazole, Kresoxim-Methyl, Flusilazole, Cyazofamid, Pencycuron,Epoxiconazole,Fenarimol, Bitertanol, Propiconazole,Boscalid,Chlorothalonil,Dicofol, Hexythiazox,Thiobencarb, Etoxazole, Flufenacet, Picoxystrobin,Tetraconazole, Spirotetramat, Quizalofop-P-EthylFluxapyroxad, Tolfenpyrad, Pyraclostrobin,Dimethomorph, Famoxadone, Chromafenozide,Pinoxaden, Sulfentrazone, Azoxystrobin, Difenoconazole, Metrafenone, FenpyroximatePropquizafop,Pyridalyl, Novaluron, Metaflumizone,Lufenuron,Chlorfluazuron, Spinosyn A, Spinosyn D,Emamectin Benzoate, Fipronil, Flubendiamide**
0.5	**Chlorpyrifos**
0.05	**DDT-o,p; DDE-p,p**
0.01	**Ethofenprox, Lamda Cyhalothrin, Atrazine, Endosulfan, Chlorpropham,**

2. Result & Discussion

By measuring multiple variables such as linearity, limit of quantification (LOQ), accuracy (in terms of recovery), and precision (in terms of repetition), the recommended method was evaluated. Every validating parameter that had been evaluated conformed with international guidelines for chromatographic analysis of pesticide residues (Colazzo et al., 2018). Resultant outcomes (Table 5 and Table 6) have been assessed utilizing the European SANTE Guideline's validation guidelines and standards (SANTE, 2019).

Table 5.The result data of LC-MS/MS instrument

Sample Name	**Detectable Analytes name**	**Detectable conc. (ppm)**	**MRL (ppm) As per FSSAI**	**Remarks**
JKP_1	Acetamiprid	0.02	0.01	> limit
	Imidaclorprid	0.02	0.01	> limit

	Metalaxyl	0.1	0.01	> limit
JKP_3	Imidaclorprid	0.01	0.01	= limit
	Metalaxyl	0.1	0.01	> limit
	Carbendazim*	0.01	0.01	= limit
	Fipronil	0.01	0.01	= limit
JKP_4	Dinotefuran	0.01	0.01	= limit
	Acetamiprid	0.01	0.01	= limit
	Imidaclorprid	0.02	0.1	> limit
	Tolfenopyrad	0.01	0.01	= limit
	Azoxystrobin	0.01	0.01	= limit
	Difenoconazole	0.01	0.01	= limit
JKP_5	Myclobutanyil	0.02	0.01	> limit
	Hexythiazox	0.02	0.01	> limit
	Lufenuron	0.01	0.01	= limit
JKP_6				
	Dinotefuran	0.10	0.01	> limit
	Imidaclorprid	0.2	0.01	> limit
	Metalaxyl	0.1	0.01	> limit
	Myclobutanyil	0.1	0.01	> limit
	Hexythiazox	0.01	0.01	= limit
	Fipronil	0.04	0.01	> limit
JKP_7	Dinotefuran	0.01	0.01	= limit
	Imidaclorprid	0.01	0.01	= limit
	Myclobutanyil	0.01	0.01	= limit
JKP_8	Imidaclorprid	0.01	0.01	= limit
JKP_9	Imidaclorprid	0.04	0.01	> limit
	Propiconazone	0.02	0.01	> limit
	Picoxystrobin	0.01	0.01	= limit
	Tolfenpyrad	0.05	0.01	> limit
	Pyraclostrobin	0.05	0.01	> limit
	Difenoconazole	0.01	0.01	= limit
	Novaluron	0.1	0.01	> limit

	Fipronil	0.01	0.01	= limit
JKP_10	Imidaclorprid	0.08	0.01	> limit
	Metalaxyl	0.07	0.01	> limit
	Fenazaquin	0.03	0.01	> limit
	Hexythiazox	0.02	0.01	> limit
	Picoxystrobin	0.02	0.01	> limit
	Fluxapyroxad	0.01	0.01	= limit
	Tolfenpyrad	0.1	0.01	> limit
	Pyraclostrobin	0.02	0.01	> limit
	Azoxystrobin	0.1	0.01	> limit
	Difenconazole	0.2	0.01	> limit
	Fipronil	0.2	0.01	> limit
JKP_11				
	Carbofuran**	0.04	0.01	> limit
	Imidaclorprid	0.2	0.01	> limit
	Metalaxyl	0.02	0.01	> limit
	Picoxystrobin	0.02	0.01	> limit
	Fluxapyroxad	0.01	0.01	= limit
	Tolfenpyrad	0.02	0.01	> limit
	Pyraclostrobin	0.02	0.01	> limit
	Azoxystrobin	0.02	0.01	> limit
	Difenconazole	0.1	0.01	> limit
	Fipronil	0.2	0.01	> limit
JKP_12	Dinotefuran	0.1	0.01	> limit
	Imidaclorprid	0.02	0.01	> limit
	Metalaxyl	0.01	0.01	= limit
	Fluxapyroxad	0.1	0.01	> limit
	Tolfenpyrad	0.5	0.01	> limit
	Picoxystrobin	0.3	0.1	> limit
	Azoxystrobin	0.02	0.01	> limit
	Difenconazole	0.1	0.01	> limit
	Fipronil	0.03	0.01	> limit

*As per2.3.1. Restriction on the use of insecticides,Food Safety and Standards (Contaminants, Toxins and Residues) Regulation

**Marked pesticides are banned notification No.2-15015/30/2010 dated the 1st August, 2011.

Table 6.The result data of GC-MS/MS instrument

Sl No.	Detectable Analytes name	Detectable conc. (ppm)	MRL (ppm) As per FSSAI	Remarks
JKP_1	*Lamda Cyhalothrin	0.005	0.01	< Limit
JKP_2	Ethofenprox	0.4	0.01	> Limit
JKP_5	**DDT-o,p′	0.2	0.05	> limit
	Chlorpyrifos	0.01	0.5	< limit
	*Lamda Cyhalothrin	0.04	0.01	> limit
	Atrazine	0.01	0.01	= limit
JKP_ 10	*Lamda Cyhalothrin	0.01	0.01	= limit
	Endosulfan I	0.02	0.01	> limit
	**DDE-p,p	0.02	0.05	< limit
	Chlorpyrifos	0.01	0.5	< limit
	*Lamda Cyhalothin	0.01	0.01	= limit
JKP_11				
	Chlorpyrifos	0.01	0.5	< limit
	**DDE-p,p	0.02	0.05	< limit
	*Lamda Cyhalothrin	0.02	0.01	> limit
	Chlorpropham	0.01	0.01	= limit
JKP_12	**DDE-p,p	0.01	0.05	< limit
	*Lamda Cyhalothrin	0.02	0.01	< limit

*As per2.3.1. Restriction on the use of insecticides,Food Safety and Standards (Contaminants, Toxins and Residues) Regulation

**Marked pesticides are banned notification No.2-15015/30/2010 dated the 1st August, 2011.

The results of this study indicate that specific pesticide residues, especially in samples JKP_1, JKP_2, JKP_6, JKP_9, JKP_10, and JKP_11, above the Maximum Residue Limit (MRL) specified by the FSSAI. Concerns regarding safety have been caused by the identification of several banned chemicals, such as carbendazim and chemicals linked to DDT. When various contaminants are found in one sample, it could suggest that there are contamination concerns.To ensure conformity to food safety laws, more research and corrections might be needed.

This study highlights a critical public health concern regarding the safety of both conventionally marketed and "organically grown" green chillis available on various ecommerce platforms. Contrary to consumer expectations, significant levels of pesticide residues were detected in both categories of chillis. This finding raises questions about the authenticity of "organic" labels and the effectiveness of current monitoring and regulatory frameworks. This study underscores the urgent need for stricter enforcement of pesticide residue limits under the Food Safety and Standards Authority of India (FSSAI) regulations.

Conclusion

The FSSAI, along with the Ministry of Health and Family Welfare, plays a vital role in ensuring food safety and protecting consumer health. Enhanced surveillance, periodic testing of both conventional and organic food products, and stricter certification processes for organic produce are essential measures. Consumer awareness and transparency in the food supply chain must also be prioritized. The findings of this study call for collaborative action from regulatory authorities, policymakers, food producers, and e-commerce platforms to ensure the integrity of food labelling claims and the safety of food consumed by the public.

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DIFFERENT DRUG ACTIVITY AND MECHANISMS USED IN NEURODEGENERATIVE DISEASES

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ABSTRACT

Neurodegenerative diseases represent a spectrum of disorders that are characterized by the progressive destruction of cells and connections that make up the nervous system. These circumstances are hindering the existence of millions of individuals around the world, at last prompting the breakdown of the brain networks that are expected for human capability. The primary risk factor for the majority of primary neurodegenerative diseases is aging. In fact, it has been long believed that age-related changes in the brain make people more susceptible to neurodegeneration. Unfortunately, research for effective treatments is compromised due to lack of knowledge on the pathologic processes comprehensive these diseases and of dependable biomarkers. Clinical trials are challenging because they necessitate huge populations that must be monitored over extended periods of time, to record potential impacts on the course of the disease. These challenges lead to frequent failures and the waste of financial and human resources. New therapies can come from three main sources: synthesis, natural products, and existing drugs. This last source is known as drug repurposing, which is the most advantageous, since the drug's pharmacokinetic and pharmacodynamics profiles are already established, like cholinesterase inhibitors, NMDA receptor antagonists, and other medications, and the investment put into this strategy is not as significant as for the classic development of new drugs. Alternative study designs can be taken into consideration to

find disease modifiers and lower the costs of clinical studies, as research in this field must continue until a thorough understanding of fundamental pathologic processes is attained.

Keywords: *Alternative trials, Disease modification, Drugs and its MOA, Neurodegenerative Diseases, Symptomatic treatment, Therapeutic approaches.*

Introduction

*As people age, the prevalence of neurodegenerative disorders i*ncluding Parkinson's disease (PD) and Alzheimer's dementia (AD) rises. As a result, they will soon place a heavy load on aging populations similar to our own. The majority of neurodegenerative illnesses have unknown aetiologies and pathophysiologies, making them a scientific challenge. It is challenging but vital to create efficient remedies for illnesses whose causes are currently unknown. Neurodegenerative disease clinical trials necessitate a large number of patients to be monitored for an extended period of time, which makes the research extremely challenging to conduct and drives up expenses. While awaitingthe elucidation of the processes underlying neurodegeneration, alternative study designs may be beneficial for identifying disease-modifying treatments.Current therapeutic approaches for the treatment of neurodegenerative diseases merely offer limited, and even worse, transient symptomatic benefits to patients, with no mitigation of the insidious loss of neuronal cells encountered in these conditions. Neurodegenerative diseases have a multifactorial pathoetiological origin, and it is not surprising that conventional drug-discovery approaches that embrace a 'one gene, one drug, one disease' philosophy may not offer the best pathway towards the development of much needed disease-modifying therapeutics for these diseases. Drugs that target only one protein are susceptible to resistance, one reason (among several others) being that even a single mutation in the target active site often substantially reduces compound binding affinity and, hence, efficacy. On the other hand, resistance to drugs that target multiple proteins would require the unlikely event of concurrent mutations appearing

in multiple protein targets. In view of these concerns, many clinicians and basic scientists have becomepersuaded that a strategy aimed at the simultaneous targeting of multiple proteins (and therefore etiologies) involved in the development of a disease should be more beneficial than the currently accepted 'silver bullet' approach. This approach may be useful in designing drug treatments for a range of diseases. Diseases of the CNS for which such an approach has been suggested include, among others, movement disorders, cognitive deficit disorders, negative symptoms in schizophrenia, Lewy body disease and depressive illness.

Ageing And Neurodegenerative Diseases

Life expectancy has increased during the past century due to improvements in healthcare and the development of better surroundings (Niccoli & Partridge, 2012). Since the primary risk factor for neurodegenerative illnesses is advanced age (Hou et al., 2019), the number of patients suffering from age-related primary neurodegenerative diseases, such as Alzheimer's disease (AD) (Hebert et al., 2010), Parkinson's disease (PD) (Poewe et al., 2017), or amyotrophic lateral sclerosis (ALS) (Talbott et al., 2016), rises sharply with any increase in the elderly population. In light of these recent shifts in the population, because primary neurodegenerative illnesses are expensive and significantly impact the quality of healthcare, they will have significant socioeconomic ramifications for healthcare systems. Life of those impacted and those who provide care, presenting a serious social emergency. It will take a more focused research strategy and innovative solutions to address these significant societal responsibilities (DiLuca & Olsen, 2014).

It makes natural that current medications are being evaluated for NDs given the increasing need for therapy and the potential benefits of drug repurposing. Drugs that have been repurposed for the most researched neurodegenerative diseases—Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS)—are included in this study (Figure 1).

The topic of drug repurposing with examples in NDs has been reviewed a few times, but no comprehensive review has been done for all of these diseases, which would provide a wider perspective and a better understanding of how these diseases might be related to one another.

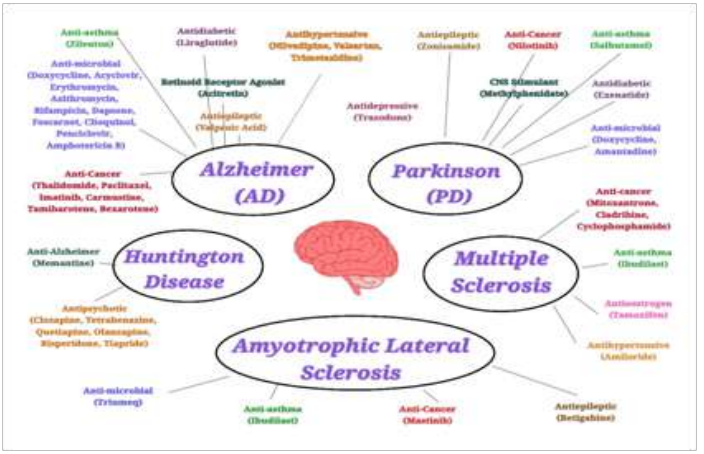

Fig. 1. Summary of the diseases and repurposed drugs presented in this review

Alzheimer's Disease

One of the most common NDs, AD accounts for 80% of dementia cases in the elderly. Progressive memory loss, learning difficulties, and a deterioration in behaviour and function are some of its signs. Although the exact origin of AD is still unknown, it is thought to be related to the build-up of amyloid plaques in the brain, which eventually causes the loss of neurons andsynapses.

Since there is currently no known cure for AD, the medications used to treat it are primarily intended to address cognitive problems or other symptoms, and they work best when taken early. Interesting enough, galantamine is one medication sold to treat AD.

Parkinson's Disease

Affecting people all around the world, PD is the second most prevalent ND after AD. Although other neurotransmitter systems also seem to be impacted, the loss of dopaminergic neurons in the substantia nigra is believed to be the primary cause of Parkinson's disease's most well-known motor symptoms. It is increasingly recognized that Parkinson's disease (PD) includes a number of symptoms that are not related to motor function, including depression, sleep disturbances, and cognitive impairment. Deep brain stimulation and dopamine replacement are two effective treatments for Parkinson's disease (PD), despite the fact that there is currently no cure for the condition.

Amantadine is another medication in the antiparkinsonian arsenal that was repurposed, much like AD. Amantadine, a mild glutamate receptor antagonist that increases dopamine and blocks its reuptake, was actually initially created to treat influenza before being used to treat Parkinson's disease.

Huntington's Disease

HD is the most prevalent monogenical neurological illness in the developed world and is autosomal dominant. Dementia, behavioural and mental impairments, and involuntary choreatic motions are its hallmarks. It is caused by a genetic mutation that results in a mutant form of the multifunctional protein huntingtin. This form of the protein is toxic and causes malfunction and neuronal death. When HD first appears in adulthood, the symptoms worsen over time and eventually cause death within a few years. The only way to treat this illness is to manage its symptoms as there is currently no recognized cure.

Tetrabenazine, which functions as a mild blocker of D2 dopamine postsynaptic neurons and a high-affinity, reversible inhibitor of monoamine uptake of presynaptic neurons, was initially created as part of research to create simple compounds with reserpine-like antipsychotic action.

Multiple Sclerosis

MS is a central nervous system autoimmune disease. It is a

long-term, inflammatory disease in which there is variable destruction of the myelin and axons. It starts with treatable neurological impairments and progresses over time, but its course is uncertain. Although MS has no known cure, there are now approved treatments to lessen its symptoms and slow its progression.

Numerous anticancer medications have been modified to treat multiple sclerosis and its associated symptoms. An example of this is the synthetic chemical mitoxantrone, an anthracenedione that has been utilized as a wide-spectrum anticancer drug to treat acute leukemia, lymphoma, and breast and prostate cancer.

Amyotrophic Lateral Sclerosis

Death of the upper and lower motor neurons that govern voluntary muscles is a hallmark of ALS. It causes muscular atrophy, a condition in which muscles progressively weaken and shrink. Additional symptoms include breathing, swallowing, and speech difficulties, as well as twitching and stiffness in the muscles. About 10% of ALS cases are genetically inherited, with the majority of cases having an unknown aetiology.

Only two medications, riluzole and edaravone, are now available to slow the progression of ALS, however they cannot reverse symptoms once they have appeared. Nevertheless, some medications are currently being researched for their potential use in treating ALS.

Dog cancer is treated with the tyrosine kinase inhibitor mastitinib. Tyrosine kinase inhibitors may be effective against ALS because of the potential sensitivity of aberrant glial cells that proliferate in the disease. In the same rat model, mastinib was shown to enhance survival and decrease glial cell activation.

Table1

Summary of the the rapeutic approaches developed to target the biological path ways contributing to the path ogenesis of primary neurode generative disorders.

