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Advances in Pharmaceutical and Biosciences Research

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First Edition

Dr. R.B. Tripathi
Dr. G. Bhavani
Dr. P. Ponmanickam

Thanuj International Publishers,
Tamil Nadu, India

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Preface

Advances in Pharmaceutical and Biosciences Research have brought about remarkable progress in various fields, including Cancer biology, Pharmacology, Endocrinology, Ecotoxicology, Botany, Agricultural sciences, Entomology and Immunology. The book presents a comprehensive collection of chapters covering diverse topics in the medical sciences, Medicines, Medicinal plants, Health care, Stem cells, Nanotechnology and Gene therapy. We aim to foster scientific curiosity, inspire further researchand contribute to the advancement of knowledge in these fields.

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Environmental Factors with special reference to Carcinogenesis

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Introduction

Cancer is one of the most serious diseases that threaten human being today. Tumor development in humans is considered to be a multistep process analogous to Darwinian evolution, during which normal cells undergo a succession of genetic alterations that results in their acquiring various growth advantages and ultimately their conversion into cancer cells (Hananan and Weinberg, 2000). Cancer is a group of complicated diseases and a genetic disease but environmental and other non-genetic factors directly or in-directly play a role in many stages of carcinogenesis. There are many harmful substrates around us, especially with the development of industry which are potential carcinogens. Major carcinogens, numerous and of varied natures, have been identified in the working environment (asbestos fibres, heavy metals, soot and tar, arsenic, benzene, aromatic amines, vinyl monochloride, ionizing radiation, etc.), and in the overall environment (residential radon, UV radiation, aflatoxin). Several viruses (EBV, HPV, HBV, HCV, HTLV1, HHV8, etc.) have been shown to be carcinogenic, as are some bacteria (*Helicobacter pylori*) and parasites (*Schistosoma*). Certain major carcinogens are of iatrogenic origin, in particular cancer treatments (ionizing radiations, alkylating agents, epipodophyllotoxin), as are, to a lesser degree, some hormone treatments. To date, apart from alcohol intake with respect to esophageal, upper aerodigestive tract, and liver cancer, few diet-related factors have been shown to be carcinogenic.

There are number of environmental factors which are directly or indirectly associated with cancer and are discussed here.

Radiation and cancer

Radiation-induced cancers are a stochastic late effect of ionizing or non-ionizing radiation. They include some leukemia and lymphoma, thyroid

cancer, skin cancer, some sarcomas and some lung and breast carcinomas (Wakeford, 2004). The most important environmental mutagen by far is the ultraviolet (UV) component of sunlight which has been rated as carcinogenic to humans by IARC (IARC Press, 1992). Exposure to UV is a dose-dependent risk factor (Kopf *et al.*, 1984; Gallagher *et al.*, 2005), which can cause skin cancers, mostly basal cell and squamous cell carcinoma. Radiation-induced cancers are a stochastic late effect of ionizing or non-ionizing radiation. They include some leukemia and lymphoma, thyroid cancers, skin cancers, some sarcomas and some lung and breast carcinomas (Wekford, 2004). Ionizing radiation induced DNA damages produce irreversible changes during DNA replication or during the processing of the DNA damage by enzymatic repair processes. Most of these changes take place during the cell cycles immediately following exposure. However, a number of evidences have implicated that the progeny of normal cells exposed to ionizing radiation, for example, Xradiation and UV-radiation, exhibit delayed response including specific gene mutations and chromosome aberrations, termed radiation-induced genomic instability (Wright, 1999). Exposure to radon and radon decay products at home and/or at the workplace are the most widely found sources of exposure to ionizing radiation (Axelson*et al.*, 2002). Breast cancer risk is most increased among girls exposed to chest radiation around the age at puberty, at a time of intense breast development. Different environmental agents responsible of different types of cancer are shown in Table 1.

Biological Agents (viruses, bacteria and other pathogens) and Cancer

The infection-attributable cancers are estimated to be about 18.0% of the global cancer burden in the year 2002 comprising 1.8 million total cases (Parkin *et al.*, 2006). International Agency for Research in Cancer (IARC) has identified six viruses: human papillomavirus (types 16 and 18), Epstein–Barr virus (EBV), hepatitis B virus, hepatitis C virus, human T-cell lymphotropic virus type I and human immunodeficiency virus type-1 (HIV-1) as group 1 carcinogens (IARC Monographs). In addition, bacteria Helicobacter pylori (H. pylori), parasite *Schistosoma haematobium* and liver fluke Opisthorchisviverrini has also been included in group 1 carcinogens. Apart from these established carcinogenic infection there are certain infection such as, polyomaviruse (SV40), Schistosomajaponicum and Clonorchissinensis, which have only sporadically been associated with cancer development. It is estimated that oncogenic viruses are involved worldwide in about 16% of neoplasia (Pisani*et al.*, 1990), with a range from less than 10% in high-income countries to 25% in Africa (Parkin *et al.*, 2000; Talbot *et al.*, 2004). In Western

developed countries, human papilloma virus (HPV), in particular HPV type 16 or 18 (HPV-16, HPV-18), and hepatitis B virus (HBV) are the most frequent oncogenic DNA viruses. These two viruses contribute differently to carcinogenesis: HPV-16 is directly mutagenic by inducing the viral genes E6 and E7 (Song *et al.*, 1999). HBV is a precursor to cirrhosis of the liver and is associated with an increased lifetime risk of developing primary hepatocarcinoma (PHC) (also called hepatocellular carcinoma, HCC), usually 21–32 years later. HBV also may synergize with environmental factors such as alcohol-associated cirrhosis in causing PIIC. Besides, HBV, hepatitis C virus may be involved in PHC, too. Recently, a novel herpes virus (IIHV-8) was isolated which is associated with three neoplastic disorders: Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), and diffuse Castleman's disease. HHV-8 has been found to encode several homologues of cellular genes, which may enable the virus to facilitate cell proliferation (Blaho and Anaronson, 1999). In nonWestern countries, in addition to the abovementioned cancers, Burkitt's lymphoma and nasopharyngeal carcinoma have been shown to be caused by EBV, and Kaposi sarcoma (KS) to be associated with HIV and the Human herpes virus type 8 (HHV-8) (IARC monographs, 1996; IARC monographs, 1997; Griffin, 2000; Pagano *et al.*, 2004). RNA tumor viruses include the hepatitis C virus (HCV) and the unique retrovirus presently known to be oncogenic in humans, the human T-cell lymphotropic virus type 1 (HTLV1). HTLV-1 is directly mutagenic, while HCV, as HBV, is thought to produce oxidative stress in infected cells and thus to act indirectly through chronic inflammation (de Maria *et al.*, 1996; Koike *et al.*, 2002). There are other microorganisms, including selected parasites such as Opisthorchisviverrini or Schistosomahaematobium and bacteria such as Helicobacter pylori may also be involved, acting as cofactors and/or carcinogens (Belpomme*et al.*, 2007). Table 2, summarizes the data related to contribution made by some other biological agents who are responsible for different cancers.

Chemical agents and cancer

There are two classic environmental carcinogens: polycyclic aromatic hydrocarbons (PAH), generated from the combustion of fossil fuels, and aromatic amines, which are present in cigarette smoke and other environmental media. Both PAH and aromatic amines are major etiologic factors in lung, bladder and possibly breast cancer. Carcinogenic residues bound to DNA or surrogate proteins (known as adducts) provide both a fingerprint of exposure and an indicator of procarcinogenic DNA damage. In general, more PAH–

DNA adducts are formed in persons who smoke or are exposed to PAH in the workplace and ambient air. PAH–DNA adducts, especially those formed by the carcinogen benzopyrene diol epoxide (BPDF), have been linked to an increased risk of lung cancer. PAH–DNA adducts in human lung tissue increase sister chromatid exchange formation, and mutagenicity of lung microsomes. Trichloroethylene has been reported to be strongly associated with kidney, liver, esophageal cancers and nonHodgkin's lymphoma (IARC, 1995; Wartenberg*et al.,* 2000; Hansen *et al.,* 2001; Wartenberg*et al.,* 2002; Raaschou-Nielsen *et al.*, 2003) and perchloroethylene with esophageal cancers (Ruder *et al*., 1994; Weiss, 1995). Similarly, smokers have more hemoglobin adducts formed by the aromatic amine 4-aminobiphynyl (4-ABP) which is associated with an increased risk of the bladder cancer (Perera, 1997). As for the malignant disease inhuman hematological system, a variety of chemicals and drugs have been suggested as possible leukegenic agents in human leukemia, but only benzol can be unequivocally implicated. Disturbances of the hematopoietic system, especially marrow aplasia with pancytopenia, in workers chronically exposed to benzol have been recognized for many years. And the overwhelming predominance of acute myelocyticleukemia (AML) or closely related syndromes, often preceded by periods of aplasia with pancytopenia, in such workers provides compelling evidence for an etiologic relationship. The documentation for a link between leukemia and other solvents is not as firm as it is for benzene. Nonetheless, evidence for a link between lymphoma and organochlorine group, such as 1,3-dichloropropene, herbicides (mostly 2,4-dichlorophenoxyacetic acid) and so on, is becoming stronger. Although some studies suggest there might be an association between exposure to pesticides and acute leukemia, MDS, lymphoma, myeloma, and myeloproliferative disorders, the risk from exposure to it appears to be greater for solid tumors than for leukemia. Exposure to chemical substances is also one of the most important conditions potentially associated with cancer development in different sites. This results from the ability of such substances to act as cancer initiators (substances able to cause DNA damage in a single cell) and/or cancer promoters (substances stimulating an altered cell to divide, thus reproducing prior DNA damage). When prevalent in the environment, such cancer promoters can cause the incipient tumor tissue to grow, making this process irreversible and enabling further clinical tumor expression (Le *et al.,* 2002).

Smoking

Smoking is the most important risk factor for cancer, with a marked role in lung cancer etiology, but also recognized as a major risk factor in other cancers also. Cigarette, cigar, and pipe smoking, chewing tobacco, snuff, and exposure to environmental tobacco smoke (ETS or secondhand smoke) are all linked to increased cancer risks. Cigarette, cigar, and pipe smoking have been associated with cancers of the lung, mouth, bladder, colon, kidney, throat, nasal cavity, voice box, esophagus, lip, stomach, cervix, liver, and pancreas, and with leukemia; smokeless tobacco has been linked to cancers of the mouth; and ETS has been implicated in lung cancer. Cigarette smoke contains more than 100 cancer-causing substances. The risk for cancers of the mouth, voice box, and esophagus is further increased among smokers who also drink more than two drinks per day (English *et al.,* 1995; IARC Press, 2004).

Occupational Chemicals

In 1775, Sir Percival Pott reported, cancers of the scrotum which was the first recognized occupational chemically-induced cancer. Now a day, occupational cancers are reported to represent 3-11% of all cancers, but this percentage is probably underestimated and may be as high as 16-22% in men. Harvard Center for Cancer Prevention (HCCP), in 1996, classified 32 substances or industries as carcinogenic in humans (Landrigan*et al.,* 1995; HCCP, 1996). Recently, 28 agents have been considered as definite occupational carcinogens in human, 27 as probable occupational carcinogens and 113 as possible occupational carcinogens (Siemiatycki*et al.,* 2004; Clapp *et al.,* 2005). Among carcinogenic substances, asbestos is a classical example. There is no doubt that asbestos is carcinogenic and induces occupational cancers, including mesothelioma and approximately 11% of lung cancer (IARC Press, 1977; IARC Press, 2002). Likewise, wood-dust-related cancers, although their occurrence is mostly limited to joiners or cabinet makers is limited, are also occupational cancers, insufficiently declared (ethmoid cancers) or even not yet declared (sinus cancers) (Hayes *et al.,* 1986; Blot *et al.,* 1997). Solvents, paints, dyes, gasoline and other petroleum products can also cause occupational cancers. After the leukemogenic effect of benzene was first recognized (Goguel*et al.,* 1967; Surralles*et al.,* 1997), the mutagenic effect of other solvents was established. Moreover mineral oils and lubricants have been associated with some types of cancers, including larynx, skin and bladder cancers (Kane *et al.,* 1984; Mackerer*et al.,* 2003). Phthalates are widely used since the last world war, due to their plasticizing and emulsifying properties. For this reason, they are added to polyvinyl chloride (PVC) in particular in