Disease	Target mechanism	Drug	Mechanisms of action
Alzheimer Disease (AD), ageing	Myelin	Clemastine fumarate	Binds to muscarinic receptor and activates OPC differentiation and myelination
Ageing		Metformin	AMPK inhibitor, restores OPC differentiation and remyelination
Ageing		LY294002	Modulator of PI3K–Akt–mTOR signalling modulator
Alzheimer Disease (AD)	Synaptic dysfunction	Levetiracetam	Inhibits calcium release intraneuronal deposits Reverses synaptic dysfunction
AD		Bryostatin 1	Activates PKC and regulates neurogenesis, axonal transport and synaptic plasticity
AD, ASL		Masitinib	Tyrosine kinase inhibitor, Mast cell inhibition, reduces secretion of toxic mediators for synapses
ASL		Riluzole	Inhibits glutamate release Up-regulation of glutamate transporter
ASL		Ceftriaxone	Blocks ADAM10 endocytosis, upregulating ADAM10 activity
AD		Cell-permeable peptides targeting ADAM10 trafficking	Inhibition of SAP97-mediated ADAM10 trafficking to synapse, decrease in ADAM10 activity
HD			

AD AD	Gene expression: Transcriptional regulation and epigenetic mechanisms	Memantine Tubastatin ACY-1215 MPT0G211 5-Aroylindoles	Contrasts NF-κB pro-inflammatory activity HDAC inhibitors: • Facilitate gene expression through chromatin remodelling • Restore memory function
Ageing, AD, ALS AD Ageing, AD, PD, HD AD	Autophagy	Rapamycin AUTEN-67 Metformin AUTOTAC	Blocks mTORC1 — Removing autophagy inhibition mTOR-dependent modulation of autophagy Activates AMPK promoting autophagy and blocking mTORC1 Removes protein aggregates using autophagy
AD PD PD, AD, ALS, HD AD AD AD, PD	Metabolic failure	MitoQ CoQ10 MitoVItE MitoTEMPOL Resveratrol Mdivi-1 Shikonin MCU inhibitors	Antioxidants: • Limit mitochondrial ROS production Antioxidant indirectly activating PGC-1α Dpr1 inhibitor limiting mitochondrial division PKM2 modulator, an apoptotic break Limit mitochondrial calcium uptake
ALS AD, PD HD AD, HD, ALS, PD	CNS inflammation	Siponimod Fingolimod Natalizumab Minocycline	Limit immune cell infiltration in the CNS Anti-inflammatory (controversial results between pre-clinical models and clinical trials)

Treatment Of Neurodegenerative Diseases: An Unmet Need:

Neurodegenerative diseases are characterized by progressive neural loss, resulting in various clinical entities. These range from common conditions, such as Alzheimer's disease and Parkinson's disease, to less frequent disorders, including amyotrophic lateral sclerosis (ALS), progressive spinal muscular atrophies, and hereditary sensory-motor neuropathies. Despite many years of research and advancements in basic and clinical research, aetiology and pathogenesis of most of those conditions are still obscure. Dementia of Alzheimer type and Parkinson's disease are the most represented within the group in terms of prevalence and socio-economic burden. This is especially true for high-income countries with longer life expectancy, since PD and AD incidence increases with aging. Epidemiologic studies show that PD has an estimated prevalence between 65.6 (age under 65 years old) and 12 500 (age above 85 years old) per 100 000 and an estimated incidence between 5 (age under 65 years old) and 346 (age above 85 years old) per 100 000/year in Europe and AD has an estimated incidence of ~ 300 to 5600 per 100 000/year in USA, significantly increasing with age groups (from 65-69 to 90+ year). Similar figures are found in Europe for AD with incidence of 240 to 7020 per 100 000/year. AD and PD are very highly disabling conditions (AD is listed among the top 20 leading disabling conditions worldwide by WHO) for the affected subjects but also have a strong impact on their caregivers and to the local health system. Parkinson's disease and AD can therefore be considered as paradigmatic of all neurodegenerative diseases.

Developing a medicinal product able to cure neurodegenerative diseases is such a huge commitment that long time and hard work are still required for its achievement. There are many open questions that need to be answered, such as identification of plausible drug candidates, of reliable animal models with good predictive value to establish neuroprotection, identification of sensitive clinical endpoints and measurement tools, validation of biomarkers and design and execution of trials. Discussing all those issues is out of

the scope of this paper. Therefore, only some aspects will be addressed thereafter regarding the requirements of an effective treatment and on clinical trial designs.

And because the illness that bears his name was first reported by Aloysius Alzheimer in the early 1900s, who first described its histopathology. However, no remedy has been discovered as of yet for various ailments as well as for every neurodegenerative illness that still exists. In fact, there are now only symptomatic treatments—that is, therapies that can reduce the disease's symptoms without halting or delaying its progression. Drugs used to treat Parkinson's disease symptoms have an impact on the disease's primary motor characteristics by reducing or normalizing tremor, bradikinesia (slowing of movements), and stiffness. Good efficacy is achieved in the initial months or years, but as time goes on, the effects of the medication diminish and nearly always, long-term treatment issues arise, making the patients' quality of life extremely poor. Memory and other cognitive abilities can be somewhat restored by symptomatic therapy of mild or severe AD. Regretfully, the impact is fleeting and has a negligible effect size. Because no specific medicines have been approved for the other neurodegenerative illnesses yet, they are all undertreated. ALS is an example, as it has been shown that the anticonvulsant riluzole "extends life or the time to mechanical breathing for individuals suffering from amyotrophic lateral sclerosis. Riluzole prolongs the survival of ALS patients, according to clinical trials. For riluzole 100 mg/day and placebo, the median survival time was 13.5 months and 16.5 months, respectively. The clinical importance of this discovery is not striking, despite the fact that a 3-month survival difference was deemed statistically significant in this quickly evolving disease.

Another limitation of symptomatic drugs is that they frequently only address one symptom of illnesses that frequently exhibit several symptoms, which are caused by the degeneration of various neurotransmitter systems a variety of central nervous system regions. For example, not just the dopamine producing cells of the substantial Ingra pair's compacts die in Parkinson's disease (PD), for which only dopaminergic

drugs are available. Numerous symptoms could be caused by the death of other cells and neurotransmitter systems that dopamine replacement treatment cannot address.

Due to their complexity, neurodegenerative illnesses are extremely challenging to treat, and it is evident that symptomatic care alone is insufficient. Consequently, the objective would be a disease-modifying medication that could significantly slow—or even stop—the progression of the illness. It should be shown that a disease modifying technique has a lasting impact on the underlying illness process. A system that can stop pathological neuronal loss, or neuroprotection, is a component of the disease-modifying process rather than a replacement for it. A disease modification should indicate long-term alterations in the pathogenic process rather than transient effects like those associated with symptomatic medications.

Therapeutic approaches to modify gene expression in neurodegenerative disorders

Since targeting transcriptional regulators would cause a broad modulation of numerous impacted downstream processes, it may be advantageous in the fight against neurodegenerative diseases. This feature, however, also poses a possible warning since altering the transcription of multiple genes may be harmful because not all of them are involved in the pathogenesis. However, attempts have been made to investigate, develop, and test therapeutic strategies with the goal of altering mechanisms linked to transcription. Because there is so much evidence that CREB's functionality or expression is reduced in many diseases, it is one of the most sought-after targets in the therapy of neurodegenerative disorders. However, there are currently no medications that specifically boost CREB-dependent signaling. To restore its expression levels, a variety of tactics could be used, such as utilizing molecular biology or genetic techniques, or influencing its upstream signaling regulators. Because many neurodegenerative disorders are complicated and multivariate, medication research is likely to focus on targets other than the conventional actors linked to a particular disorder. In the treatment of AD, NF-κB is an example of

these kinds of targets. Memantine, an FDA-approved therapy for AD, appears to inhibit NF-κB's pro-inflammatory effects. Other anti-inflammatory medications are being evaluated in preclinical or clinical settings for their ability to disrupt NF-κB (see to Sun et al., 2022).

A lot of work has gone into investigating HDAC inhibitors as potential treatments for neurodegeneration. By making the chromatin more conducive to transcription, HDAC inhibitors may widely promote gene expression by allowing for higher expression of maybe advantageous genes. Amyloid clearance is one instance of an AD-relevant mechanism that may be favorably controlled through HDAC regulation. In fact, amyloid clearance was enhanced in humanized cultured astrocytes or several animal AD models by pharmacological inhibition or genetic targeting of HDACs (Prasad & Rao, 2018; Su et al., 2021). Additionally, non-selective HDAC inhibitors effectively corrected neuronal structural abnormalities and memory performance in a mouse AD transgenic model (Ricobaraza et al., 2012). However, given the possible adverse consequences of non-selective inhibition as demonstrated by clinical research, caution should be exercised when following this course (Prince et al., 2009).The fact that all HDACs have a lot of structural similarities and are expressed in diverse organs and cell types has made it difficult to design and create selective HDAC inhibitors. However, given improved technological possibilities, it is highly probable that blocking a particular HDAC could be a promising treatment approach (Gupta et al., 2020). The selective inhibition of HDAC6 by tubastatin A, ACY-1215 (ricolinostat), MPT0G211, or 5-aroylindoles is a promising example. These drugs have demonstrated good outcomes in animal models of AD (Fan et al., 2018; Lee et al., 2018; Onishi et al., 2021) and are being evaluated in clinical trials for conditions other than neurodegeneration.

Cutting The Costs:

The persistent inability to create a novel treatment for neurodegenerative diseases is costly and discouraging. After a protracted and expensive development procedure, the vast majority of compounds under investigation rarely reach

signing up. No one has yet to hit the market out of the 509 compounds mentioned in a paper by Myoung-Ok Kwon and Paul Herrling that were in any stage of development up until June 2006. All of the products that were in Phase II or III at the time were probably discontinued because they were ineffective. Consequently, all of the financial and human resources that were invested were squandered. An average of $1 billion is thought to be spent on clinical trials required to launch a new medication. The high expenses and potential for failure may make pharmaceutical companies hesitant to engage in this area.

The so-called adaptive design is a novel study design paradigm that has recently been proposed. It is believed to have the potential to significantly lower trial expenses while also improving trial outcomes. What makes it unique is in that, depending on the analysis of interim data, planned changes to one or more specific parts of the research design and hypotheses are permitted. A practical approach in this situation would be to "kill" the therapeutic compounds that don't show promise in the early clinical stages before they move on to more costly stages of research. Patients' safety would increase if useless medications were eliminated since they would be exposed to fewer ineffective therapies, the length of the research, and ultimately significantly reduce the expenses. However, determining what is and is not useless is essential since failing to do so could result in the irreversible discarding of agents that were mistakenly deemed useless. In contrast to standard efficacy studies, which test the null hypothesis that treatments are equivalent and reject it if one treatment is likely to be more effective than the other, so-called futility studies are intended to determine whether a treatment exhibits promise and will, therefore, result in outcomes that surpass a significant threshold. The main focus of these investigations is the threshold. If the threshold for futility is appropriately established, medications that surpass it may be deemed "non-futile drugs" and may move forward with confirmatory trials, whereas ineffective treatments shouldn't be investigated further. Studies on futility have long been effectively employed in cancer research, but more lately, they have been given consideration in the field of

neurodegeneration. These are Phase II trials that typically compare one treated arm over a respectably brief length of time to a predetermined threshold value that represents a consistent clinical assessment.

Conclusions:

Neurodegenerative disease treatment presents difficult problems. These difficulties stem in part from the intricate pathophysiology that underlies the genesis of these illnesses. The effectiveness of a new strategy that claims to employ a single polypharmacological drug molecule—that is, one that affects many drug targets in the disease's etiological pathway—may offer a new hope in treatment of many neurodegenerative diseases. Provide fresh promise for treating a variety of neurodegenerative illnesses, including those linked to cognitive loss. Future treatment plans for neurodegenerative disorders and the cognitive decline they cause may require a shift similar to an all-out strategy. The focus of drug development efforts will need to change from creating selective agents that only target one pathophysiological pathway to creating agents (like DMLs) that work through several mechanisms and are meant to address the disease state's inherent complexity.

The main unmet medical need in neurodegenerative illnesses is a disease-modifying treatment that slows or prevents the progression of the illness. While aetiology or pathophysiology of these conditions are not entirely understood, efforts to create therapies that can influence the course of the disease must go on.

Innovative study designs like the delayed start and randomised withdrawal designs may be used in human clinical trials to assist distinguish between a medicinal agent's symptomatic and disease-modifying effects. Another effective method to promote research in the field is through futility studies. Initiate neurological field by reducing the expenses associated with drug development programs.

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AMELIORATION OF SALINITY STRESS IN RICE CULTIVATION OF WEST BENGAL

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ABSTRACT

West Bengal a prominent rice-producing state in India, faces significant challenges related to soil salinity, particularly in its coastal regions and the Sundarbans delta. Salinity adversely impacts rice productivity by affecting soil structure, nutrient availability, and water uptake by plants. This is a critical issue in saline- prone district like south 24 parganas, North 24 paraganas, and Purba Medinipur, where tidal intrusion and rising sea level exacerbate the problem. Research highlights that salinity stress reduces germination rates, plants growth, and grain yield in rice, a staple food crop for millions in the state. This issue has included introducing salt- tolerance rice varieties such as Swarna sub1, Nona Bokra, and CSR 43, along with improved agronomic practices like proper drainage system. The study undergoes the importance of adaptive strategies to mitigate salinity effects, including sustainable water management, genetic enhancement of rice varieties, and community engagement. These measures the essential to ensure food security and the economic stability of farmers in the salinity – affected regions of west Bengal. Further research and policy investigations are required to address the long-term impacts of salinity of rice cultivation in the state.

Keywords: *Rice variety, Rice cultivation, Sustainable water management, Salinity stress, West Bengal.*

INTRODUCTION

Rice is the major food for the millions of people. Rice is also the main food crop for the people in coastal flood-prone areas of southeast Asia. In India, these areas are distributed over nine states (Andhra Pradesh, Goa, Gujrat, Karnataka, Kerela,

Maharashtra, Odisha, Tamil Nadu, and West Bengal) and four union territories(Andaman and Nicobar, Lakshadweep, Daman and Diu, and Puducherry) but the state of WB has the highest area under the coastal saline lands (0.82 million hectares) and as a consequence of climate change, rice production in recent years is highly variable due to the increase of flood or drought, besides widespread occurrence of saline soil with variable saline levels. High monsoon rainfall, poor soil and water quality and natural weather adversities like coastal storms and cyclones make agriculture in these areas more complexrisky.

Rice exhibits enormous genetic variability in their salt sensitivity. The indica varieties Pokkali and Nonabokra (NB) are classified as highly salt-tolerant ecotypes, while the high yielding varieties like M-1-48, IR-29, IR-72, IR-36 and Taichung Native-1 are salt-sensitive (Roychoudhury et al., 2008b). Alternatively, the aromatic indica rice varieties, which are highly demanding in the global market, because of their pronounced fragrance and elegant flavour, suffer huge crop losses due to their susceptibility to biotic and abiotic stress conditions. Our earlier observations (Roychoudhury et al., 2008a, Roychoudhury et al., 2009a, Roychoudhury et al., 2009b) showed that the aromatic rice Gobindobhog (GB) behaves as highly salt-susceptible variety suffering formidable oxidative damages with lower expression of stress-inducible genes in response to salinity and abscisic acid (ABA)-mediated stress injuries, meaning that the improvement of stress tolerance in this variety is a matter of serious concern. We focused on the exogenous application of PAs, Spd and Spm, as a means to study their protective potentiality in salt tolerance of M-1-48 (salt-sensitive), NB (salt-tolerant) and

particularly GB (highly sensitive) rice varieties. The root and shoot lengths, chlorophyll and endogenous ion content, lipid peroxidation (LP), protein carbonylation and protease activity in the three varieties, subjected to NaCl solution, either in presence or absence of Spd/Spm were analyzed. The defence response mechanism, in the form of some enzymatic and non-enzymatic antioxidant assay or compatible solute measurements were performed in the leaves of the three NaCl-treated varieties and their differential modifications in relation to exogenous Spd or Spm has been studied extensively and documented in this communication.Polyamines (PAs), including the diamine putrescine (Put2+), triamine spermidine (Spd3+) and tetramine spermine (Spm4+), are ubiquitous, low-molecular-weight, straight chain, aliphatic amines, ranging in concentration from 10 µM to mM and involved in various biochemical and physiological processes related to the regulation of plant growth and development. Because of their polycationic nature at physiological pH, PAs are able to interact with proteins, nucleic acids, membrane phospholipids and cell wall constituents, thereby stabilizing these molecules. In recent years, much attention has been devoted to the involvement of PAs as second messengers in response to different environmental stresses (Alcazar et al., 2006, Liu et al., 2007) like osmotic stress, salinity, drought, heat, chilling, high light intensity, mineral nutrient deficiency, heavy metals, pH variation, UV-irradiation and exposure to pollution stress.

Salinity Stress

Saline soils are defined as having an electrical conductivity (EC) of 4 dS/m in a saturated soil-paste extract (Richard, 1954). However, Bresler et al. (1982) considered soil to be affected by salt when EC reaches 2 dS/m, as many fruit, vegetable, and ornamental plants experience salt toxicity between 2–4 dS/m. A soil is considered salinized if salt levels exceed certain limits: (1) 3–5 g/L of salt in the soil solution (Vargas et al., 2018), (2) 0.05–0.15% toxic salts in water extracts (Vargas et al., 2018), or (3) an EC of 2–4 dS/m in saturated soil-paste extracts (Bresler et al., 1982).In Eurasian countries, soil salinity is often measured using a soil/water extract ratio of 1:5 (Vargas et

al., 2018). However, preparing soil pastes and their extracts is time-consuming, so it is common to assess salinity using soil/water suspensions at ratios of 1:2.5, 1:5, or 1:10 and then adjust results using empirical formulas (Jahn et al., 2006).The equation ECSE = 250 × EC(2.5) / WCSE helps convert EC from a 1:2.5 suspension to a saturated paste extract, where ECSE is the EC of the saturated paste extract, EC(2.5) is the EC of the 1:2.5 suspension, and WCSE is the moisture content of the soil paste.