common medical devices and cosmetics. Di(2-ethylhexyl)phthalate (DEHP) and butyl-benzyl-phthalate have been suspected to be carcinogenic (Shea, 2003). In addition, vinyl chloride monomer, but not PVC, is mutagenic and thus can cause liver angiosarcoma and hepatocellular carcinoma (HCC). A major concern is the risk of childhood cancers following either parental or child exposure to occupational pollutants. Several studies, evaluating the effect of parental exposure to solvents, paints, and gasoline exhaust, showed an increased risk of leukemia and brain tumors in children (Feingold *et al.,* 1992; Smulevich*et al.,* 1999).

Biocides and pesticides

Many of biocides especially those belonging the organochlorines, carbamates and carbinols groups are rated as probable or possible carcinogens, according to the US EPA and the IARC classification (IARC Press 1991) while several are recognized as carcinogens in humans. In children, several epidemiological studies revealed an increased relative risk of cancers associated with parental exposure to pesticides, be it occupational or non-occupational (Zahm*et al.,* 1998). Paternal exposure to pesticides is associated with an excess relative risk of leukemia (Ma *et al.,* 2002), and of central nervous system tumors (Feychting*et al.,* 1998; Cordier*et al.,* 2001) as well as of Wilm'stumors (Fear *et al.,* 1998). A positive link between pesticides and breast or prostate cancers has been put forward in some studies (Charlier*et al.,* 2003; Muir *et al.,* 2004; Ibarluzea*et al.,* 2004; Mills and Yang, 2006). However, a strong association between pesticides and the relative risk of sarcoma (Dich *et al.,* 1997), Hodgkin and NHL (Hardell*et al.,* 1999; Zheng *et al.,* 2001; Hardell*et al.,* 2003) has been found for 1,1,1-trichloro-2,2-bis(pchlorophenyl) ethane (DDT), chlorophenols and phenoxyherbicides. Pesticides can contaminate the body not only through ingestion, but also through air inhalation and skin contact, accumulate in adipose tissue (Lassiter and Hallam, 1990; Geyer *et al.,* 1997), more specifically in fatty breast tissue (Muscat *et al.,* 2003), pass through the placenta (Simonich and Hites, 1995) and accumulate in the milk of nursing mothers (Sharpe and Irvine, 2004).

Food contaminants and food additives

Nitrates, pesticides and dioxins can contaminate drinking water and food. Nitrates are used in intensive farming. They are not intrinsically carcinogenic, but can be endogenously transformed into nitrites by the digestive bacterial microflora, which in turn can be further transformed into N-nitroso compounds (NOC), i.e. into alkylnitrosamines and nitrosamides

through nitrosation (Tannenbaum*et al.,* 1980; Ward *et al.,* 2005). These are highly mutagenic molecules. Secondary or tertiary amines and amides are found as common dietary contaminants (Ward *et al.,* 2005). Long-term exposure to food additives, including nitrite preservatives and artificial azodyes, may be also involved in chemically-induced carcinogenesis, due to their mutagenic properties (Palmer and Mathews, 1986; Weisburger, 1986; Sasaki *et al.,* 2002). In addition, bisphenol A, a xenoestrogen used in plastic food containers, because it can migrate in food and be repeatedly ingested, has been recently suspected to be carcinogenic in humans on the basis of results obtained from animal studies aiming at reproducing breast (Durando*et al.,* 2007) and prostate cancer genesis (Ho*et al.,* 2006; Prins*et al.,* 2007).

Metals and metalloids

Several metals and metalloids have been rated as certain or probable carcinogens by the IARC (IARC Press 1980). Inhalation of arsenic oxides can cause lung cancer, but if swallowed, cancer can develop in the bladder, kidney, liver and lung (SzymanskaChabowska*et al.,* 2002). Thus exposure to arsenic oxides has been reported to be associated with a very large spectrum of common cancer types. Aside from arsenic oxides' exposure, lung cancer has been also reported to be associated with exposure to many metals, including lead, hexavalent chromium and nickel (IARC Press 1990). Furthermore, exposure to hexavalent chromium or nickel has been found to be associated with nasopharyngeal carcinoma, exposure to lead or mercury to brain tumors, exposure to lead or cadmiumto kidney cancer and exposure to cadmium to prostate cancer (Hayes, 1997; Wesseling*et al.,* 2002; Waalkes, 2003).

Diet

There are few definite relationships between food and cancer, several studies has shown that heavy consumption of red and preserved meats, salt-preserved foods, and salt probably increase the risk of colorectal and stomach cancers. There is also evidence that a diet rich in fruits and vegetables may decrease the risks of esophageal, stomach, and colorectal cancers. Being overweight or obese appears to be one of the most important modifiable causes of cancer, after tobacco. Large population studies show a consistent association between obesity and certain kinds of cancer. The strongest links between diet and cancer are in-case of breast cancer in older women, cancers of endometrium, kidney, colon and esophagus. There is strong evidence that physical inactivity increases the risk for colon and breast cancer. The beneficial effect of exercise is greatest among very active people. Together, it is estimated

that inactivity and obesity account for 25 to 30 percent of the cases of several major cancers—colon, breast (postmenopausal), endometrial, kidney, and cancer of the esophagus. Fruits and vegetables rich in antioxidants and other micronutrients have a protective effect against diverse cancers, including lung, esophageal, oral, laryngeal, cervical and breast.