Soil salinity reduces crop production and threatens food security. In dry regions, salinity has caused yield losses of 18% to 43% (Vargas et al., 2018), worsening land degradation and climate change impacts, which can negatively affect local communities.

Hormone Regulation of Salinity Tolerance

ABA is an important plant hormone that helps plants cope with stress. When there is a shortage of water in the soil, ABA levels increase in the roots and shoots. Salinity stress, which happens when there is too much salt, also raises ABA levels. This hormone helps reduce the negative effects of salt on photosynthesis, plant growth, and the movement of nutrients. ABA helps plants tolerate salt by increasing the levels of important nutrients like potassium (K+), calcium (Ca2+), and protective substances like proline and sugars in the roots, which reduce the harmful effects of sodium (Na+) and chloride (Cl−). ABA also controls the activity of certain genes that help plants survive in salty and dry conditions. Studies by Fukuda and Tanaka showed that ABA affects genes responsible for important cell functions in barley (Hordeum vulgare) under salt stress. In wheat, ABA increased the activity of specific genes (MAPK4-like, TIP 1, and GLP 1) that help plants deal with salinity.

Some compounds with hormone-like effects, such as salicylic acid (SA) and brassinosteroids (BR), help plants cope with stress caused by harsh environmental conditions. When rice seedlings face salty conditions, their levels of SA increase, along with the activity of enzymes that produce SA. Research has shown that SA improves salt tolerance in

Arabidopsis by stabilizing cell membranes and preventing the loss of potassium (K+) through a specific channel (GORK). Arabidopsis seedlings treated with SA had higher H+-ATPase activity, which helped them retain K+ during salt stress.

While SA did not stop sodium (Na+) from accumulating in the roots, it reduced Na+ levels in the shoots.In barley, SA improved salt tolerance by increasing chlorophyll and carotenoid content, maintaining membrane stability, and promoting K+ and sugar accumulation in the roots under salty conditions. Studies by Nazar et al. suggest that SA helps plants like mung beans withstand salt stress by boosting photosynthesis, nitrogen and sulfur absorption, and antioxidant activity.

Similarly, BRs help reduce salt stress by increasing the activity of antioxidant enzymes (SOD, POX, APX, and GPX) and raising the levels of antioxidants like tocopherol, ascorbate, and glutathione. Both SA and BRs are found in many plants and play important roles in growth and stress tolerance. Researchers have studied how applying SA and BRs externally can help plants withstand salt stress by improving biochemical and physiological processes.

The physiological and biochemical alterations under salinity stress

Effect on germination and seedling stage

Seed germination is a step-by-step process influenced by internal and external factors. Salinity stress can slow down germination by reducing water absorption and decreasing the activity of α-amylase, an enzyme that breaks down starch into sugars. High salt levels in the soil make it harder for seeds to absorb water, delaying germination and reducing the overall germination rate. Even after germination, excess salt can harm the embryo by accumulating toxic Na+ and Cl- ions. Salt stress also increases harmful reactive oxygen species (ROS), which damage important molecules in the seed. As a result, lower α-amylase activity reduces the sugar supply needed for embryo growth.

Under normal conditions, seed germination happens in three phases. In Phase I, dry seeds quickly absorb water (imbibition). In Phase II, metabolic activities resume, but water uptake slows down. Phase III is the post-germination stage, where water absorption continues until full germination. In salty conditions, Phase I faces osmotic stress, and Phase II experiences ionic stress, both of which delay germination. Even after sprouting, seedling growth in Phase III is affected by salt stress.

Salinity reduces seed germination by lowering gibberellic acid levels, increasing abscisic acid, altering membrane permeability, and reducing water absorption. Germination depends on enzymes like α-amylase, which break down starch into sugars that provide energy for the growing embryo and radicle. While seeds may still germinate under moderate salinity, the process takes longer. Delayed germination is mainly caused by slow water absorption and reduced α-amylase activity due to high salt levels. Salt-sensitive seeds experience a greater drop in α-amylase activity than salt-tolerant ones, which limits sugar movement and affects embryo development.

Ionic imbalance and salinity mediated nutritional deficiencies

Some ions, like K+ and $SO4^{2}$-, are important nutrients for plants, but Na+ is not essential. That's why soil salinity is often measured by the levels of Na+ and Cl−. Salinity affects plants in three main ways. First, salt lowers the soil's water potential, making it harder for plants to absorb water, causing stress that slows growth and reduces yield. Second, when plants take in too much Na+ and Cl−, these ions become toxic, leading to leaf damage, stunted growth, and even plant death. Third, high Na+ levels compete with other essential nutrients like K+, Ca2+, and Mg2+, leading to nutrient deficiencies. When salt levels rise, plants absorb more Na+ than K+, which disturbs the balance of ions, disrupts nutrient uptake, and damages cell membranes. Research on rice has shown that salinity stress reduces nutrient levels (Fe, K, Mn, Mg, P, and Zn) in roots and shoots. It also decreases root surface area by reducing root hair density and length, which lowers nutrient

absorption. Essential elements like Ca2+, Mg2+, Fe2+, and Zn2+needed for

Oxidative stress caused by salinity

Salt stress harms plants in many ways, including causing an excessive build-up of reactive oxygen species (ROS). These harmful molecules can damage important cell components like DNA, proteins, and lipids, leading to enzyme inactivation, hormonal imbalances, and nutrient deficiencies. ROS are mainly produced in the chloroplasts, mitochondria, endoplasmic reticulum, cytosol, and peroxisomes.

Salt stress also causes stomatal closure, reducing the amount of carbon dioxide in leaves, which slows down photosynthesis. The light reactions in chloroplasts are a major source of ROS, including superoxide (O2 •-), hydrogen peroxide (H2O2), hydroxyl radical (OH•), and singlet oxygen (1O2).

Salt stress, combined with water shortage, disrupts plant metabolism and increases ROS production, causing further damage. Studies on crops like rice (Oryza sativa), maize (Zea mays), mung bean (Vigna radiata), and tomato (Solanum lycopersicum) have shown that salt stress lowers the ascorbate (ASA) to dehydroascorbate (DHA) ratio and reduces the activity of protective enzymes like superoxide dismutase (SOD) and catalase (CAT). It also increases methylglyoxal (MG), leading to oxidative damage. In rice seedlings, salt stress raised hydrogen peroxide (H2O2) and malondialdehyde (MDA) levels, while lipoxygenase (LOX) and SOD activity increased, but CAT activity decreased.

Effect on yield and yield components

Salinity stress reduces crop yields by affecting plant growth and key processes during the reproductive stage. The high-affinity K+ transporter (HKT) family helps remove excess sodium (Na+) from leaf blades, maintaining balance under salt stress. Studies show that grain dry matter and the K+/ Na+ ratio are closely linked to grain filling rate and duration. Salinity also disrupts water movement, transpiration, nutrient balance, and stomatal function, leading to oxidative damage and lower yields. It affects leaf thickness, cell size,

and turgidity, reducing the plant's ability to produce food. Many crops, including moong bean, cabbage, tomato, and broccoli, experience lower yields due to salt stress.

Salt Stress Mitigation in Rice by Microorganisms

Some microorganisms can help plants grow better in salty conditions by reducing the harmful effects of salt stress. Recently, studies have found that using salt-tolerant microorganisms like Pseudomonas multiresinivorans, Microbacterium esteraromaticum, and Bacillus subtilis, along with organic fertilizers like farmyard manure, can help rice plants cope with salt stress and grow better.

Arbuscular mycorrhizal (AM) fungi also play an important role in helping plants deal with salt stress. These fungi improve plant growth by changing root structure and expanding the network of tiny filaments (hyphae) that absorb nutrients. AM fungi help plants tolerate salt by improving nutrient absorption, protecting enzyme activity, boosting photosynthesis, and making it easier for plants to take in water. They also help balance ions in the plant, reducing harmful sodium (Na+) levels while increasing potassium (K+) absorption. This balance prevents damage to important plant processes like enzyme function and protein production, helping the plant stay healthy even in salty conditions.

Salt Stress Mitigation in Rice by Plant Nutrients

Plants use different minerals like nitrogen (N), phosphorus (P), potassium (K), zinc (Zn), iron (Fe), and silicon (Si) to cope with environmental challenges. When crops face salt stress, their growth is affected because salt disrupts the movement and balance of nutrients. High levels of sodium (Na^+) and chloride (Cl^-) can interfere with essential nutrients like potassium (K^+) and calcium (Ca^{2+}), causing an imbalance. This stress harms plant growth due to toxic ions and nutrient deficiencies. Many fertilizers have high salt content, so they should not be applied too close to young plants. Adding gypsum can help protect soil structure, improve water absorption, and reduce sodium buildup. The next sections will discuss how minerals like calcium, manganese, and silicon help plants tolerate salt stress.

Salt Stress Mitigation in Rice by Phytohormones Application

It is identified that the phytohormones such as ethylene, gibberellins, abscisic acid, cytokinins, auxins, and brass in steroids all regulate plant growth and development traits. The Abscisic acid (ABA) typically guards plants against different environmental stress conditions, analysed ABA's positive role in reducing the Cl- and Na+ concentrations and reducing transpiration in order to maintain water via switching the stomata of the plant. In addition, in rice plants, Na+, Cl, and Na+, K+ were decreased due to exogenous application of ABA. Some other studies reported the role of exogenous use of ABA in increasing the rice salt stress tolerance. however, exogenous application of 100 µM of ABA in rice increased grain yield through more efficient accumulations of soluble sugar and proline as well as maintaining K+ and Ca2+ equilibrium.

Alteration of salt-responsive genes, transcription factors, and protein synthesis

Plants regulate gene expression at different levels—before, during, and after making proteins—to respond to stress like salt. Studies on rice show that many genes change their activity under salt stress. Certain transcription factors, zinc finger proteins, and RNA processing enzymes play a role in this response. For example, proteins involved in RNA splicing, like tRNA splicing protein and AAR2, increase under salt stress. RNA-binding proteins (RBPs) also change their expression, suggesting they help regulate gene activity after transcription. Additionally, some translation initiation factors (eIFs) decrease in rice roots under salt stress, which may slow down protein production. Other proteins, such as poly A binding proteins (PABPs) and a nascent polypeptide-associated complex (NAC), also decrease, while some ribosomal proteins increase. This suggests that salt stress affects the process of making new proteins in rice, with different genes being activated or suppressed.

Proper folding and processing of proteins

Proteins can lose their shape and clump together under stress, which can make them stop working properly. To prevent

this, plants use heat-shock proteins (HSPs) and molecular chaperones (MCs) to help proteins fold correctly and stay functional. These proteins also help fix damaged proteins and keep cells healthy during stress. Studies show that proper protein folding is important for root function under salt stress. For example, a protein called rHsp90 is activated in rice roots under salt stress, and 23 other MCs (including HSPs and TCP1) increase in response to salt. Some MCs respond differently in genetically modified rice. The quick activation of HSPs and MCs helps keep proteins stable, which improves rice plants' ability to handle salt stress.

Conclusion and future prospective

Rice has been an important food for humans since ancient times. Changes in the environment can create stress conditions that harm rice crops, leading to major economic losses. Many fruits, vegetables, and ornamental plants face salt stress at electrical conductivity (EC) levels of 2–4 dS/m. In agriculture, salt stress limits crop growth and affects food security.Rice is especially sensitive to salinity, though it can tolerate some salt during germination, tillering, and maturity. However, high salt levels are harmful at the early seedling and reproductive stages. Salt stress affects rice growth by causing physiological and biochemical changes, reducing dry matter and grain yield.Researchers are working on ways to help plants survive stressful environments. Some promising methods to improve rice tolerance to salt stress include seed priming, beneficial microorganisms, applying nutrients like calcium, manganese, and silicon, using organic compounds like trehalose, and plant hormones such as abscisic acid.

Using biological approaches to improve rice under salt stress looks promising. It is important to identify key plant traits that help manage stress and find ways to enhance overall plant growth in salty conditions to maintain or increase rice production. Future research should focus on genetic, metabolic, and physiological changes in salt-stressed plants and how agricultural techniques—like priming, microorganisms, nutrients, organic compounds, and plant hormones—can help. Further studies in these areas will improve rice farming under salt stress in the near future.

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ADVANCES IN STEM CELL BIOTECHNOLOGY AND REGENERATIVE MEDICINE: APPLICATIONS, CHALLENGES, AND FUTURE PERSPECTIVES

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ABSTRACT

Stem cell biotechnology and regenerative medicine are rapidly advancing fields with substantial therapeutic potential. Stem cells, capable of differentiating into various cell types, play a crucial role in tissue engineering and developmental biology. They are increasingly applied in drug testing, disease modelling, and cell-based therapies for conditions like arthritis, Parkinson's disease, type 1 diabetes, and coronary diseases. Mesenchymal stem cells (MSCs) show promise in regenerative therapies for heart failure, wound healing, and tooth regeneration, although clinical outcomes remain variable. Advances such as lab-grown blood stem cells offer revolutionary possibilities for bone marrow transplants, particularly for children with blood disorders. Furthermore, regenerative medicines are being developed to grow new tissues and organs, offering potential cures for chronic conditions like heart disease and fibrosis. These innovations highlight the transformative potential of stem cell biotechnology, offering hope for long-term, effective treatments for a variety of debilitating diseases and improving overall human health and longevity.

Ethical considerations in stem cell biotechnology include debates over the use of embryonic stem cells, the regulation of gene-edited stem cells, and ensuring equitable access to advanced therapies. Addressing these challenges involves developing universal donor cells and immune-evasive stem

cell lines to minimize ethical and immunological concerns. Additionally, there is a growing focus on strategies to enhance the long-term safety, viability, and functional integration of transplanted stem cells into host tissues. Robust regulatory oversight and public engagement are essential to build trust, ensure safety, and establish ethical guidelines for clinical or medical applications.

Keywords: *Cell-derived Therapy, Pluripotent, Regenerative Medicine, Stem cell Biology, Transplantation.*

INTRODUCTION

Stem cells are cells with the ability to differentiate into a large number of different body cell types. They have the power to replenish their tone and can be incorporated into any type of creature's cell. Both adult cells and embryos can develop stem cells. There are numerous exciting options for specialization. Because the experimental energy decreases with each stage, a pluripotent cell can differentiate into a smaller range of cell types than a unipotent stem cell. Certain types of stem cells exist and are essential to regenerative medicine. Human embryos are the source of embryonic stem (ES) cells, whereas mature organs and tissues are the source of adult stem cells. The body uses adult stem cells primarily to repair and restore damaged tissue. Additionally, stem cells obtained from the placenta are known as placental stem cells, stem cells discovered in amniotic fluid are known as amniotic fluid stem cells, and stem cells extracted from the umbilical cord following childbirth are known as cord blood stem cells (O'Brien et al., 2009). Children with blood malignancies are treated with cord blood stem cells (Ancans, J. et al. 2012). One excellent source of fetal mesenchymal stem cells (MSCs) is placental stem cells. Each stem cell in the body has a niche, which is a unique home. When external stressors like injury or transplanting change their niche, stem cells have the capacity to adapt to new settings. As a result, they can grow into a wider variety of cells that their new environment needs. For instance, skin sweat gland stem cells can grow into glandular structures that make milk when they are put into breast fat pads. Along with nearby stem cells filling a void, committed progeny can also dedifferentiate and assume the same role

within the body. The fifth Making induced PSCs (iPSCs), or PSCs derived from adult stem cells, is an additional choice. According to Kuriyan et al. (2017) and Biehl et al. (2009), they can be used in place of ES cells. Individual cells that comprise the entire organism can be produced by the division and differentiation of totipotent stem cells. totipotency is the capacity to differentiate into both embryonic and extra-embryonic tissues. A zygote, which is produced when a sperm fertilizes an egg, is an example of a totipotent cell. These cells may eventually become a placenta or any of the three origin layers. The blastocyst's inner cell mass becomes pluripotent about four days later.

Pluripotent cells develop from this framework. Pluripotent stem cells, or PSCs, are able to differentiate into cells of any origin layer, but they are unable to create extraembryonic structures such as the placenta. One example is embryonic stem cells, or ESCs. ESCs originate from the inner cell mass of preimplantation embryos. Another example is the use of induced pluripotent stem cells (iPSCs) derived from the epiblast estate of implanted embryos. Their pluripotency varies from lower-energy representations, which are multi-, oligo-, or unipotent cells, to fully pluripotent cells, which are similar to ESCs and iPSCs. One technique to assess their range and effort is the teratoma conformation test. iPSCs, which are artificially generated from actual cells, can also be advantageous for PSCs. Their operations and culture have enormous potential for regenerative medicine, both today and in the future.

Compared to PSCs, multipotent stem cells have a smaller range of insulation, but they can differentiate into particular cell lineages. Hematopoietic stem cells, which can differentiate into several kinds of blood cells, are one example. A hematopoietic stem cell develops into an oligopotent cell following insulation. Moreover, its insulating qualities are unique to cells from its predecessors. Because some multipotent cells can differentiate into unrelated cell types, pluripotent cells have been postulated. Numerous cell types can be differentiated from oligopotent stem cells. The ability to differentiate into white blood cells but not red blood cells is

known as myeloid stem cell.