These micronutrients may act through a variety of mechanisms to block DNA damage, mutation and carcinogenesis by oxygen radicals, PAH, and other chemical carcinogens (Perera, 1997). Mycotoxins are toxic fungal metabolites, which are structurally divers, common contaminants of the ingredients of animal feed and human food. Mycotoxins with carcinogenic potency in experimental animal models include aflatoxins, sterigmatocystin, ochratoxin, fumonisins, zcaralenone, and some Penicillium toxins. Among them Aflatoxin B1 and fumonisins are worth paying particular attention to. Aflatoxin B1 (AFB1) is the most potent genotoxic agent. It is mutagenic in many model systems and produces chromosomal aberrations, micronuclei, sister chromatid exchange, unscheduled DNA synthesis, and chromosomal strand breaks and also forms adducts in rodent and human cells. Food contaminated with AFB1 is one of the major risk factors for HCC (Wang, 1999). Most of these carcinogenic mycotoxins, just like AFB1, are genotoxic agents with the exception of fumonisins, which is currently believed to act by disrupting the signal transduction pathways of the target cells.

Fumonisins, fungal toxins produced by Fusariummoniliforme, contaminate maize-based food and feeds throughout the world. Many parts of the developing world rely on maize-based foods as a major staple of their diet, and these populations can be chronically exposed to highly contaminated food. Ecological studies have linked consumption of fumonosin contaminated maize with esophageal cancer in human populations in South Africa and China. The development of biomarkers and their applications in epidemiological studies should be a priority for research on this kind of toxin (Turner *et al.*, 1999).

Environment, pregnancy and cancer

Leukemia is reported to be the second most common congenital malignancy, exceeded only by neuroblastoma. Nevertheless, it has a mortality rate considerably higher than any other congenital cancer. Therefore, many studies focus on the mechanism of catching this kind of disease. A preponderance of evidence, including the presence of high leukemic cell burdens at birth, autopsies of stillborn infants with leukemia, and the diagnosis of leukemia with identical abnormalities in monozygotic twins, supports for in utero-leukemogenesis. Investigation of the association of in utero exposure to

marijuana and other substances found an 11-fold increased risk of AML developing in children whose mother smoked marijuana during pregnancy (Sande, 1999). Pediatric acute leukemia can also be initiated prenatally by illegitimate recombination and fusion gene formation in fetal haematopoiesis, it shows distinct characteristics. Up to 75 % of all leukemia diagnosed under 12 months of age show the gene-recombination involving MLL (mixed lineage leukemia) on chromosone 11q23 (Djabali*et al.,* 1992), but it is far less frequent in leukemia diagnosed in older persons. Taking children acute lymphocyte leukemia (ALL) with TEL-AMLI, fusion gene as example, twin studies suggest that although they share the identical TEL-AML1 genomic fusion sequence, the concordance rate for all in non-infant twins is low (about 20%), which means that they show protracted and variable latency of ALL after TEL-AML1, gene fusion in utero. The striking difference in the postnatal latency period in the present twin pair most probably reflects that necessary secondary postnatal events required for the development of leukemia within the clone of preleukemic cells spawned prenatally were independently acquired at very different times. This situation could arise if such events occur entirely by chance or if they acquire promotion by particular patterns of exposure, such as infection, that can occur intermittently (Greaves, 1999; Ford *et al.,* 1998; Wiemels*et al.,* 1999).

Climate

Climate is one of the elementary and important ecologic environment in which we live, so it is important to study the influence of climatic factors on human diseases. Climatic factors persistently interacted with other ecologic and social factors, and exaggerated the influence of human life on diseases (Acuna-Soto *et al.,* 2002). Recent studies with respect to the relation between climate and cancer mostly focused on global warming, ozone depletion, ultraviolet radiation exposure and their influences on prostate cancer, skin cancer and retinoblastoma etc (Jemal*et al.,* 2000; Bodiwala*et al.,* 2003, Diffey, 2004). Peng *et al*. reported that there were significant relations between average temperature, precipitation and Environmental cancer (EC) and climate (Peng *et al.,* 2003). Studies conducted by Li *et al*. showed that there were positive relations between ultraviolet radiation, temperature and lung cancer, while there were negative relations between temperature, air pressure and liver cancer (Li *et al.,* 2005). Kinoshita et al. suggested that low solar radiation and low temperature might relate to the increasing risk of malignant neoplasm of the pancreas (Kinoshita *et al.,* 2007). There are many ecological studies concerning elevation and cancer, and many of them focused on the increase of

ultraviolet exposure and hypoxemia along with the increase of altitude and their relations to cancer. Moehrle*et al*. believed that in the high altitude areas the increased exposures to ultraviolet were linked with the increased incidence of cutaneous melanoma (Moehrle and Garbe, 1999). Krain*et al*. also suggested that high altitude exposure and/or aviator status correlated significantly with cancerous conditions of the skin, testicles, bladder, and thyroid; other less significantly associated conditions included leukemia, lymphosarcoma and Hodgkin's disease (Krain, 1991). A study conducted in northeastern Italy indicated that male residents living above 200 m were more possibly suffered from cancer of the oral cavity and pharynx, stomach and larynx, while less possibly suffered from cancer of colorectum and brain; women in locations above 200 m seemed significantly at risk for stomach cancer, but protected from cancer of the colorectum and kidney (Bidoli*et al.,* 1993). Our study suggested a negative relationship between altitude and EC, which is accorded with the result of Akhtiamov's study (Akhtiamov and Kairakbaev, 1983). Altitude might also influence other geographic environment such as geology, climate and social environment, thereby affected the EC mortality.

Conclusion: Cancer is a preventable disease

Cancer is largely caused by gene–environment interactions, it is a preventable disease. Furthermore, prevention methods should be just applied to the people who are at high risk, which is economical and effective. There are a number of different genetic and acquired susceptibility factors that modulate individual responses to environmental carcinogens. Modifiable risk factors are given in Table 3, just by changing some habits one can help in prevention of cancer. The prevention measures include (1) purifying the environment, mainly air, water and food supply, which is the most essential to life. However, as the rapid development of industry, pollution is getting more and more serious. Putting the policy of ‘sustainable development’ into practice is essential not only to maintain and restore the ecological balance, but also to improve the public health conditions including those related to cancers; (2) education to mass population to help individuals modify hazardous life styles, such as tobacco smoke, dietary constituents and so on. Smoking is not only the important carcinogen for lung cancer and bladder cancer, but also is linked strongly with the pediatricleukemia that is developed in utero or just postnatal. Tumors most often found associated with maternal smoking in pregnancy or with postnatal exposure to environmental tobacco smoke are childhood brain tumours and leukemia-lymphoma (Sasco and Vainio, 1999). (3) Molecular and genomic biomarkers that can be used for risk assessment and as surrogate end

points in clinical studies; chemo-preventive and dietary agent drug discovery and development; animal carcinogenesis models that mimic human disease.

Table 1: Different environmental agents responsible for different types of cancer

Agents (Environmental)	Associated Tumor Types
Radiations Ultraviolet (UV) rays	Leukemia, lymphoma, thyroid cancers, skin cancers, sarcomas, lung and breast carcinomas skin cancers, mostly basal cell and squamous cell carcinoma, melanoma
Benzene, carbon tetrachloride, chloroform, dichloromethane (methylene chloride), tetrachloroethylene, trichloroethylene and Benzidine	Leukemia, Skin and Lung cancers
Silica dusts, Tobacco carcinogen (Nicotine), Asbestos, Vinyl chloride	Lung cancer
Polycyclic aromatic hydrocarbons (PAHs)	Lung, Skin and Urinary cancers
Metals compounds Arsenic compounds Beryllium, Chromium and Cadmium compounds Lead compounds Nickel compounds	 Skin, Lung, Bladder, Kidney and Liver cancers Lung cancer Kidney and Brain cancers Nasal cavity and Lung cancers
Lead acetate and Lead phosphate	Kidney and Brain cancer
Vinyl chloride	Lung cancers, angiosarcomas (blood vessel tumors), liver and brain cancers
Tamoxifen, Fenretinide	Breast Cancer
Long-term use of oral contraceptives	Breast and Liver cancers
Diethylstilbestrol (DES)	Cervical, Vaginal and Breast cancers
13-cis-retinoic acid	Head/Neck cancer

Vitamin E , Selenium	Prostate cancer
Calcium and Non-steroidal anti-inflammatory (NSAID)	Colon cancer
Cyclophosphamide, chlorambucil, melphalan	Increase the occurrence of second cancers, including leukemia
Cyclosporin and azathioprine	Lymphoma

Table 2: Biological agents associated with Human Cancers

Agent	**Human tumors**
Human papilloma virus	Cervical cancer, anogenital cancer, skin cancer, oral cancer, esophageal cancer, oropharynx cancer
Epstein–Barr virus (EBV)	NHL, HD, Nasopharyngeal carcinoma, Burkitt's lymphoma, Hodgkin's lymphoma
Kaposi's sarcoma-associated herpes virus	Kaposi's sarcoma(KS), primary effusion lymphoma
HTLV-1	Adult T-cell leukemia (ATL)/ lymphoma (ATL)
HIV/HHV-8	KS, NHL, cervical, testicular, Lung conjunctival SCC, multiple myeloma, leiomyosarcoma
Hepatitis B virus	Liver cancer
Hepatitis C virus	Liver cancer
Helicobacter pylori	Stomach cancer, Lymphoma
Schistosomahaematobium	Bladder carcinoma
Opisthorchisviverrini	Cholangiocarcinoma
Schistosomajaponicum	Liver, colorectal cancer
Clonorchissinensis	Cholangiocarcinoma

Table 3: Modifiable Risk Factors

Primary Prevention	Secondary prevention
Healthy Diet	Breast self-examination
Exercise	Testicular self-examination
Tobacco, beetal use	Mammography
Alcohol	Skin examinations
Rest	Pap smears
Sunscreen use/safety	Digital rectal examinations, PSA
Safe sex practices	Colonoscopy
Genetic testing	Fine Needle Aspiration Cytology
Health maintenance: regular check-ups	Other screening tests, e.g., thyroid and liver function, cardiac function (EKG)

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The triangle concepts: Linking your Brain and Gut-Microbiota

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Introduction

The Gut microbiota plays an important role in digestion, absorption of nutrients in humans.(1)It has been found that trillions of bacteria in the gut proves to be essential component for our immune system and for metabolic health, but also on the other hand it influence the development and disease of the enteric and central nervous system, including motility disorders, behavioural disorders, neurodegenerative disease, cerebrovascular accidents and neuroimmune-mediated disorders(1,2). The microbes present in the gut can alter the bioavailability and bioactivity of the drug (3). Recently, neuroscientists have developed adequate tools with which to reveal the bi-directional links between gut physiology and brain function to determine how these links operate under normal and stressful conditions. The complex and multifaceted system of gut-brain communication not only ensures proper maintenance and co-ordination of gastrointestinal functions to support behaviour and physiological processes, but also permits feedback from the gut to exert profound effects on mood, motivated behaviour, and higher cognitive functions(3,4). The linkage between gut functions on the one hand and emotional and cognitive processes on the other is afforded through afferent and efferent neural projection pathways, bi-directional neuroendocrine signaling, immune activationand signalling from gut to brain, altered intestinal permeability, modulation of enteric sensory-motor reflexes and entero endocrine signalling(3). Gut microbiota have emerged as a critical component

potentially affecting all of these neuro-immuno-endocrine pathways. In this review, we discuss the common drugs that distrubts the gut microbiota and eventually alters the well being.