Two characteristics that set unipotent stem cells apart are their limited capacity for insulation and their unusual ability to divide continuously. They are a good choice for the restorative application of regenerative medicine because of their last point. Dermatocytes are an example of a unipotent cell; these cells can develop into them. The potential of using mesenchymal stem cells (MSCs) to regenerate different tissues, including bone, cartilage, and other human tissues, has been investigated by several researchers in recent years. In order to improve the use of the biomimetic method in biomedical devices and human prosthetics, new materials have also been investigated and mixed with MSCs (O'Brien et al., 2009). The challenges of using MSCs in bone and dental applications have been addressed by using stem cell types such as dental pulp, human-periapical odontogenic cysts, and periodontal ligament (Mousaei et al., 2022). The coupling of MSCs with advanced biomaterials has made it possible to apply regenerative medicine to organs and tissues that have either been damaged or have experienced degenerative processes (Kuriyan et al., 2017 & Biehl, et el., 2009). But stem cell transplantation has also been researched for cancer and other severe diseases. (2014; Srijaya et al.).

As a result, the field of regenerative medicine has recently placed a strong emphasis on novel biomaterials and stem cell functions. The enormous quantity of research that has been done on these subjects has allowed for significant advancements to be made thus far.

Application Of Stem Cell in Human Disease

Heart disease

Since the remedial uses of stem cell- grounded treatments for cardiac conditions have lately been completely covered in reviews (Bolli et al., 2021) and (Liu et al., 2021) this study will make on them in the corridor that follow, with an emphasis on hPSCs and MSCs. A substantial quantum of preclinical and clinical exploration generally supports the safety biographies of stem cell- grounded curatives, particularly adult stem cell remedy (e.g., MSC- grounded products).

Clinical trials, still, haven't yet produced substantiation of the treatment's effectiveness because, indeed in phase III trials, several studies have produced antithetical findings and no statistically significant variations in infarct size, cardiac function, or clinical issues (Zhang, J. et al., 2021). A meta- analysis's findings demonstrated that stem cells from colorful sources demanded any remedial function. The given cells may have a remedial effect through immunological regulation rather than regenerative function, which could regard for the dismal issues of the clinical trials to far. thus, to ascertain the effectiveness of the treatments, precisely planned, randomized, and placebo- controlled phase III studies with proper cell medication ways, patient selection, follow- up schedules, and applicable clinical assessments must be carried out. The stylish cell source and lozenge, the delivery system and timing of administration, the distribution of cells after administration, and the medium of action are more issues that must be addressed.

Liver Disease

The largest critical organ in the mortal body, the liver carries out vital natural tasks similar as metabolism, abetting in digestion, detoxifying the body, storing vitamins, and further(Si-Tayeb et al., 2010). Liver failure, cirrhosis, cancer, alcoholic liver complaint, non-alcoholic adipose livercomplaint(NAFLD), and autoimmune liver complaint(ALD) are among thepathological conditions that may arise from a disturbance of liver homeostasis and function. Although there are veritably many applicable and available patron organs, orthotropic liver transplantation is the only effective treatment for serious liver diseases. At the moment, liver ancestor cells, hPSCs, MSCs, and HSCs are linked to stem cell- grounded treatments for liver illness.

Liver Cirrhosis

In liver cirrhosis, the hepatic vascular structure defeats due to parenchymal extermination after expansive nodular regrowth with thick fibrotic septa, which is one of the primary causes of morbidity and mortality encyclopedically(Arroyo et al., 2016). Actually, unless a liver transplant is done, liver cirrhosis is

allowed to be the final stage of liver illness that eventually results in death. currently, stem cell- grounded remedy further specifically, MSCs is showingpledge as a treatment for liver cirrhosis. The MSC group(n = 30) and the control group(n = 15) were resolve up among 45 habitual hepatitis B cases with decompensated liver cirrhosis in a clinical trial employing umbilical cord deduced MSCs(UC-MSCs)(Zhang et al., 2012). The MSC group's ascites volume was much lower than the control groups, according to the data. The MSC groups also showed a significant enhancement in liver function, as seen by a drop in the sodium model for end- stage liver complaint score, a drop in total blood bilirubin situations, and an increase in serum albumin attention(Zhang et al., 2012). Results from a phase II trial that used BM- MSCs in 25 cases with liver cirrhosis caused by HCV also showed analogous issues(El- Ansary et al., 2012). In line with theseexaminations, three further clinical trials that concentrated on alcoholic and hepatitis B- convinced liver cirrhosis were carried out and vindicated that the administration of MSC bettered and restored liver functioning(Xu et al., 2014) and(Fang et al., 2018). The safety and possible remedial benefits of MSC- grounded curatives might be further corroborated and the viability of treating contagion- related liver cirrhosis established by the big cohort study as the clinical trial carried out by Fang(Fanget al., 2018) 49 According to a randomized controlled phase 2 trial, cases with liver cirrhosis do n't parade remedial benefits from systemic preface of BM- MSCs(Mohamadnejad et al., 2019) 50. Liver cirrhosis is n't solely caused by MSCs. An open- marker clinical trial that was lately carried out in 19 children who had liver cirrhosis as a result of biliary atresia following the Kasai operation demonstrated the approach's safety and viability by demonstrating the enhancement in liver function following the administration of bone gist mononuclear cells(BMNC), as determined by biochemical tests and pediatric end- stage liver complaint(PELD) Scores(Nguyen et al., 2022). Another trial that used BMNCs in 32 cases with decompensated liver cirrhosis demonstrated the safety and efficacity of administering BMNCs in discrepancy to the control group(Bai et al., 2014). A recent long- term disquisition of cases who entered supplemental blood- deduced stem cells showed that

the chance of developing hepatocellular melanoma did n't rise, and the long- term survival rate was significantly better than in the control group(Guo et al., 2019). Cases with liver cirrhosis shared in a multicentre, open, randomized controlled phase 2 trial that involved CD133 HSC infusion; nevertheless, the issues didn't show enhancement of liver conditions, and cirrhosis continued(Newsome et al., 2018). specially, these issues are harmonious with previous randomized controlled exploration that set up no remedial pledge in bone gist-deduced stem cells and G- CSF administered through the hepatic roadway(Spahr et al., 2013).

Cancer

Stem cell remedy is a delicate conception that should be used and handled precisely while treating cancer. In addition to shielding cancer cases from expensive, potentially dangerous, or hamstrung stem cell- grounded curatives, clinicians and experimenters should also shield those without a cancer opinion from the possibility of developing cancer. For the treatment of cancer, untested stem cell conventions generally used three cell- grounded curatives autologous HSCTs, stromal vascular bit(SVF), and multipotent stem cells, like MSCs. A miracle known as" graft- versus- excrescence goods" occurs when allogeneic HSCTs produce patron lymphocytes that aid in the repression and retrogression of hematological malice and certain solid excrescences(Barisic et al., 2022). Still, stem cell conventions give allogeneic cell-rested remedy for the treatment of solid malice despite limited scientific confirmation supporting the safety and effectiveness of the treatment. High- quality confirmation from the Cochrane library shows that gist transplantation via autologous HSCTs in combination with high- cure chemotherapy does n't ameliorate the overall survival of women with metastatic bone cancer. In addition, a study including further than 41,000 bone cancer cases demonstrated no significant difference in survival benefits between cases who entered HSCTs following high- cure chemotherapy and cases who passed conventional treatment(Mello et al., 2001). therefore, the use of autologous T- cell transplants as monotherapy and advertising stem cell- rested curatives as

if they're medically approved or preferred treatment of solid excrescences is considered untrue statements and needs to be advised to cancer cases(Sissung et al., 2020). Because of their special rates, multitudinous preclinical studies conducted over the once many decades have shown the pledge of MSC-grounded remedy in the treatment of cancer. Through ingrain tropism regulated by growth factors, chemokines, and cytokines, they give the capacity to resettle toward damaged regions. In order to reply to external cues, MSCs must express certain C – X – C chemokine receptor type-4(CXCR4) as well as fresh chemokine receptors(similar as CCR1, CCR2, CCR4, CCR7, etc.)(Fu et al., 2019). likewise, the MSC face expresses particular adherence proteins, including as CD49d, CD44, CD54, CD102, and CD106, which enable the cells to cleave, rotate, resettle, and enter the blood vessel lumen in order to insinuate the injured towel(Zachar et al., 2016). Like injured apkins, excrescences release a variety of chemo attractants that, through the CXCL12/ CXCR4 axis, also draw MSC migration. Diffusible cytokines like interleukin 8(IL- 8) and growth factors like transubstantiating growth factor- beta 1(TGF- β1), platelet- deduced growth factor(PDGF), fibroblast growth factor 2(FGF- 2), vascular endothelial growth factor(VEGF), and extracellular matrix motes like matrix metalloproteinase 2(MMP- 2) have also been shown in earlier exploration to tightly regulate MSC migration toward the cancer point(Cronwright et al., 2005). The main issues with MSC- grounded remedy are the protumor and antitumor goods that arise from the commerce between MSCs and cancer cells after they've effectively migrated to nasty towel. The regenerative parcels of MSCs, which control towel form and recovery, are extensively honored. The protumor conduct of these cells are also responsible for this special capability. According to a previous study, MSCs cache chemokine(C – C motif) ligand 5 (CCL- 5) in response to bone cancer cells, which controls the excrescence irruption process(Aldinucci et al., 2020) and(Karnoub et al., 2007). According to other exploration, MSCs release a variety of growth factors that help cancer cells from dying, including VEGF, introductory FGF, HGF, PDGF, and others.85 likewise, MSCs can change into cancer- associated fibroblasts, a particular type of

cell that lives in the excrescence medium and can promote carcinogenesis, in response to signals given by cancer cells, similar as TGF- β,(Schmohl et al., 2020) and(Mishra et al., 2008). The development of cancer rectifiers has made use of MSCs' strong excrescence repression capabilities, despite their demonstrated involvement in protumor conditioning.

About 25 clinical trials that use MSCs as a remedial treatment for cancer have been registered on ClinicalTrials.gov therefore far(Liang et al., 2020). The maturity of these trials are phase 1 and 2 examinations that assess the treatment's efficacity and safety. MSCs and an oncolytic contagion strategy have been coupled in studies that take advantage of MSC- grounded remedy. Oncolytic contagions are particular kinds of contagions that can be set up naturally or through inheritable engineering. They've the capacity to specifically infect cancer cells and destroy them without harming the healthy cells around them(Cao et al., 2020). A finished phase I/ II exploration that used BM- MSCs infected with the oncolytic adenovirus ICOVIR5 to treat resistant and metastatic solid excrescences in both adult and pediatric cases showed that the treatment was safe and offered early substantiation of its eventuality as a treatment(Melen et al., 2016). In one pediatric illustration, the same experimenters reported that three times after administering MSC- grounded remedy, all suggestions of malice had fully faded(Garcia- Castro et al., 2016). According to a 2019 study, adipose-deduced MSCs fitted with the vaccinia contagion have the capacity to exclude resistant excrescence cells by combining strong contagion modification with excrescence cell senitization to contagion infection(Draganov et al., 2019). Advances in stem cell-grounded remedyand further conventional treatments like chemotherapy, radiation, and surgery have steered in a new and instigative period in cancer exploration and treatment. Although stem cell- grounded remedy has been viewed as a new and charming remedial system for the treatment of cancer, preclinical exploration has shown disagreeing results regarding the protumor and antitumor goods. The use of stem cell- grounded remedy, particularly MSCs, gives cancer cases new stopgap despite this disagreeing reality by offering a new and more effective tool in acclimatized drug.

Arthritis

Arthritis: The broad term for cartilage diseases that affect in common discomfort and inflammation is arthritis. The most current type of arthritis brought on by cases is osteoarthritis(OA). Declination and shy articular cartilage rejuvenescence(Martel- Pelletier et al., 2016). analogous to bitsy joints at the hand and large joints at the knee and hips, OA affects one or further diarthrodial joints, performing in excruciating pain and a posterior loss in mobility. Primary OA, also known as idiopathic OA, and secondary OA are the two types of OA that are brought on by analogous unproductive factors similar as trauma, surgery, and aberrant common development at birth(Olsson et al., 2021). Due to the fact that traditional OA treatments are ineffective and may affect in excruciating pain and lengthy recuperation. thus, stem cell remedy has gained a lot of attention in the regenerative sector and has recently surfaced as a pivotal system for OA(Mahmoudian et al., 2021). It has been demonstrated that administering HSCs can lessen bone lesions, promote bone rejuvenescence, and accelerate the vascularization process in degenerative cartilage. Three intraarticular injections of redundant blood stem cells were used in ten OA cases inatrouble to gauge their efficacity. Analysis conducted after injection showed a significant drop in all parameters and a drop in the WOMAC score. Every case finished the 6-nanosecond walk tests, adding by further than 54 measures. An enhancement in cartilage density was shown by MRI examination, indicating thatpost-administration cartilage declination was lessened. CD34 stem cells were suggested as a way to ameliorate the restorative eventuality of HSCT indeed further(Kubsik-Gidlewska et al., 2016). Due to their immunoregulatory parcels, MSCs are presently the subject of a significant number of studies aimed at treating OA. anti-inflammatory parcels. MSCs have been the primary cell source in a number of small- and large- scale studies, demonstrating their safety profile and wordless efficacity in lowering cartilage declination, easing pain, and, in certain situations, promoting the rejuvenescence of cartilage shape and structure. There's still important to learn about the ideal source of MSCs for the treatment of OA, whether it comes

from the umbilical cord, adipose towel, or bone bones. The salutary goods of MSC- grounded remedies on OA cases were verified by a routine evaluation that examined 61 out of 3172 papers with roughly 2390 OA cases; still, the study also regarding BM- MSCs, clinical trials proved clinical problems at the 1- time follow- up, with of those trials reporting clinical problems that were noticeably better than those in the control group. still, a strong examination of the data set revealed that while the issues of the remaining eight trials were extremely low, those from six trials were low. also, after one injection, the positive confirmation from the MRI analysis was low to truly poor strength of confirmation. The outgrowth examination of autologous adipose towel- deduced MSCs(AT- MSCs) likewise produced similar results. As a result, the review set up that the restorative eventuality of MSC treatment on clinical enterprises and MRI analysis wasn't well validated. redundant blood tube, and so forth), as well as clinical outgrowth assessments and controlcuratives in between randomized clinical studies. likewise, the information of the superior source of MSCs for the treatment of OA couldn't be demonstrated by routine analysis(Molnar et al., 2022). The authors support the idea that stem cell-rested remedies could be regarded as a necessary treatment for OA when first- line treatments, similar as education, exercise, and body weight operation, have failed, indeed though they've been demonstrated to be safe and doable in the operation of OA.

Diabetes

Diabetes treatment Clinical experiments have employed a variety of stem cell types to treat diabetes (Path et al., 2019). Diabetes mellitus may result from autoimmune reasons (type 1 diabetes), lifestyle decisions and genetic inheritance (type 2 diabetes), or even pregnancy-related hormonal changes (gestational diabetes). Insulin injections are a common treatment for diabetes, but they provide challenges because of their high cost and transient nature24. By directly repairing the pancreatic cells, stem cells can address this issue. Additionally, stem cells are being used to treat diabetes-related ailments such wounds that don't heal (Lopes et al., 2018).

Wound healing

Healing wounds Stem cells have antibacterial qualities because they secrete antimicrobial substances, aid in immune response regulation, and encourage cell proliferation and differentiation at the wound site. Autologous stem cell usage eliminates the risk of immunological rejection (Ayavoo et al., 2021).

Tooth regeneration

Regeneration of teeth Dental reconstruction with MSCs has been the subject of numerous investigations. Dental pulp-derived stem cells (DPSCs) may encourage periodontal regeneration in a canine model, according to a study by Khorsand et al., 2013. Additionally, it was demonstrated that canine DPSCs could be isolated successfully and that they were capable of multi-lineage differentiation and fast proliferation.

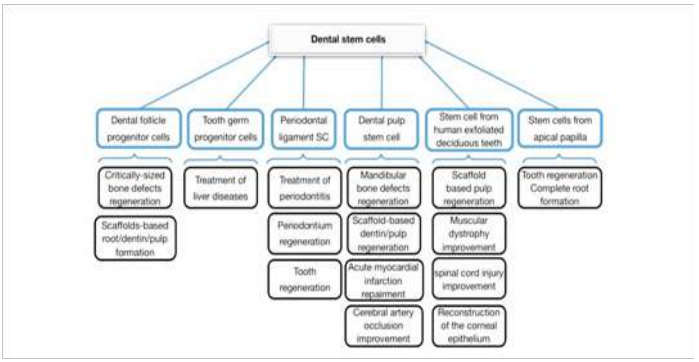

Fig.1 illustrates further uses for dental-derived stem cells.

Limitations, Ethical Concern and Future Perspective

The difference between categorizing embryonic stem (ES) cells as embryos or as specialized body tissues significantly affects their ethical considerations. This classification shapes individuals' perspectives, with some prioritizing the protection of embryos while others assign minimal moral value to early-stage embryos.

Currently, there are three potential sources for stem cells: adult stem cells from donors, ES cells derived from surplus preimplantation embryos, and embryonic stem cells (EG cells) sourced from aborted foetuses.

Harvesting stem cells from naturally abandoned foetuses raises smaller moral issues but faces practical challenges due to the difficulty of segregating functional EG cells from spontaneously abandoned tissue. Roughly 60% of robotic revocations affect from fatal anomalies, with chromosomal abnormalities linked in 20% (Boue et al., 1975).

The use of ES or EG cells attained from optional revocations raises specific moral questions for those opposing revocation or the destruction of early embryonic life. This dilemma leads to enterprises of conspiracy with perceived wrongdoing, particularly in religious surrounds.

When the potential for reproduction of a physical cell is contrasted with the implied growth of an embryo, it becomes clear that the former may have more experimental potential. The source of stem cells, embryonic fragment cells, naturally aid in the formation of new tissue. Nevertheless, these cells are pluripotent rather than totipotent as they are unable to generate necessary structures for additional development when isolated from the embryo.