Gut microbiota and its importance.

It has been found that gut microbiota plays an important role in maintaining our metabolic health and immune health(4). Many studies have also recorded that the composition of one's gut microbiota is individually specific and also influenced by genetically(5,6). 1018 microorganisms were estimated to be present in the gut and mostly anaerobes, they are responsible for multiple functions in bowel movement, digestion of food and absorption of nutrients. In a bi-directional manner, the brain and the gut works that could affect each other's function and significantly impact stress, anxiety, depression and cognition(4,5).

In the past few years, the relationship of gutdysbiosis to disease conditions were established. A connection is noted between microflora leading to depression and any permutations that trigger the gut microbiome composition leads microbial lipopolysaccharides production. This, in turn activate the inflammatory response and cytokines send the signals to vagus nerve that links the process to the hypothalamic-pituitary-adrenal axis and causes behavioural effects(6,7).

Drugs that affect the gut microbiota and well being

Proton pump inhibitors are commonly preferred in peptic ulcer, gastroesophageal reflux disorder and stress ulcers. The main mechanism of PPIs is to inhibit the H+K+ATPase at the apical canaliculi of the parietal cell and thereby completely suppress the gastric acid in a dose dependant manner. PPIs are well-established group of drugs in managing the acid-related disorders, but on the other hand it has a potential impact on the gut microbiome and well being. The acid in the stomach serves as a barrier against ingested pathogens and when this barrier is disrupted by PPIs, it affects the balance in microbial communities in the gastro intestinal tract. Many workers suggest that prolonged use of PPIs may lead to a reduction in microbial diversity and potentially favours the over growth of certain bacteria, while diminishing the prevalence of others. PPIs induces changes in the gut microbiome contributes to clinically important diseases. Many studies have proved that changes in the microbiome leads to a decreased colonisation-resistance to enteric infections, including *Clostridum difficile, Camphylobacter and Salmonella*. Increased use of PPIs in early childhood may induce long-term changes in the developing gut microbiome, which can lead to obesity in later life (8, 9,10,11).

Certain bacterial infections like *C.difficile* develop in an altered gut microbial environment following the administration of antibiotics. Antibiotics like Doxycycline, a broad spectrum of antibiotic used in bacterial infections and Metronidazole, is an anti amoebic agent used in amoebiasis and anaerobic infections, when these groups of drugs are used for a prolonged period of time, it causes an imbalance in the gut microbes. Studies have suggested that alterations in the gut microbiome can alter the production of neurotransmitters. Neurotransmitters like serotonin and dopamine play an important role in regulating mood and emotional states (9,11).

Metformin is an oral hypoglycaemic agent used in the treatment of type-2 diabetes. Many studies suggest that some of its beneficial effects are mediated by the gut microbiota. On the other hand, it is clinically well known that up to one-third of patients taking metformin report gastro intestinal sideeffects like diarrhoea, bloating and nausea. It is identified that these type of metformin-induced changes are due to an increase of virulence factors and gas metabolism genes, which is mainly derived from an increase in *E.coli* species(11,12,13,14).

Levodopa, used for the treatment of Parkinson disease, is an example of microbial impact on drug efficacy. Levodopa after oral administration gets absorbed via the small intestine reaches the systemic circulation and crosses the blood brain barrier, where its gets converted to active dopamine in the presence of the human enzyme aromatic amino acid decarboxylase. The bioavailability of levodopa to the brain is a key factor for drug efficacy. In recent studies, it has shown that the microbial decarboxylases produced by the microbes in the gut metabolizes levodopa. Novel bacterial L-dopa metabolism by tyrosine decarboxylases (tyrDCs) has been identified, dominantly driven by *Enterococcus faecalis*. Gut bacterial metabolism of L-dopa not only decreases drug bioavailability, but it also induces adverse effects like hypertensive crisis due to pheripheral conversion of bacterial influenced L-dopa to dopamine and further conversion to m-tyramine(15).

Conclusion

These studies show a complicated interaction between drugs and gut microbes. It also emphasize the need to control the use of drugs to over come the adverse effects. A higher dosage of drugs like PPIs, metformin, antibiotics seems to be associated with large microbial changes that affects the health of an individual. In contrary, for a better bioavailability of drugs like levodopa, the microflora should be suppressed. A balanced Gut microbiome is very

essential for an healthy well being. The use of supplemental probiotics has an promising effects on the balance of microbes in the Gut.

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Mammalian Pheromones

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Abstract

Animals facilitate olfactory communication via pheromones. These are chemical substances released by an individual and perceived by another individual of the same species which evoke specific reactions in the perceiver. Both secretion and excretion of animals act as sources of pheromones. Among the sources, urine, feces, and scent glands are predominant. In this chapter, the classical studies on pheromones particularly, the Bruce effect, Vandenberg effect, Whitten effect, and Lee-Boot effect were discussed. Further, the classification and functional significance of pheromones were also given.

Keywords: Pheromones, chemical communication, types, pheromone effects

1.0 Introduction

Pheromones are chemical signals that are used by members of the same species to communicate information and elicit particular reactions or behaviors in the recipient (Brennan and Keverne, 2004). Martin Luscher and Peter Karlson originally used this word in 1959. This term was derived from the Greek word "pherin" to carry "horman" to excite/ stimulate. These chemical substances have the suitable chemical and physical properties to interact favorably with chemoreceptors of main olfactory and accessory olfactory systems by which they evoke responses in perceivers (Dominic, 1991; Archuman, 2003).

2.0. Classification of pheromones

Three categories such as signaling, priming, and imprinting, have been classified for pheromones based on responses of recipients. Releaser pheromones, or signaling pheromones, cause recipients to behave immediately, usually via the central nervous system. These include marking territory, attracting opposite sex, identifying an individual, aggression, and mother-young interaction. Primer pheromones influence a delayed response to prolonged stimulation in the recipients mediated through the neuroendocrine system (eg. Estrus induction, pregnancy failure, estrus synchronization) (Dominic, 1991).

3.0. Sources of pheromones

All mammals release the chemical messages through urine, feces, saliva, vaginal mucus, and exocrine gland secretions (preputial, clitoral, armpit, interdigital, pedal gland, preorbital gland, flank gland, etc.). Pheromones are emitted by the pheromonal sources that vaporize into the surrounding atmosphere (air-borne). The efficacy of pheromones depends on several factors, including the compound's volatility, stability in the atmosphere, rate of diffusion, receiver olfactory efficiency, and wind currents. Pheromones with high vapor can reach long distances and mediate long-distance communication (Archunan, 2003).

4.0. Pheromone perception

Chemosensory systems such as major olfactory and accessory systems are well developed in mammals for detecting pheromones. The vomeronasal organ (VNO) contains receptors for the accessory olfactory system, which project to the accessory olfactory bulb. The nasal epithelium has receptors for the main olfactory system, which project to the olfactory lobe (Gomez *et al*., 2005). Among these, the accessory olfactory system (AOS) is mainly involved in pheromonal transmission, and the main olfactory system (MOS) is in charge of odor discrimination (Tirindelli *et al*., 1998; Baxi *et al*., 2006). According to Thompson *et al*. (2007) and Baum and Kelliher (2009), the AOS deals with non-volatile chemicals that come into touch with the animals, while the MOS manages somewhat volatile chemical compounds that are airborne.

5.0. Classical Reports on pheromones

i. Bruce effect

The Bruce effect, or pregnancy block, when a recently impregnated female mouse encounters a male mouse other than the one with which she

mated, the pregnancy is aborted. It is caused by a pheromonal substance secreted in the urine of intact males- but not of castrated males (https://quizlet.com/245554776/phermones-flash-cards/?funnelUUID=72bda419-efd2-47b1-ac4b-736da24dd506).

This effect was first noted by Hilda M. Bruce in 1959 and has been mainly studied in laboratory mice (*Mus musculus*). In mice, pregnancies can only be terminated before implantation of the embryo, whereas in other species even late pregnancies are interrupted. The Bruce effect has also been observed in deer mice, grassland voles, and necked lemmings (https://academic-accelerator.com/encyclopedia/bruce-effect).

ii. Lee Boot effect

Lee Boot effect describes how housing females in groups and separating them from males can either inhibit or prolong the estrous cycles of mature female mice (and other rodents) (Van der Lee and Boot, 1956). It is due to the actions of an estrogen-dependent pheromone (2,5-dimethylpyrazine) in the urine of female mice that acts on the vomeronasal organ. According to Jemiolo and Novotny (1994), this pheromone synchronizes or ends the recipient's cycle by lowering the concentration of luteinizing hormone and raising prolactin levels. Spontaneous pseudopregnancy in mice also occurs in the same way. When separated females come into contact with bedding containing urine from other females' cages, they also react the same way. The adrenal glands are required for the production of the urine pheromone which is responsible for this effect.

iii. Whitten effect

Induction of synchronous estrus in a female population through the action of male pheromones is known as Whitten effect. All mammals experience reproductive effects from social signals or stimuli. This is an example of male-to-female pheromonal effects in mice as noted by Wesley K. Whitten (1956, 1966, 1968).

When a male mouse's urine is exposed to a group of female mice, it causes the Whitten effect. The specific pheromones found in male mice urine that influence female reproductive status-controlling hormone systems. The action depends on sex hormones like testosterone produced by sexually mature males. The urine of sexually immature mice failed to evoke this effect (https://www.wikiwand.com/en/whitten%20effect).

If groups of females are exposed to the pheromones of a male, they begin cycling again, and their cycles will be synchronized. A recent study also

strongly proved the role of pheromones in the Whitten effect and rejected the hypothesis that male vocalizations induce female estrus (Wolfl *et al*., 2023).

iv. Vandenberg effect

The Vandenbergh effect is a phenomenon initially described by J.G. Vandenbergh *et al.* in 1975. It is characterized by the early induction of the first estrous cycle in prepubertal female mice as a result of exposure to the pheromone-rich urine of a dominant (or sexually mature) male mouse (Vandenbergh *et al*., 2015).

In a physiological sense, the first estrus is triggered by the production of GnRH after being exposed to male urine. Exposure to adult female mice has also been observed to exhibit the Vandenbergh effect. A prepubertal female mouse experiences a delay in estrus when it comes into contact with the urine of a mature female mouse. It is due to the suppression of GnRH in the prepubertal female mouse (Carlson, 2013).

Pheromones in male urine are the source of the Vandenbergh effect. Urine alone is enough for this effect. It is not necessary of presence of male animals on the site. The vomeronasal organ located in the septum of the female nostril detects these pheromones. This effect happens because a female's biology will only initiate puberty in response to the presence of potential mates. If there's no chance of finding a mating partner, female mice won't waste the energy going through puberty (Sapolsky, 2004).

6.0. Role of pheromones in mammals

6.1. Sex pheromones

It plays a significant role in successful reproduction. When a female releases the sex pheromones, it signals the readiness to mate. In non-primate species, it commonly happens during the estrus period. Males in non-primate species are only permitted during the estrus phase when they release pheromones through their urine, feces, vaginal mucus, and other exocrine glandular secretions (Table 1).

5 alpha-androst-16-en-3-one, which is present in boar saliva, attracts and induces sows to mate. Some of these, like the 5a-androst-16-en-3-one and 5a-androst-16-en-3-ol secreted by boars in their saliva, can operate as classical pheromones in evoking a specific behavioral response (Brennan and Keverne 2004). Because these hormones are volatile, sows' main olfactory system detects them at a distance, prompting females to approach and induce lordosis to ease mating (Dorries *et al*., 1997; Brennan and Kendrick, 2006).