Advanced technology might potentially render these cells totipotent, as demonstrated in a 1993 Canadian study using genetically altered embryos and mouse stem cells to grow and colonize an entire organism(Nagy et al., 1993).

An endless supply of specialized cells for medicinal applications is now possible because to the creation of iPSCs, which have made it possible to extract stem cells from a patient's cells. IPSCs can be genetically matched to the patient, which significantly reduces the risk of immunological rejection and allows for personalized treatment for a variety of diseases.

New treatment options for hereditary illnesses and genetic disorders are made possible by combining stem cell technology with gene editing methods like CRISPR-Cas9.

Gene-edited stem cells can be used to treat genetic diseases and lessen the chance that dangerous mutations would be passed on to future generations.

Conclusion

In conclusion, after decades of research, the treatment of stem cells has become an astounding medical revolution. The possibilities for stem cells increase with each experience, but there are still many challenges to solve. In any case, stem cells have a major impact on transplantation and regenerative medicine. Currently, stem cell treatment provides prospects for handling neurodegenerative disorders. The use of patient cells is possible via induced pluripotency. The organizational banks are gaining popularity as they collect cells, the source of regenerative therapy in the fight against current and future diseases. We are more capable than ever of extending human life thanks to stem cell therapy and all of its restorative advantages.

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MICROBIAL BIOREMEDIATION OF METALLOID-CONTAMINATED ENVIRONMENTS: A SHORT REVIEW ON REMOVAL BY MICROORGANISMS

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Abstract

The permanence, bioavailability, and toxicity of metal ions make heavy metal contamination a serious environmental problem. Even though chemical and physical remediation methods have been around for a while, they frequently have drawbacks. The application of living organisms for environmental cleanup, or bioremediation, has drawn more attention. Microorganism-based biotechnological methods have previously demonstrated notable progress and are still being developed; different microbial strains can efficiently eliminate heavy metals. The main traits of heavy metals and how they interact with microbes are examined in this research. It describes the fundamentals and varieties of bioremediation techniques, including successful pilot-scale bioreactor investigations, and highlights the mechanisms underlying microbial resistance. Due to their innate resistance mechanisms and natural adaptation to pollution, special attention is paid to native bacteria that have been isolated from contamination. Additional benefits of using bacterial biofilms or consortia include increased resilience and the capacity to utilize several metabolic pathways, which allows for the remediation of multiple heavy metals at once. It also explores how emerging technologies like metagenomics, genetic engineering, and nanotechnology hold promise for enhancing bioremediation

procedures. To sum up, bioremediation is a very viable and sustainable way to deal with heavy metal pollution and improve the health of the ecosystem.

Keywords: *Bioremediation, contaminated, heavymetal, microorganisms.*

Introduction

Industrialization has significantly contributed to environmental pollution, introducing various hazardous was tesintoeco systems. Microorganisms shows an important role in determining the fate of these contaminants by influencing their characteristics, transformation, distribution, transportation, and mineralization. They show efficacy in cleaning up a variety of environmental pollutions, like heavy metals (HMs) and organic chemicals.While organic pollutants can been tirely broken down and removed from the environment through microbial metabolism, because they are not biodegradable, heavy metals continue to exist. Rather, microbial activity can change heavy metals into forms that are more mobile, bioavailable, or less poisonous. Furthermore, metals can be more easily removed from contaminated settings because microorganisms can adsorb and concentrate them within their cells. This makes the macritical tool in addressing pollution and offers significant practical applications. 22 heavy metals havesome biological significance out of 53 heavymetals present.Because of their poor solubility, elements such as cerium (Ce), tin (Sn), zirconium (Zr), thorium (Th), and gallium (Ga) have little biological impact (Fashola MO et al., 2016). Biochemical, physiological, and metabolic activities depend on trace metals like molybdenum (Mo), manganese (Mn), and iron (Fe) functioning as enzyme cofactors, micronutrients, and osmotic regulators. Even though they are hazardous, metals like vanadium (V), nickel (Ni), copper (Cu), chromium (Cr), cobalt (Co), wolfram (W), and zinc (Zn) are nonetheless important trace elements. Others, likemercury (Hg), uranium (U), lead (Pb), arsenic (As), cadmium (Cd), silver (Ag), cadmium (Cd), and antimony (Sb), are mostly hazardous and offer little advantages. Lead (Pb), As, Cd,Cr,Cu,mercury (Hg), and Zn are common pollutants in contaminated areas (Isakovski MK

et al., 2020). Because of pedogenetic weathering processes, heavy metals are present as insoluble formsat trace levelsin the environment naturally.But because of the disruptions caused by human activity to these natural geochemical cycles, metal buildup has accelerated to levels higher than background values. Heavy metal-containing wastes are released directly intotheenvironment by industrial processes such as mining, surface finishing, energy production, metallurgy, and electroplating. Additionally, trace levels of hazardous metals like Cd and Pb are frequently present in soils along with metals like Mn, Cu, Co, Fe, Ni, Zn, and Mo that are introduced by fertilizers. Animal wastes used as fertilizers in agriculture frequently include elevated levels of Zn and Cu from growth promoters in pig and poultry farming, and historically, pesticides containing Cu, Hg, Mn, Pb, or Zn were commonly utilized(Volarić A et al.2018). This chapter aims to discuss heavy metals present in various formsin the environment.Further describe the general as well as microbial mechanism used to removethe heavy metal-polluted water.

Heavymetals present intheenvironment

Although heavy metals (HMs) naturally arise in the environmentand are essential for various biological processes, their accumulation in living organisms can be harmful. Common environmental contaminants include As,Cd, Hg,Ni,Cr,Pb,and Cu(Harischandra et al., 2019). Cd enters the atmosphere through both humanand natural activities, with exposure occurring via contaminated food, air, or water. It accumulates in soils and sediments due to industrial waste, absorption, and surface runoff but provides no benefits to plant growth or metabolism while posing severe health risks (Hayat et al., 2018).

Mercury is one of the most toxicHMs present in the biosphere, with its levels rising due to human activities. When it interacts with aquatic sediments, it forms methylmercury, a highly toxic compound that contaminates the food chain through fish and seafood, leading to neurological disorders in humans upon ingestion (Gworek et al., 2020). Similarly, lead, though naturally occurring in trace amounts, is increasingly released into the environment due to mining, manufacturing, and

fossil fuel combustion. Excessive lead exposure, particularly in children, causes severe health complications when ingested through lead-contaminated dust or other sources (Loh et al., 2016).

Manganese (Mn), which exists in multiple oxidation states, is abundant in nature. The combustion of gasoline additives such as methylcyclopentadienyl manganese tricarbonyl (MMT) results in the emission of manganese oxides. While Mn is crucial for physiological functions, excessive exposure leads to toxicity (O'Neal and Zheng, 2015). Cr is primarily found in the environment as Cr (III) and Cr (VI), with the latter being highly toxic.

Industrial activities can convert Cr (VI)in to the lessharmful Cr (III). Cr industrial use contributes significantly to environmental contamination, with ferrochrome industries being a major source (Coetzee et al., 2020). Co is widely distributed innature, foundin plants, soil, rocks, and water, and use dinalloys. However, tiny amountsofCo are generally harmless, Excessive exposure can be deadly and extremely poisonous.Ni is a naturally abundant element;both natural and man-made sources release it into the atmosphere. Prolonged nickel exposure can result in allergies, respiratoryissues, and diseases like lung can cerand cardiov as cular problems (Genchietal.,2020; Lu et al., 2005). Cu is a vital micronutrient that plays a key role in essential plant functions like chlorophyll production, protein metabolism, and photosyn thes is.However,Cu deficiency disrupts metabolic functions, excessive exposure leads to toxicity.

Zinc is an essential metal that is needed as a cofactor in many enzyme activities. Depending on exposure amounts and techniques, its toxicity varies. Mining and smelting are primary sources of zinc emissions, negatively impacting ecosystemsandlivingorganisms. A poisonous substance called antimony is released into the atmosphere by both man-made and natural activities, such as volcanic eruptions. Workers in industrial areas face antimony exposure, leading to respiratory issues, cardiotoxicity, and even carcinogenic effects. Thorium is discharged into the environment through natural water systems and industrial processes. Because it is

more poisonous than the majority of heavy metals, exposure to it can cause serious health issues (Figure1).

Figure1: Diagrammatic description of environmental heavy metals.

Microbialmechanismfor removal of HMspolluted water

Microorganisms usenumeroustactics to remediate HMs-contaminated soils and play animportant role in metal oxidation, as demonstrated by Thiobacillus ferrooxidans, which promotes the oxidation of metal sulfides, resulting in the release of heavy metals. Through sulfur oxidation, microbes facilitate the conversion of sulfide ores into metal ions via biological leaching. Additionally, some microbes contribute to metal reduction, thereby lowering their toxicity. For example, the removal efficiency of HM-nFeS for Cr(VI) was 12–20% lower than that of DM-nFeS due to variations in their reduction capabilities. The following section delves deeper into the specific microbial pathways involved in heavy metal remediation.

Bioleaching

Bioleaching is a key microbial mechanism for remediating polluted soils. Chemolithotrophs and acidophilesfacilitate the oxidation of Fe(II) to Fe(III) and the reduction of sulfur (S) to sulfuric acid (H2SO4), aiding in the dissolution of metal oxides and sulfides, which subsequently releases heavy

metals.Microbesactasreducingagentsinbioleaching and can also recover and extract heavymetals. Research has indicated that recovery rates are high, such as 98.7% Cd recovery using Annona squamosa-based absorbents, 100% Cu recovery with Pseudomonas putida, and over 90% Cu and Pb recovery with Enterobacter. Acidophiles and chemolithotrophs contribute by producing acids and oxidizing sulfur compounds, making bioleachingeco-friendly,energy-efficient,and effective at lowertemperatures(Adetunji et al., 2023).

Bioaccumulation and Biosorption

Bioaccumulation and biosorption are essential microbial strategies for remediating contaminated soils. In biosorption, microorganisms utilize their cellular structures to adsorb and sequester heavy metal ions on binding sites located on their cell walls. Various microbes, including Bacillus subtilis, Rhizopus arrhizus, Magneto spirillum gryphiswaldense, and microalgae, generate biosorbents that facilitate heavy metal removal. Bacteria, in particular, are highly effective because of the large surface-to-volume ratio and also the existence of diverse chemosorption sites. Dead bacterial cells, like Bacillus sphaericus, often show even higher biosorption capacity compared to living cells. Bioaccumulation, however, involves actively transporting Ions of heavy metals across the cell membrane into intracellular regions via ionicchannelsandcarrier-mediated transport. Microbes have been shown to bio accumulate metalssuchas Ni, Pb, Hg, Cr, and Cd.Forinstance, Microbacteriumsp. Reduced Cr (VI) up take by 88%, and Microbacterium liquefaciense liminated 90–95%of Cr.These microbes mayuseheavy metalsin the irmeta bolismor detoxify them through enzyme production, making them effective in heavy metal remediation. (Naskar et al., 2020).

Biotrans for mation

Biotransformation enables microorganisms to convert toxic metal ions into less harmful forms. Bacteria employ various mechanisms, such as oxidation, reduction, isomerization, hydrolysis, demethylation, andmethylation to adapt to environmental changes. For instance, Micrococcus and

Acinetobacter oxidize the toxic arsenic species As(III) convert into a less soluble and non-harmful form, while Bacillus species capable of tolerating Cr(VI) reduce it to the less toxic Cr(III). Additionally, processes like biofilm formation and biological chelation aid microbes in mitigating heavy metal toxicity, making biotransformation a crucial strategy for effective remediation (Adetunji et al., 2023).

Biovolatilization

Bio-volatilization is a microbial method in which toxic heavy metals are enzymatically transformed into volatile, less harmful compounds, thereby reducing their presence in soil and water. This mechanism relies on enzymatic reduction and methylation, involving enzymes such as arsenic methyltransferase, mercury reductase, and antimony methyltransferase to convert metals like arsenic (As), mercury (Hg), and antimony (Sb). For example, bacterial methyltransferases modify arsenate As(V) into volatile methylated forms that are released into the atmosphere. Similarly, enzymes like mercurial lyase and MerAin bacteria and archaea facilitate mercury volatilization. Additionally, fungi such as Scopulariopsis brevicaulis efficiently convert As(V) and Hg(II) into less toxic forms (Boriová et al., 2014).

Biomineralization

In biomineralization, microorganisms playanimportant role in mineral formation, aiding in the immobilization of heavy metals. Certain bacteria, such as Sporosarcina ginsengisoli, facilitate the production of calcite, aragonite, and vaterite, while Bacillus subtilis promotes lead (Pb) mineralization by releasing phosphates. Fungi like Penicillium chrysogenum also contribute by mineralizing lead (Pb) and chromium (Cr). Additionally, Pseudomonas putida aids in the precipitation of cadmium (Cd) by forming carbonate and phosphate minerals. Microbes generate extracellular polymeric substances (EPS) and organic acids, while siderophores bind to metals, forming stable complexes that limit metal uptake by plants and immobilize them in the soil, thereby reducing their toxicity(He et al., 2019).

Generalmechanismofheavymetalremoval

Various processes have been proposed to address metalloid pollutionacrossdifferentenvironments,likechemical,physical, and biological approaches. Although all these techniques aim to detoxify and remediate pollutants, bioremediation stands out as an effective option for minimizing the toxic effects of metalloids while enabling their recovery for industrial applications. In recent years, this approach has gained significant attention due to its affordability, eco-friendliness, and ease of implementation.Additionally, bioremediation requires no specializedtechnicalequipmentorskillsandusually doesn't generate harmful byproducts or extra garbage. Among the micro bialagents involved, bacteria playa keyrole in bioremediation because of their small size, high population density, rapid growth, adaptability to diverse environments, and ease of cultivation and management. Keyfactors influencing thee fficiency of bioremediation include soil type, organometallic ions, nutrient availability, the nature and concentration of pollutants, moisture levels, redox potential (Eh), microbial community dynamics, solubility, pH,temperature, and electron acceptors. Bioremediation techniques can be broadly classified into in-situ andex-situ methods. Mycoremediation, which involvesusing fungi, represents an effective strategy for removing metalloids (Zhang et al., 2020). Another approach, known as phytore mediation, utilizesalgae, microalgae, or pollutanthy perac cumulator plants to remediate contaminated areas. A related method, phytobial remediation, combines plants with endophytic bacteria, offering an additional biological approach for addressing pollution in affected regions. Bioremediation utilizes living organisms to decrease the bioavailability of pollutants in the environment and minimize their toxic effects. Metals and metalloids can be removed from contaminated sites through various mechanisms, including bioleaching, bioaccumulation, biotransformation, biomineralization, biosorption, and microbial involvement in chemisorptions (Talukdar et al., 2020). Conversely, bioaccumulation is an active, energy-dependent, and often irreversible process where ions are transported into the cell and retained intracellularly through specific proteins and channels.Recent interest has shifted toward

biotransformation, a process driven by oxidation-reduction reactions.The oxidation states of metalloids, which can be changed by electron gain or loss, have an impact on their toxicity (Sher et al., 2019). Figure 2 shows an overview of the main mechanisms underlying the resistance of bacteria to metals.Their potential in bioremediation applications is being investigated more and more (Table 1).

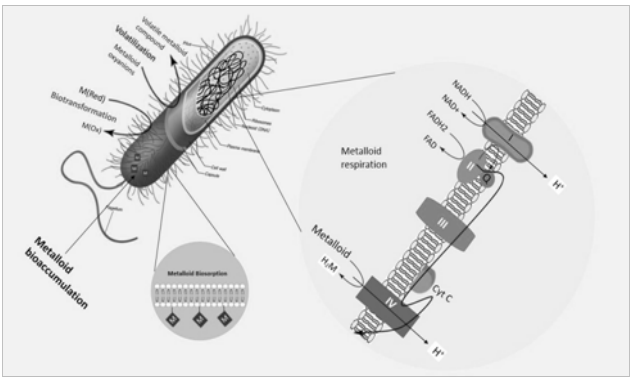

Figure 2: General mechanism of heavy metal removal

Table1: Examples of micro organis msstudied and strategically treated for Heavy metal bioremediation methods

Sl. No.	Speciesname	Heavy metals	Ref.
1	Rhizobium	Zn, Pb, As, Cu, Cd, Hg, and Se	(Pajueloetal., 2008)
2	Rhodococcus,	Cr,Cd,and Pb	(Goswamietal.,2017)
3	Ochrobactrum pseudinter medium	Cr	(Tandonet al., 2020)
4	Rhodococcus	Dye	(Nazarietal., 2022)
5	Lactobacill us plantarum	Cr	(Ameenetal., 2020)
6	Pseudomona saeruginosa	Zn andCr	(Awasthietal., 2015)

Conclusion

Even while research on the bioremediation of metalloids has advanced significantly, little is known about the mechanisms and the particular needs of the microbes. Advanced techniques such as proteomics and metabolomics offer promising avenues for uncovering the detailed processes involved in bioremediation. Additionally, specialized software could be developed to predict the optimal combination of electron shuttles, enzymes, cofactors, nutrients, microbial strains, and physicochemical conditions needed for effective ex-situ bioremediation of contaminated sites. The connection between nitrogen and sulfurcycles and metalloid decontamination, as well as the role of microbes engaged in these cycles in bioremediation, is an intriguing area that warrants further investigation. Furthermore, proteomic and transcriptomic analyses could provide valuable insights into the synergistic interactions among microbes that diminish metalloids, ultimately mitigating their poisonous effects. These approaches could pave the way for more efficient and targeted bioremediation strategies.

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A SHORT REVIEW ON THE SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING PLANT ISOLATE AND ITS ANTIFUNGAL POTENTIAL

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ABSTRACT

Plant pathogenic fungi can be a serious hazard to agriculture worldwide, lowering crop production yields, causing health problems, and having a substantial negative economic impact. Various conventional management strategies are used to control fungal diseases before and after harvest. However, because of their shortcomings and negative impacts on the environment, the manufacture of plant extracts-based metal oxide nanoparticles (NPs) is a viable alternative to the conventional approach. The objective of the study was to use a green method to biosynthesize zinc oxide (ZnO) NPs from different plant components and evaluate the antimycotic activity of these bioinspired ZnONPs against a range of harmful fungi. Because of their small size and unique biological activity, ZnO NPs have garnered a lot of attention from researchers. A straightforward one-step synthesis of ZnONPs can be facilitated by the potentiality of various plant components to decrease and encapsulate biomolecules. The characteristics of ZnO NPs are effectively altered by temperature, pH, and reagent concentrations. Characteristics including morphology, structure, and content are determined

using a variety of analytical methods, including FT-IR, TEM, EDX, zeta potential, and UV-Vis, etc. The antifungal efficacy of NPs against a plant pathogenic fungus was assessed, and microscopic analysis revealed that it damaged the hyphae and spore of a fungus. Future goals embrace the creation of sustainable methods, a greater variety of biological uses, and safety guidelines for the responsible use of ZnONPs.

Keywords: *antifungal potentiality, biosynthesis, plant pathogenic fungus, ZnO NPs*

Introduction:

Due to the recent rapid expansion in the world's population, raising agricultural production (70%-100%) is urgently needed to meet the demands of a persistently growing population (Ghosh et al.,2025). However, economically significant crops can suffer critical harm from plant-pathogenic fungi, reducing agricultural productivity worldwide. Food safety problems and significant pre- and postharvest losses are still prevalent in several developing nations, mainly as a consequence of fungal activity, including the generation of mycotoxin (Nxumalo et al., 2024). For instance, the pathogen Botrytis sp. causes gray mold, which causes large losses in crops including tomatoes, strawberries, and grapes. Blue mold rot, caused by Penicillium sp., damages crops including apples, pears, and citrus and causes significant financial losses during transportandstorage.Mucor sp. is responsible for a considerable quantity of postharvest degradation caused by the soft rot of numerous vegetablesand fruits (Luciano-Rosario et al.,2020; Nxumalo et al., 2024). The long-standing usage of synthetic fungicides is more costly to protect the crops from fungal diseases either before or after harvest. However, overutilization of them has led to increased drug resistance, contamination of the food chain, financial losses, prolonged persistence, residual toxicity, environmental pollution, and negative side effects like diarrhea, liver damage, nausea, elevated body temperature, and renal failure (Lushchak et al., 2018).

Nanotechnology advancements are believed to be able to fix and treat these issues through different kinds of conventional

methods; nevertheless, these technologies have several disadvantages and adverse effects on the environment. According to the literature review, the biosynthesized metal oxide nanoparticles (NPs) have demonstrated remarkable antifungal action against spore-forming plant-pathogenic fungi and effectively inhibit fungal growth, which could pave the way for sustainable agriculture henceforth (Ghojavand et al., 2020). Zinc oxide has drawn a lot of interest due to its potency as an antifungal agent among other nano metal oxides. The key motive for this efficacy is its distinct physicochemical characteristics, which include its capacity to fabricate reactive oxygen species (ROS) in cells, such as hydroxyl groups, hydrogen peroxide, andsuperoxide anions; release zinc ions; and interrelate with fungal cell membranes to disrupt cellular functions. The outside of the microorganism exposed to the ZnONPs directly correlates with the collection of ROS produced (Djearamane et al., 2022; Hernandez et al., 2024). Consequently, plant-based biosynthesis methodologies have been explored as viable substitutes. The presence of several phytochemicals, including amides, terpenoids, phenolic compounds, polysaccharides, polyphenols, and flavonoids, in plant extracts influences particle size, shape, and stability, making them both reducing and stabilizing agents. Theprocess has several benefits, such as being non-toxic, economical, ecologically benign, and generating stable nanoparticles with improved characteristics (Alprol et al.,2023; Ashour et al.,2023; Alprol et al., 2024; Nxumalo et al., 2024).

Thus, the chapter intends to review the capability of environmentally friendly ZnO nanoparticles as a successful and sustainable antimycotic agent in agriculture. The chapter also discussed produced nanoparticles that were described using several methods, such as UV-Vis, zeta potential,FT-IR, SEM, TEM, XRD, etc. Finally, the antifungal characteristics of ZnO nanoparticles derived from plants and their activity against a few plant-pathogenic fungi were described.

Biosynthesized nano zinc oxide:

Numerous investigations on the synthesis of various inorganic metallic oxides, along with TiO2, ZnO, and CuO, have been reported. ZnO has drawn the most attention among these

due to its inexpensive production costs, ease of synthesis, and safe handling. n-type semiconductormetal oxide that has significant antimicrobial qualities and a wide variety of nanostructures (Niser et al., 2019). Moreover, having a higher surface-to-volume ratio and diverse morphologies, ZnO nanoparticles can stop any microbial growth. As stated in studies, while subjected to UV light, zinc oxide nanoparticles can generate significant amounts of H2O2, which causes oxidative stress that is detrimental to fungal cells. Ultraviolet (UV) light may also be absorbed by ZnO, and its nanoform can exhibit antifungal properties by producing Zn2+ ions in microbial conditions. This mechanism has a significant effect on the metabolism of amino acids and disturbs the fungal enzymatic systems (Ghosh et al., 2025). The thorough examination and understanding of materials and structures at the nanoscale,typically between 1-100 nm in diameter, is known as nano-characterization. Accurate characterization is pivotal for various scientific, commercial, and technological applications because the properties and behaviors of materials at this scale might differ significantly. This has been accomplished through the development of a variety of advanced tools and methods, together referred to as nano-characterization instrumentation, including Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), X-ray diffraction (XRD), transmission electron

Plant	**Plant parts used**	**Zn precursor**	**Shape**	**Size (nm)**	**Causative agent**	**Disease**	**Ref.**
Nigella sativa	Seed	Zinc sulfate	Wurtzite Hexagonal structure	-	*Cercospora canescens*	Disease in mung bean plants (*Cercospora* leaf spot)	Aftab et al., 2025
Trachyspermum ammi	Seed	Zinc sulphate	Hexagonal wurtzite structure	15-20	*Cercospora canescens*	Disease in mung bean plants (*Cercospora* leaf spot)	Rafiq et al., 2022

Terminalia bellerica	Leaves	-	Spherical to hexagonal	20-30	*Alternaria brassicae*	Blight disease in *Brassica juncea*	Dhiman et 2021, 2022 2023
Ocimum americanum	Leaves	-	Wurtzite structure	45–50	*Aspergillus niger*	Black mold, fruit rot	Vidhya et 2020
Eucalyptus globules	Leaves	Zinc nitrate (with hexahydrate)	Mostly spherical	52–70	*Diplodia seriata, Alternaria mali, and Botryosphaeria dothidea*	Diseases in Apple Orchards	Ahmad et 2020
Syzygium aromaticum	Flower bud	Zinc nitrate hexahydrate	Triangular and hexagonal	30-40	*Fusarium graminearum*	*Gibberella* ear rot diseases in cereals and *Fusarium* stalk rot, head blight	Lakshmee et al., 2019

microscopy (TEM), etc. (Mohammed et. al., 2024). The way through which zinc oxide nanoparticles (ZnONPs) interact with microorganisms is dependentonnumerouspoints, including:

Surface Area and Particle Size: Because of their smaller size ZnONPs (10–50 nm) withlarger surface area-to-volume ratio interact more with microbial cells.

Shape & Morphology: In general, spherical and rod-shaped ZnONPs work better than irregular ones.

Surface Charge & Functionalization: Cell disruption of fungi results from the greater interactions of positively charged ZnONPs with negatively charged fungal membranes.

Concentration: More reactive oxygen species (ROS) are produced at higher ZnONP concentrations, which boosts antifungal action.

Plant extracts contain a variety of active biomolecules that stabilize and decrease nanoparticles. Earlier investigation has revealed that synthesis has used several plant extracts,

such as Azadirachta indica, Aloe vera, Vitex trifolia, Moringa oleifera, and many more extracts that have been shown to exhibit antifungal action in ZnO nanoparticles made from them. Plant parts like flowers, leaves, roots, stems,seeds,fruits, barks, rhizomes, peels, and shells have been utilized in this process, demonstrating the adaptability and variety of green synthesis techniques (Verma et al., 2025).

Antifungal potency of plant-moderated ZnONPs:

The antifungal capabilities of phytofabricated ZnONPs have attracted a lot of attention as a result of their increased bioactivity, eco-friendly synthesis, surface functionalization, surface activity, bigger area, size, shape, and structure. Since nano zinc oxide exhibits antifungal qualities, different kinds of fungal strains, Penicillium sp., Fusarium sp., Colletotrichum sp., Aspergillus sp., Rhizoctonia sp., and others, have been used to study its antifungal potential in vitro (Table 1). There is still much debate on the antimycotic mechanism of metallic oxide nanoparticles.

Table1: Antifungal Potentiality of Plant-Based ZnO nanoparticle against phytopathogenic fungi

However,the mechanisms of antifungal activity of ZnO (Figure 1) are as follows(Nisar et. al., 2019; Ghorbani et. al., 2022; Gharpure et. al., 2022):

Creation of Reactive Oxygen Species (ROS):Producing ROS (hydroxyl radicals, superoxide anions, and hydrogen peroxide) via ZnO NPs disrupts the normal operation of numerous intracellular organelles and causes cell damage.

Disruption of Cell Membrane:ZnO NPs may interact with the fungal cell membrane, enhancing its permeability, which induces the leakage of vital biomolecules and, ultimately, to the cell death.

Inhibition of Enzymes, Proteins, and DNA: ZnONPs can influence the function of intracellular organelles and damage all biomolecules, including proteins and DNA, in the fungal cell by causing oxidative stress. By disrupting the electron transfer chainand destroying cellular enzymes, nanostructures can also lead to fungal death.

Release of zinc ions:Fungal metabolism is disrupted by the gradual release of Zinc cations from the dissolution of nano ZnO on the cell membrane surface, affecting enzyme activity and cellular processes.

Figure 1: Mechanism of antifungal activity of ZnO NPs

Future prospects:

The employment of bioinspiredZnO NPs in antifungal applications is gaining popularity because of its strong antimicrobial capabilities, biocompatibility, and environmentally benign manufacturing.

Future studies can focus on:

- Enhancing the bioavailability and stability of ZnO NPs for usage in medicine and agriculture.
- Optimizing synthesis techniques to produce consistent ZnO NP size and shape, which has an immediate consequence antifungal efficacy.
- The occurrence of ecological friendly nano-fungicides to fight fungal diseases in crops.
- Toxicity issues related to ZnONP accumulation in living things.
- More investigation is mandatory to find out the long-term impacts on both the nature and human beings.

• ZnO nanoparticles may be more successful while incorporated with other antifungal agents.

• Cost-effective production requires the development of large-scale green synthesis techniques.

Conclusion:

Green-synthesized ZnONPs hold promise as an efficient and environmentally friendly way to support biological activities. Their cost-effective, straightforward, and environmentally benign nature makes them a desirable substitute for conventional methods. A sustainable method of producing ZnO-NPs is offered by the usage of plant extractions as reducing and stabilizing representatives, which also removes the necessity for hazardous chemicals. These NPs showed great potential against plant-pathogenic fungi. The ZnONPs to preserve crops before and after harvest can satisfy the growing demand for food by managing new diseases and reducing waste in an environmentally conscious way. Thus, this strategy can greatly increase food security by reducing losses before and after harvest and securing the quality andsafety of goods during storage and transit. Consequently, our results suggest that utilizing plant extracts to produce ZnO NPs offers a viable way to counteract resistant and developing plant diseases. Additionally, this strategy might be seen as a favorable way to deal with the current environmental problems.

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ECOTOXICOLOGICAL IMPACT OF BIOINSPIRED SILVER NANOPARTICLES: A SHORT REVIEW

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ABSTRACT

The exponential progress of nanotechnology has garnered a huge technological and scientific interest due to its many uses in devices and functional materials. Owing to various attractive physicochemical properties, nanoparticles differ from bulk material, leading to the reaction with everycomponent of the environment to a great extent.As a result,their production and release into the environment increase simultaneously. However, there isa deficiency of knowledge of the potential risk of nanoparticles because many researches are either in early stages or are technology-dependent. Very few frameworks with a globally unified policy are modeled around the environment and living organisms. A holistic way of addressing the potential risk of nanoparticles ought to be accounted for. Considering the fact,the objective of our studies is to asses the potential risk of biogenic nanoparticles in the environment. It also examines the state of the art in the future application of bioinspired AgNPs without any environmental risk.This critical review is based on literature. However, a pool of such discourses and their contextual evidence are rarely available in the literature. This review article describes the impact ofbio-inspiredsilver nanoparticles on every component of the environment. This discourse also

focuses on the assessment of the toxicity of sustainable green synthesized AgNPs. It is centered on recent advances and future considerations for the application of AgNPsalongside the probable challenges associated with their use.

Keywords: *Bioinspired Nanoparticles, physicochemical property, ecotoxicity, environmental risk, risk assessment.*

Introduction:

In the last three decades, a huge expansion of nanotechnology has been creating intense curisity, spreading novel application in myriad sectors and becoming pivotal in research. Among various types of nanoparticles, silver nanoparticles (AgNPs) are vastly used in medicinal biology, agricultural applications, and the industrial sector.The sustainable application ofnanoparticles in the formulation of agrochemicals is responsible for revolution in agricultural fields by reducing the necessity of pesticides. These particles are also attributed to minimizing environmental stresses, controlling nutritional loss, improving crop yield, protecting plants from pathogens, and altering their life cycle patterns. Moreover, AgNPs help in the drug delivery system by treating cancer cells with little side effects. These particles also act as protective shields in food preservation and against ultraviolet radiation. AgNPs contribute to the diagnostic procedure and purification of heavy metals from wastewater.

To meet the increasing demand for nanoparticles, various chemical and physical synthesis processes like electrochemical reduction, microwave irradiation, heat-induced evaporation, etc, have been evolved. These methods are mostly energy-intensive, complicated, and expensive andrelease a large number of toxic byproductsinto the environment.However,the green synthesis of nanoparticles has drawn popularity as an alternative to traditional methods, becoming economically beneficial, eco-friendly, and simple (Awasthi et al., 2022).

Due to the fast and wide progress of 'Nanotechnology' with huge production and diversity of nanomaterials application, there is important to concern about its biological effects, cellular interaction and toxicity for plants, animals and ecosystems.The ecotoxicological effectof AgNPs on

ecosystemsis not clearly explained yet (Tsepina et al., 2024). Therefore, this articlebriefly overviewed the interaction of AgNPs at a molecular level with every component of the ecosystem for a long period. It is essential to explore the existing gapconcerning the risk assessment of exposure to silver nanoparticles.

The swift commercialization of nanomaterials is anticipated to increase the release of silver nanoparticles into the environment, potentially posing a health risk to humans(Wong et al., 2023). AgNPs provide a dual-edged sword – they may be applied to mitigate environmental pollution,but they may also be responsible for toxicity to the environment. These particles manifest themselves in the ecosystem through several pathways –

a) Intentionally anthropogenic silver nanoparticles

b) Unintentionally, nanoparticles from industrial process

c) Naturally generated nanomaterials

The process and trajectory of generation determine how nanoparticles affect biological things. Additionally, these are controlled by their physicochemical characteristics and environmental accumulation potential(Arora et al., 2024).

Around the world, ongoing researchisattempting to minimize the toxicity level associated with nanoparticles. This chapter reviewed the eco-toxicity of plant-based AgNPs on the ecosystem.This chapter also delves into the direct interaction of AgNPs with living beings. To explore the impact of AgNPs on components of the environment, we elaborate on the detailed stability and potentiality of nanoparticles through this chapter. Future research should maintain a balance in the application of nanoparticles for the well-being of the ecosystem by several eco-friendly norms.

Positive Impact of silver nanoparticles on the environment:

Some characteristics, such as huge potential of reactivity and especially nanoscale (1-100 nanometers) dimension, high surface ratio to volume, etc, enable AgNPs to be suitable for

applications in catalysis, antimicrobial activity, drug delivery, and imaging (Cheng et al.,2023).

(1)As an antimicrobial agent:

Silver nanoparticles exhibit both broad–spectrum antimicrobial properties and synergistic effects in combination with certain antibiotics. The following steps are followed inproteomic mechanisms of antimicrobial action of AgNPs:

- Reactive Oxygen Species (ROS)production
- DNA damage
- bacterial cell rupture
- protein synthesis inhibition

The application of AgNPs in new therapeutics to combat bacterial resistance represents a substantial advancement in Nanotechnology.' Current applications of nanomedicine benefit patients in terms of safety, infection control, and efficacy.

(2)As an antibiofilm agent:

Bacteria in the planktonic state are dispersed in search of nutrients in the microenvironment in a community or attached to surfaces together, known as biofilm. In biofilm, bacteria can tolerate the harsh conditions by an extracellular polymeric matrix that helps in adhesion.These biofilms infectthe surgical site of patients as well ascause a longer recovery time that increases treatment costs or sometimes results in death (Bajire et al.,2023)

Asbiofilm agents, AgNPs commercially act as a coating material of various medical substances like catheters, wound dressings, etc, to prevent the spreading of infection (Al-Sawarees et al., 2024). Three distinct steps are governed by AgNPs for controlling biofilm growths:

(a) AgNPs being charged interact with the surface of biofilm.

(b) AgNPs enter into the biofilmsliable on the surface chemistry, charge,size, and concentrationof nanoparticles

as well as the surface composition of biofilm.

(c) AgNPs subsequently penetrate the biofilm and engage with its various components. (Chauhan et al., 2023).

On the other hand, from the literature, bacterial resistance to AgNPs is also noted. Some mechanisms, such as chemical detoxification mechanisms, active efflux systems, etc., contributeto bacterial resistance to AgNPs. Bacterial resistance to AgNPs involves multiple strategies to counteract silver toxicity. Bacteria induce AgNP aggregation and reduce Ag^{+} to Ag^{0}, minimizing its reactivity. They prevent silver entry by modifying membrane permeability and expel Ag^{+} ions via efflux pumps. Defense mechanisms are activated to neutralize toxicity while cell damage is repaired. Biofilm formation acts as a shield, surface charge alterations reduce silver binding, and stress responses enhance bacterial survival.

According to Li and Xu (2024), this mechanism involves a series of interrelated systems. When cells are exposed to sublethalconcentrations of AgNPs,oxidative stress and damaged DNA trigger the development of bacterial resistance phenotypes.

(3)As a dye removal agent from wastewater:

The world is grappling with challenges related to the contamination of water with dyes. Recently, dyes have beenuniversally recognized as carcinogenic substances, contributing to elevated BOD (Biological Oxygen Demand), reducing photosynthesis activity, hampering the growth of plants, and decreasing light penetration into aquatic ecosystems (Ardila-Leal et al., 2021). The bioinspired AgNPs exhibit remarkable catalytic properties in degrading dyes where NaBH4 (Sodium hydroborate) is a reducing agent. In this process, electrons are moved from the borohydride anion (BH4-) to the dye molecules. This electron triggers the degradation of dyes by targeting the chromophore ring and making it colorless.

NegativeImpact of Silver Nanoparticleson the Environment:

Up to a certain level,AgNPs are not regarded as toxic but beneficial in many aspects. Beyond the permissible limit, silver nanoparticles are regarded as a toxic compound to

the ecosystem, though the toxicity of AgNPs and Ag+ is not the same.The vast application of AgNPs raises the risk to the environment and the ecotoxicological effect on living organisms over the last decades. The toxicity level of AgNPs conditionallydepends onthe source of exposure, their highest level of accumulation in every taxon, and the biological community that consumes it.The Environmental Protection Agency (EPA) and the World Health Organization (WHO) have set the Maximum Contamination Limit (MCL) at 0.1 mg/L for silver. However, the Predicted Environmental Concentrations (PECs) of AgNPs typically fall within the range of 0.03- 0.08 mg/L.Moreover, the L(E) C50 value of AgNPs is 0.1mg/L, whichmakes it a deleterious toxic compound in the environment (Ihtisham et al., 2021). After being releasedinto the environment, AgNPs either may be oxidized to generatea more reactive ionic form (Ag+) or are transformed into more toxic species like silver nitrate, silver chloride, etc. Ag of AgNPs can be converted into Ag2S by a sulfidation reaction, while sulfide accelerates the aggregation of AgNPs and forms a chain-like structure.

Ecotoxicological impacts on animals:

The cytotoxicity of AgNPs leads to the generation of huge amounts of ROS, affecting thecytoskeleton, interferingwith DNA repair enzymes, and inducing cell adaptosis (Figure 1). Amr et al. (2023) explained the cytotoxicity potential of AgNPs against human skin fibroblasts.

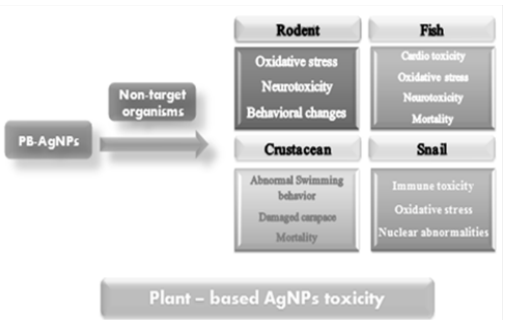

Figure 1: Ecotoxicity of plant-basedAgNPs on animals.

- **On humans:**

The toxic effects of silver nanoparticles are multifaceted and include genotoxicity, cytotoxicity, and effects on the immune system. Therapeutic applications of AgNPs in the human body lead to the damage of erythrocytes (hemolysis, membrane damage, and agglutination).The systemic system can also be affected by deposited nanoparticles in the respiratory tract with the help of the phagocytosis process. Elfsmark et al. (2022) demonstrated that metal–based nanoparticles generate a huge amount ofpro-inflammatory cytokinesand ROS in epithelial cells of human lungs.

- **On fish:**

Studies indicate that exposure to AgNPs leads to a decline in the levels of essential amino acids, including L-histidine, L-isoleucine, and L-phenylalanine, in fish gills. This disruption in amino acid metabolism results in metabolic dysfunction and reduced energy production in the gills.AgNPs also disrupt the citrate cycle as well as energy production by altering the citric acid level. Marinho et al. (2021)observed that activities of acetylcholin esterase (AChE) in both the brain and muscle of zebrafish are substantially reduced by AgNPs.Exposure to AgNPs in adult zebrafish has been linked to hepatotoxicity, increased mortality, DNA damage, histopathological alterations in the gills, and nuclear abnormalities.

Ecotoxicological impacts of AgNPs on plants:

Mitochondria, chloroplasts, and peroxisomes are related to oxidative metabolic activity and electron flow that generateROS in plant cells. The ecotoxicological impacts of AgNPs on plantsare summarizedin Table 1. These ROS alter membrane fluidity and permeability, lipid peroxidation, and bring difficulties in nutrient acquisition. In this way, after introducing into the plant cells, metal-based nanoparticles alter the plant metabolic process by affecting the intercellular organisms. Silver nanoparticles also damage plant cells and DNA, especially breaking DNA strands andcausingchromosomal aberration.

Table 1: Summary of phytotoxic impacts of AgNPs in plants

Nanoparticles	Species Name	Ecotoxicological Impacts	References
Silver nanoparticles	Corbicula fluminea	Severe tissue damageinduced oxidative stressimbalance antioxidant enzyme	
Activities(SOD, MDA, CAT)	Li et al., 2024		
	Oryza sativa	reduces the mitochondrial membrane potential	Ihtisham et al., 202I
	Pennisetum glaucum	induced antioxidative responses	Khan et al..,2020
	Arabidopsis thaliana	affected thylakoid membrane of chloroplasts	Kruszka et al.., 2020
	Zea mays	induced activity of antioxidative enzymereduction of plant growth	Yilmaz et al..,2021
	Stevia rebaudiana	induce in gene expressions of KaurenoicAcid Hydroxylase (KAH) that leads toglycoside's biosynthesis	Ramezani et al., 2020
	Vicia faba	induced a series of changes on leaves likenecrotic spot-on leaf, reduced photosynthetic rate,decreased photosynthetic pigment, inducenon-photo chemical quenching	Falco et al., 2020
	Prosopis juliflora	Reduce photochemical efficiency	Valdez-Salas et al.,2020

Perspectives:

- Conduct studies on the toxicokinetics of plant-based AgNPs in living organisms.
- Confirm the sustainable and safe application of AgNPs from one health perspective.
- Develop robust studies on the biomagnification and trophic transfer of AgNPs.
- Develop new clean technology to fruitfullydecrease the ecological risk related to AgNPs.
- Determine various ecotoxicological matriceslike Effective concentration (EC50), LC50, Lowest-Observed-Effect Concentration (LOEC), and No-Observed-Effect Concentration (NOEC) to accurately calculate the concentration for safe using of PB-AgNPs.

Conclusions:

The biological, chemical, and physical properties of nanoparticles make them demandable in every field of our day-to-day life. Nanoparticles maintain a life-cycle where chances exit to release in the environment at every stage. Despite having advantageous properties of bioinspired AgNPs, these nanoparticles are not exempted from ecotoxicity.Therefore, this bibliometric and systematic reviewprovidesa current understanding of potential risks in deliberately using AgNPs. The plant based AgNPs induced toxic impacts on non-target animals, and safe concentrations of these NPs for living organisms in the one health context should be accurately framed. A lot of advancement has been made in investigating ecotoxicity, but an integrated ecotoxicity assessment approach essentially needs to be developed to support the research on interactions between AgNPs and living organisms. Quantitivelystructured-activityrelationships, integrated biomarker responses, and read-across techniques are some effective tools of computational toxicology applications that will revolutionize the research on the ecotoxicity of silver nanoparticles in the future.

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A COMPREHENSIVE INVESTIGATION OF PUBLIC HEALTH PROBLEMS AND PREVENTIVE STRATEGIES FOR INDOOR AIR POLLUTION

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ABSTRACT

The quality of indoor air (IAQ) is one of the primary factors influencing people's health and wellbeing. The measurement, identification, and potential health impacts of IAQ are currently not well understood by the general public. Microbial spores, pollen, allergens, sulfur dioxide (SO2), carbon monoxide (CO), nitrous oxide (NO), volatile organic compounds (VOCs), particulate matter (PM), microbial spores, pollen, and other airborne pollutants are the main causes of IAQ degradation. The origins of the main indoor air pollutants, their molecular toxicity mechanisms, and their impacts on neurological, cardiovascular, ophthalmic, and fetal health are all covered in this study.Modern approaches and environmentally friendly techniques for controlling and lowering pollution levels are also highlighted, and ongoing projects to improve and manage IAQ are examined, along with their special benefits and possibilities. Children and women are more vulnerable to poor indoor air quality because of their unique physical features and longer exposure durations. A variety of health problems can be brought on by indoor air pollution because it sets off several damage pathways, such as oxidative stress, DNA methylation, epigenetic changes, and gene activation. Exposure increases the risk of early mortality, SBS, acute

lower respiratory tract infections, and low birth weight in exposed populations. However, lung cancer, chronic obstructive pulmonary disease, and cardiovascular problems are the primary causes of disability and early mortality.

Key words: *Indoor Air Pollution, Public Health, Preventive Measures*

Introduction: Due to modern lifestyles and increasing urbanization, people are spending more time indoors—whether at workplaces, theaters, restaurants, retail stores, or homes where they are exposed to indoor air pollutants. Air pollution is one of the biggest environmental hazards to human health, according to the WHO, because of the connection between air quality and health. Up until now, a lot of focus has been on lowering personal exposure to indoor air pollutants, which necessitates examining interior sources and the potential for lowering emissions from them. Consequently, indoor air quality (IAQ) has become a critical concern, significantly impacting human health and well-being in both living and working environments (Cincinelli, A., & Martellini, T., 2017). Numerous scientific studies have established a clear connection between improved air quality and positive health outcomes. Indoor air pollution can have both immediate and long-term effects on health, with severe cases potentially leading to life-threatening conditions. In addition to interior activities and materials, outside influences can affect the concentration of contaminants in indoor air. The interior environment is an extension of the exterior atmosphere, meaning that the quality of the air outside has a direct impact on the quality of the air within, regardless of the level of insulation, even in areas that are mechanically conditioned and ventilated or naturally ventilated. Carbon monoxide (CO) and other non-reactive pollutants can enter an interior space and be exacerbated by unvented gas burners, flawed cooking and heating appliances, fireplaces, tobacco smoke, and automobile emissions from adjoining garages (Vardoulakis, Set al. 2020). However, reactive pollutants that normally originate outside, including ozone (O3) and sulfur dioxide (SO2) rapidly diminish once they reach an interior environment. The presence of people indoors and

their metabolism are linked to carbon dioxide, which is regarded as an indication of air quality in non-industrial indoor contexts including homes, workplaces, and schools. Indicators of the presence of additional contaminants are also found in it. Despite having little to no toxicological effects on humans at low doses, CO2 directly affects human health at greater quantities. CO2 produces respiratory acidosis and hypercapnia at concentrations above 5%, and it can induce convulsions, coma, and even death at values over 10% (Wolkoff, P., 2018).

The majority of human exposure to indoor air pollutants comes from a variety of sources, including emissions from heating appliances, cooking activities, furniture, flooring, wall coverings, paints, glues, waxes, polishes, cleaning supplies, personal care items, and tobacco smoking. House age and size, air exchange rates, door and window openings, building improvements, and external pollutant levels can all have an impact on interior air pollutant concentrations. The volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs) included in cleaning solutions and personal care items are often partly oxidized and condensed, resulting in the formation of small particles.

1. The indoor air quality problems

Indoor surroundings may be at least twice as contaminated as outside ones, according to the most recent research on human exposure to indoor pollution (European Commission, 2003). In fact, compared to the air in a living room, the air on an urban street with typical traffic may be cleaner. Particularly in highly industrialized or heavily populated places, interior air pollution has historically gotten far less attention than outside air pollution (Moreno-Rangel, A et al 2020). The European Environment Agency (2013) notes that as buildings are increasingly walled off from the outside world to save energy costs for heating and cooling, the dangers of prolonged exposure to indoor air pollution have been more obvious in recent years. In many buildings, interior pollutants build up because mechanical ventilation is the only method used to circulate internal air with a significantly lower amount of external air dilution. A recent commission study estimates that nearly three billion people worldwide are exposed to poor indoor air quality (IAQ) daily due to the use of

solid fuels for heating, cooking, and lighting. According to the Royal College of Physicians (2016), household air pollution has a profound impact on the respiratory and cardiovascular systems, significantly contributing to global morbidity and mortality rates(Cincinelli, A., & Martellini, T., 2017).The research also emphasized the substantial role that low IAQ has in the overall number of premature deaths.

The first explicit IAQ recommendations were released by the WHO in 2010 (World Health Organization and WHO Regional Office for Europe, 2010), and other organizations in a number of nations have since embraced them. Although these recommendations were the first attempt to create a broad indoor air quality standard (World Health Organization, 2010) they only addressed a small number of priority indoor air contaminants that were known to have harmful health impacts (Baeza Romero, M. T.et al, 2022) The intrinsic complexity of indoor air pollution and the paucity of data about the long-term health impacts of each pollutant make it difficult to implement a comprehensive quantitative regulation that covers all physical, chemical, biological, and particle exposures.Particulate particles, biological pollutants (algergens, bacteria, mold, fungus, spores, etc.), physical agents (temperature, electromagnetic fields) as well as more than 400 distinct chemical constituents, primarily inorganic and volatile organic chemicals (VICs and VOCs, respectively). Organic emergent compounds released by plastics, medications, or personal hygiene items have been found in interior settings more recently. Apart from the diverse range of indoor pollutants, their prevalence and concentration are influenced by several elements, including outside air quality, the nation's socioeconomic progress, activity types, occupancy, environmental variables, time of year, etc. Furthermore, pollution sources and emission rates are subject to rapid change over time (Luengas et al., 2015). While outdoor pollutants are largely influenced by human activities such as road traffic and industry, indoor pollutants originate from both permanent and occasional sources. Permanent sources include building materials, adhesives, paints, and varnishes, while occasional sources encompass furniture, cleaning and disinfection products, cooking, personal care items, and even human metabolism. Furthermore, indoor gas-phase interactions from other indoor airborne chemicals may result in secondary pollutants (Weschler, C. J, 2006)

IAQ and human health have been shown to be negatively impacted by a number of indoor air contaminants. Toxic metals, microbes, PM, radon, SO2, O3, CO, NOx, and volatile and semi-volatile organic compounds (VOCs) are the primary indoor air pollutants.

1. The primary pollutants found in indoor air

IAQ and human health have been shown to be negatively impacted by a number of indoor air contaminants(Vardoulakis, Set al. 2020). Toxic metals, microbes, PM, radon, SO2, O3, CO, NOx, and volatile and semi-volatile organic compounds (VOCs) are the primary indoor air pollutants(Cincinelli, A., & Martellini, T., 2017).

2.1Particulate matters: Particulate matter (PM) consists of carbonaceous particles combined with reactive metals and adsorbed organic compounds. Its primary components include sulfates, nitrates, endotoxins, polycyclic aromatic hydrocarbons, and heavy metals such as iron, nickel, copper, zinc, and vanadium. PM is typically classified into three categories based on particle size: (i) coarse particles (PM10) with a diameter of less than 10 μm, (ii) fine particles (PM2.5) with a diameter of less than 2.5 μm, and (iii) ultrafine particles (PM0.1) with a diameter of less than 0.1 μm. PM is particularly concerning because it can be inhaled, posing serious health risks to the heart and lungs. Studies have shown that indoor PM levels often exceed outdoor levels. Indoor PM sources include both particles that infiltrate from the outdoor environment and those generated by indoor activities. PM is mostly released into houses as a result of cooking, burning fossil fuels, smoking, using machinery, and domestic hobbies. Due to its capacity to enter both the alveoli and the tiny airways, PM0.1 produced by burning fossil fuels poses a larger health risk than PM10 and PM2.5. Research on the concentration of main indoor pollutants has shown that the biggest contributors of indoor air pollution are cooking and smoking cigarettes, whereas cleaning activities typically contribute less to indoor PM. Smoking is a recognized cause of indoor PM2.5, with concentrations in winter being higher than in summer and estimates of increases in households

with smokers ranging from 25 to 45 μg/m3 (Cincinelli, A., & Martellini, T., 2017).

2.2 VOCs: Volatile organic compounds (VOCs) are gases emitted from liquids or solids and encompass a wide range of substances. One of the most common VOCs is formaldehyde, a colourless gas with a strong odour, which is released from particleboard, plywood, glues, and other building materials. Indoor VOC concentrations are at least ten times higher than outdoor levels, regardless of a building's location. There are four primary sources of indoor VOCs; firstly, human activities such as cooking, smoking, and the use of cleaning and personal care products; secondly, indoor chemical reactions; thirdly outdoor air infiltration through ventilation systems, and fourthly emissions from building materials.Several factors influence VOC concentrations, including building modifications, the frequency of door and window openings, outdoor VOC levels, air exchange rates, and the age and size of the structure. Research has identified approximately 50 different VOCs released during a 90-minute cooking period. Due to their low boiling points and ease of volatilization even at room temperature, the World Health Organization (WHO) has categorized VOCs into four groups. 1st, VVOCs (very volatile organic compounds) with Tb between 50 and 100 °C; VOCs (volatile organic compounds) with Tb between 100 and 240 °C; SVOCs (semi-volatile inorganic compounds) with Tb between 240 and 380 °C; and POM (particulate organic matter) with Tb more than 380 °C.There are typically three major ways to be exposed to volatile organic compounds (VOCs) emitted from consumer products: ingestion, inhalation, or skin contact. Short-term exposure to low concentrations of VOCs causes no significant harm to most individuals, but long-term exposure to certain VOCs is thought to pose a major danger to human health and may even cause cancer. Regarding SVOCs, direct transdermal absorption from the air contributes more than inhaled consumption(Wolkoff, P., 2018).

2.3 Nox: The primary nitrogen oxides, nitrogen dioxide (NO_2) and nitric oxide (NO), are closely associated with combustion sources such as heaters and cooking stoves. Ambient NO and NO_2 concentrations are significantly influenced by local

sources and sinks. Indoor concentrations of these pollutants often exceed outdoor levels when gas stoves and heaters are in use, whereas in buildings without combustion activities, indoor NO_2 levels are typically about half of those found outdoors. NO_2 is considered a major pollutant because NO readily oxidizes to form NO_2 under ambient conditions. When NO_2 reacts with water, it produces nitrous acid (HONO), a strong oxidant and a common indoor pollutant (Soulou, I., 2021) Both indoor and outdoor sources contribute to indoor NO_2 levels, meaning high outdoor NO_2 concentrations from combustion processes or traffic emissions can impact indoor air quality. Studies indicate that proximity to roads significantly affects indoor NO_2 levels, and the extent of air exchange between indoor and outdoor environments also plays a crucial role. Key indoor sources of NO_2 include smoking and the use of combustion-based appliances such as stoves, ovens, water heaters, fireplaces, and equipment that burn wood, gas, oil, coal, or kerosene.

2.4 Ozonethe atmospheric photochemical interactions of O2, NOx, and VOCs mostly create ozone, a potent oxidizing agent. However, because it reacts slowly with the majority of airborne contaminants, it cannot be employed to remove other chemical pollutants from interior environments. Ozone allows for a quick response with a number of indoor contaminants;however, the byproducts of the reaction can harm materials and annoy people. The external environment and electrical device functioning are the primary causes of indoor ozone.Devices that often release indoor ozone gas include photocopiers, air purifiers, disinfection equipment, and other office supplies. These devices' ozone emission processes may be separated into two groups: photochemical and corona discharge. Multiple variables have been found to influence indoor ozone levels: (i) outdoor ozone levels; (ii) indoor emission rates; (iii) air-exchange rates; (iv) surface-removal rates; and (v) interactions between airborne ozone and other chemicals. Depending on the air exchange rate, indoor ozone levels typically range from 20% to 80% of outside ozone levels. Although skin exposure is a known vector, breathing is the main way that humans are exposed to ozone.

2.5SO2: The most prevalent gas in the category of sulfur oxides (SOx) found in the atmosphere is sulfur dioxide (SO2). The main source of SO2 is the burning of fossil fuels, which it mixes with PMs and aerosols to create a complicated mixture of different air pollutants. Tobacco smoke, kerosene heaters, coal or wood stoves, oil furnaces, and vented gas appliances are indoor sources of SO2 emissions. Furthermore, outside air is thought to be a major source of SO2 indoors. Compared to outside levels, indoor SO2 levels are frequently lower. Because SO2 is easily absorbed by interior surfaces and reactivated, indoor emissions are typically low. The hourly SO2 concentration in buildings is reported to frequently be less than 20 parts per billion. Only inhalation exposes humans to SO2, which might affect respiratory function(Wolkoff, P., 2018).

2.6 Biological pollutants: Indoor biological pollutants include house dust, cockroaches, mites, pollen, and allergens such as animal fur and cat saliva, as well as microorganisms like bacteria, fungi, and viruses. These biological allergens, also known as antigens, can be produced by various insects, animals, mites, plants, and fungi. When they interact with specific immunoglobulin E (IgE) antibodies, they can trigger allergic reactions. Both indoor and outdoor environments contribute to allergen exposure. Indoor sources include molds, plants, insects, rodents, furred pets (such as dog and cat dander), and house dust mites, while outdoor allergens originate from sources like plants and airborne molds. Bacteria and viruses are often introduced or carried by humans and animals. Exposure to biological allergens has been linked to sensitization, respiratory infections, allergic respiratory disorders, and wheezing. Additionally, indoor exposure to bacteria and viruses can lead to a range of infectious and non-infectious health issues(Wolkoff, P., 2018).

3. Indoor Air Pollution's Impact on Human Health

3.1 Building-Associated Illness: Over the past decades, declining indoor air quality (IAQ) in buildings and homes has been associated with various symptoms and illnesses. Exposure to inorganic, organic, physical, and biological contaminants indoors—though often at low levels—is

widespread, continuous, and unavoidable. As a result, the impact of indoor air pollution (IAP) on human health has been a significant concern. According to the World Health Organization (WHO), building-associated illness refers to any health condition caused by indoor environmental factors. These illnesses are typically categorized into two main groups: Sick Building Syndrome (SBS) and Building-Related Illness (BRI).3.1.1 Sick Building Syndrome (SBS) (Tiotiu, A.,2020)

A collection of symptoms that are connected to the physical surroundings of particular buildings are frequently referred to as SBS. It is challenging to pinpoint the exact reasons of SBS's acute health and comfort consequences, which manifest when patients spend a specific period of time in a building. In a structure, these impacts might be pervasive or restricted to certain locations. Symptoms have been shown to get worse with the amount of time spent in buildings and to go away with more time spent outside of them. The WHO has classified SBS symptoms brought on by IAP into four groups: (i) irritation of mucous membranes, such as the eyes, throat, and nose; (ii) neurotoxic effects, such as headaches, irritability, and exhaustion; (iii) asthma and symptoms similar to asthma, such as tightness in the chest and wheezing; and (iv) dryness and irritation of the skin, gastrointestinal issues (such as diarrhea), and other issues. Infants, the elderly, those with chronic illnesses, and the majority of urban inhabitants of any age have increased health risks connected with IAP-related SBS symptoms, according to research by the International Labour Organization (ILO). High room temperatures, humid buildings, and insufficient ventilation rates all appear to raise the risk of SBS prevalence. Furthermore, the prevalence of SBS symptoms is significantly influenced by other risk variables, including gender, atopy, and psychosocial factors. (Lin, C. C., at al,2022)

3.2 Acute Respiratory Infection: Because pollutants generally enter the human body by breathing, the respiratory system is often the primary focus of IAP effects. Acute respiratory infections are divided into acute lower respiratory infections (ALRIs) and upper respiratory infections (URIs) based on the region of the respiratory tract that is impacted. URIs are upper

respiratory diseases that are frequently moderate in nature and are brought on by biological pollutants (viruses, bacteria, fungus, fungal spores, and mites). Common symptoms include cough, sinusitis, and otitis media. In contrast, ALRI, an acute lung infection, is brought on by bacteria or viruses and causes inflammation of the lung.A million children under the age of five die each year from childhood ALRI, which has been proven to be 78% more likely to occur in children with IAP. The comparatively greater lung surface area of youngsters may be the reason of this. The risk of having ALRI is two to three times higher for children who live in buildings that use solid fuels than for those that use clean fuels, according to research. IAP negatively impacts the respiratory tract's host defenses against infections, both specific and nonspecific. Additionally, cooking moms have a higher prevalence of chronic bronchitis and respiratory infections that are more severe due to IAP. (Murphy, J., et al,2023)

Cardiovascular Diseases (CVDs): Solid fuel consumption in households releases various contaminants, including particulate matter (PM), polycyclic aromatic hydrocarbons (PAHs), carbon monoxide (CO), heavy metals, and other organic pollutants, all of which have been linked to cardiovascular diseases (CVDs). Exposure to PM2.5 is particularly associated with acute cardiovascular conditions such as atrial fibrillation, myocardial infarction, cardiac arrhythmia, heart failure, and ischemic stroke. PM contributes to CVDs by inducing oxidative stress, systemic inflammation, increased blood coagulability, and autonomic and vascular imbalance. It also plays a key role in the release of endothelins (potent vasoconstrictors), fibrinogen, platelet activation, and increased plasma viscosity, all of which can negatively impact cardiovascular health. Additionally, carbon monoxide (CO) in indoor air influences the production of carboxyhemoglobin, which significantly affects cardiovascular function by reducing oxygen delivery to tissues. Exposure to PAHs and lead (Pb) from cooking fuel has been shown to increase oxidative damage, activate the renin-angiotensin system, and reduce nitric oxide levels, ultimately leading to increased vascular tone and peripheral vascular resistance—both of which contribute to cardiovascular strain. (Radbel, J., et al. 2024)

3 Present-Day Techniques for IAQ Monitoring and Control:

Two-dimensional (2D) nanostructured materials have attracted significant attention over the past decade due to their unique physical and chemical properties, making them highly suitable for gas-sensing applications. These materials exhibit exceptional characteristics, including high surface sensitivity, a large surface-to-volume ratio, and superior semiconducting capabilities. A promising approach for developing high-performance indoor air quality (IAQ) monitoring sensors is the integration of 2D nanostructured materials with materials of different dimensions. This strategy has led to four distinct IAQ monitoring designs by combining 2D nanostructures with 3D bulk materials, other 2D nanostructures, 1D nanostructures (such as nanotubes, nanowires, nanofibers, and nanorods), and 0D nanomaterials (such as quantum dots, nanoparticles, nanocrystals, and nanoclusters). These materials offer advantages in photonic, electrical, and chemical sensing applications due to their high surface-to-volume ratio, unique morphology, and rapid electron-transport capabilities. In recent developments, Van der Waals heterostructures, formed by stacking multiple 2D materials, have been explored for IAQ monitoring (Tran, V. et al, 2020) . Examples include graphene/$MoSe_2$, graphene/ black phosphorus/$MoSe_2$, and graphene/WS_2/graphene. Additionally, layered 2D-nanostructured semiconductors can be integrated with conventional 3D bulk semiconductor substrates, either by direct growth or transplantation, to create innovative gas-sensing devices. These heterostructure-based devices leverage the superior properties of their components, making them highly effective for IAQ detection. A key trend in IAQ monitoring is the development of low-cost sensor networks and systems, with metal oxide (MOx) sensors emerging as one of the most effective technologies. These sensors are valued for their affordability, high sensitivity, ease of use, and compatibility with modern electronics, making them ideal for portable and remote IAQ monitoring. Metal oxide semiconductor gas sensors have shown promising results in detecting volatile organic compounds (VOCs) and carbon monoxide (CO) in indoor environments. Various

metal oxides, including SnO_2, ZnO, TiO_2, In_2O_3, Fe_2O_3, MoO_3, Co_3O_4, CuO, NiO, and CdO, have been successfully utilized in IAQ-sensing applications (Dai, X., et al,2023).

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ENVIRONMENTAL DNA (eDNA) METABARCODING'S POTENTIAL AND DIFFICULTIES FOR MANGROVE RESTORATION IN SOUTHEAST ASIA

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ABSTRACT

Mangrove forests in Southeast Asia are crucial for coastal protection, carbon sequestration, and biodiversity. However, rapid deforestation and habitat degradation necessitate efficient restoration strategies. Environmental DNA (eDNA) metabarcoding offers a non-invasive and cost-effective method for assessing biodiversity and monitoring ecosystem recovery. By analyzing genetic material from water and sediment samples, eDNA provides insights into species composition, including cryptic and rare organisms, which traditional surveys might overlook. This technology enables real-time tracking of restoration progress and ecosystem health. Despite its potential, eDNA metabarcoding faces challenges such as environmental degradation of DNA, taxonomic reference gaps, and data interpretation complexities. Factors like salinity, temperature, and microbial activity influence DNA persistence, potentially biasing results. Additionally, Southeast Asia's diverse yet under-documented biodiversity limits the accuracy of genetic reference databases. Standardization of sampling protocols and bioinformatics pipelines is essential to ensure reliability across studies. Integrating eDNA with conventional monitoring can enhance mangrove restoration efforts by providing comprehensive biodiversity assessments. Addressing methodological challenges will be key to leveraging eDNA metabarcoding as a powerful tool for conservation and ecological restoration in

Southeast Asia's threatened mangrove ecosystems.

Keywords: *Conservation, eDNA, Metabarcoding,Mangrove Restoration, Southeast Asia*

Introduction:

The technique of environmental DNA (eDNA) metabarcoding is used to discover and track biodiversity by analysing genetic material found in environmental samples (such as soil, water, or air). The possibilities and difficulties of employing environmental DNA (eDNA) metabarcoding to track and improve mangrove restoration initiatives in Southeast Asia are covered in the research(Naputo, C. F. P, 2024). It draws attention to the advantages of eDNA as a non-invasive, reasonably priced way to monitor species presence, measure biodiversity, and analyse ecosystem health in mangrove habitats --- areas that are sometimes challenging to sample using conventional techniques. (Wee, A. K, 2023) The potential of eDNA in mangrove restoration lies in its capacity to identify a variety of species, including cryptic and uncommon organisms, and its use in tracking the effectiveness of restoration initiatives. eDNA can shed light on how well restoration methods work and how quickly biodiversity is returning. But the study also addresses a number of issues, such as the requirement for standardized procedures for data collecting, DNA extraction, and sample collection.

Application of Environmental DNA in mangrove restoration:

The study investigates several uses of environmental DNA (eDNA) metabarcoding in the conservation and restoration of mangroves(Naputo, C. F. P, 2024). By making it possible to conduct quick, economical, and consistent assessments of biodiversity across several sites, eDNA can improve tracking functional recovery(Wee, A. K, 2023). It makes it possible to track changes across the entire community, find rare or cryptic species, and gain a better knowledge of ecosystem connections and species mobility. By tracking key species, their habitat boundaries, and movement ecology, eDNA also supports the design of restoration sites and helps prioritize conservation areas (Naputo, C. F. P, 2024). Furthermore,

eDNA offers early warnings for dangers like invasive species, dangerous plants, and algae that could impair mangrove ecosystem function and biodiversity. Through species-specific qPCR assays, it provides an affordable, repeatable way to identify the existence and abundance of vulnerable taxa, including enigmatic species like dugongs and proboscis monkeys (Foster, N. R, 2020). In order to support adaptation efforts, eDNA also monitors how climate change is affecting mangrove communities. Additionally, by facilitating local engagement in data gathering with little training, eDNA promotes community-based restoration by guaranteeing greater participation and more thorough monitoring. By encouraging early hazard detection and enhancing ecological results, this cooperative strategy, supported by molecular ecologists, promotes a more efficient and sustainable mangrove restoration process.

Challenges:

There are various obstacles to overcome when using environmental DNA (eDNA) metabarcoding for mangrove conservation and restoration. Risks of contamination during sample collection and eDNA degradation in tropical regions, which impacts amplification success, are examples of technical hurdles (Xing, Q,2024). A number of variables, such as pH and suspended particles, affect eDNA filtration techniques, and PCR inhibitors in murky waters make analysis more difficult (Basyuni, M,2021). Furthermore, the design of particular primers is crucial for effective amplification, particularly in biodiverse mangrove habitats. Methods still need to be standardized because variations in water types and sampling strategies impact taxon detection and make it more difficult to compare research. Data on biodiversity in Southeast Asian (SEA) mangroves is uneven due to the lack of a standard approach(Foster, N. R, 2020. Delineating sampling limits is made more challenging by the dynamic nature of mangrove fauna, which varies depending on factors including salinity and tidal conditions. Furthermore, nothing is known about how eDNA is released and removed in these intricate settings. One of the data disadvantages is the lack of an extensive regional reference database, which

makes it more difficult to accurately identify species. Current databases lack worldwide applicability and are frequently region-specific. Furthermore, further research is needed to develop trustworthy connections when measuring species biomass or abundance using eDNA(Helfer, V., 2018). Lastly, because molecular analyses are expensive and frequently only available in high-income nations, there are still issues with finance, infrastructure, and capacity-building. Widespread eDNA implementation in SEA is hampered by a lack of resources and experience, underscoring the need for improved infrastructure development and training.

Future Prospective and discussion

The possibility of eDNA metabarcoding as a biomonitoring technique for mangrove restoration in Southeast Asia (SEA) is covered in this paper. In order to fill in data gaps, increase capacity, and create an extensive reference database, it emphasizes the necessity of international cooperation. Initiatives that have been successful at the national level, such the Atlas of Living Australia, are mentioned as examples of regional collaboration. International collaborations, such as the Japan-Philippines-Indonesia initiative, show how beneficial teamwork is for improving eDNA research. In order to inform biomonitoring methods, the study emphasizes the significance of standardizing methodology, developing a cooperative platform for data exchange, and carrying out baseline investigations(Cahyani, N. K. D.2024). The study comes to the conclusion that eDNA metabarcoding can help data-driven coastal management and conservation initiatives, particularly in SEA, where cooperation is necessary to achieve restoration objectives and shared coastal resources. While eDNA metabarcoding has great potential to improve mangrove biomonitoring and restoration efforts, its successful implementation will necessitate overcoming a number of obstacles, according to the paper's conclusion on the prospects and challenges of environmental DNA (eDNA) metabarcoding in mangrove restoration in Southeast Asia. Promoting regional and global cooperation to overcome data constraints, increase capacity, and create an extensive reference database are important components. Furthering the application of eDNA

in mangrove ecosystems also requires baseline investigations, the development of cooperative platforms for data sharing, and the standardization of procedures. The study comes to the conclusion that, with consistent international cooperation and funding, eDNA metabarcoding can be incorporated into mangrove restoration projects to support data-driven coastal management, enhance conservation results, and advance the ecological and socioeconomic objectives of the area, such as coastal defense and food security(Helfer, V., 2018).

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