Table 1. Sex pheromones in different mammals

S.No.	Name of the animal	Source of pheromone	Compound	Reference
1	Bovine (*Bos taurus*)	Faeces	Acetic acid; propionic acid and 1-iodo undecane	Sankar and Archunan, 2008
2	Murrah buffalo (*Bubalus bubalis*)	Urine	1-chlorooctane, 4-methylphenol and 9-octadecenoic acid.	Rajanarayanan and Archunan, 2011
3.	Blackbuck (*Antelope cervicapra* Linn. 1758)	Urine	2-methyl-3-butyn-2-ol, 3,7-dimethylnonane, 3-phenyl-2-propen-1-ol and 2-hydroxybenzoic acid	Archunan and Rajagopal, 2013
4.	Domestic dogs (*Canis familiaris*)	Urine	Methylketones, e.g., 2-octanone, 2-pentanone and 3-hexanone	Dzięcioł *et al*., 2018
5.	Mice (*Mus musculus*)	Urine	1–Iodo-2 methylundecane	Achiraman *et al*., 2010

6.2. Social dominance

Dominance hierarchy is a fundamental social phenomenon that affects fitness and health in many mammalian species. There are social groups in many species where hundreds or even thousands of individuals live. An essential component of group dynamics is the establishment of dominance hierarchies, in which an individual's physiological state, vulnerability to illness, capacity for reproduction, and life expectancy can all be strongly influenced by their place in the hierarchical structure (Zilkha *et al*., 2023).

Thoβ *et al*. (2019) reported that the regulation of volatile and non-volatile pheromones is influenced by the social status of male mice. Following the establishment of a territory and the achievement of social dominance, male

mice produce major urine proteins (MUPs) at a pace more than twice as fast. After assuming social and territorial dominance, male house mice release an increased quantity and variety of MUPs. This increases the intensity of the volatiles and attracts estrus-producing females with its urine.

The pheromones associated with reproductive success in a competitive environment were demonstrated by Luzynski *et al*. (2021). MUP and VOC excretion are influenced by social status, and females find dominating males' urine odor. In both sexes, territorial dominance was also associated with successful reproduction; however, no association was discovered between urine chemicals and either social standing or successful reproduction in females.

Mammalian reproduction relies heavily on chemical communication, while male scent signaling has received the most attention. In addition, female chemical signals have a significant impact on parental care, sexual attraction, and the regulation of female-to-female reproductive cooperation and rivalry. In addition to announcing a female's position and sexual receptivity, female odors can have significant physiological priming effects on male growth and sperm production. In a variety of social systems, female odors have been linked to the suppression of reproduction in young or inferior females; females with lower competitive ability may profit by postponing reproduction until more favorable circumstances (Coombes *et al*., 2018).

6.3. Hormone dependency

Variations in sex hormones and the reproductive cycle affect the release of sex pheromones, scent-marking behaviors, and scent gland growth. Specific sex pheromone production and sex-related behaviors are eliminated by gonodectomy. Once sex hormones are supplemented externally, these events resume. All male-specific chemicals produced by the flank glands of golden hamsters (*Mesocricetus auratus*) were eliminated by castration, but just one compound in females was impacted by bilateral ovariectomies. These ovariectomized females' glandular chemicals resembled those of males after receiving testosterone treatment, indicating that androgen is primarily responsible for these compounds (Liu *et al*., 2010).

The development of the preputial gland of rats (*Rattus norvegicus*) and four compounds—geranyl linalool isomer, oxirane, farnesol, and lanosterol—was shown to be dependent on testosterone, according to a study that involved castration and castration with testosterone supplementation. Likewise, it has been demonstrated that a low molecular mass protein with a molecular weight of 18 kDa, which is thought to be a pheromone carrier, is likewise dependent

on testosterone (Ponmanickam *et al*., 2010). In bioassays of inter-male aggressive behavior, two volatile components of male mouse urine—dihydro-exo-brevican and 2-(sec-butyl)-dihydrothiazole—have been discovered to be active. When added to castrated male urine, the two synthetic chemicals work in concert, but not when added to water. They also cause fighting that is both quantitatively and qualitatively similar to the fighting caused by intact male urine (Novotny *et al*., 1985).

6.4. Aggression

Animals in the animal kingdom frequently attack other members of their species when they perceive a threat. Usually, male animals take this action to defend both their partners and their territory. According to this source on pheromones and aggression, they will attack other males who trespass into their territory, but they will not harm other females or, in laboratory settings, neutered males (https://www.thinkib.net/psychology/page/45731/pheromones-and-aggression).

Chamero *et al* (2007) attempted to isolate pheromones in mouse urine. The identified compounds swabbed the backs of neutered male mice and then introduced him as an intruder into the cage of a healthy male mouse (bioassay). Using this technique, they were able to narrow it down to a protein that may be a pheromone that provokes aggressive behavior (https://www.thinkib.net/psychology/page/45731/pheromones-and-aggression).

Male urine contains (S)-2-sec-butyl-4,5-dihydrothiazole (SBT) and (R, R)-3,4-dehydro-exo-brevicomin (DB), which induce estrus in female mice and aggression in other males. According to Chamero *et al*. (2007), Major Urinary Protein (MUP) in mouse urine functions as a pheromone carrier that mediates male-male aggression via the accessory olfactory neuronal circuit.

For successful reproduction, females are attracted to male-derived chemicals. But lactating dams attack male intruders with great vigor during a specific period following parturition. A pregnant mouse has significant alterations in her behavior and physiology. Dam tends to her pups and constructs a nest for them. Moreover, dams protect the nest from conspecific intruders, particularly by assaulting gonadally intact males. Since a functioning olfactory system is necessary for maternal behavior, chemosignals may have a role in the development of maternal behavior. Depending on the female reproductive status, the same chemosignal, darcin, either stimulates aggression or attraction (Martín-Sánchez *et al*., 2015).

6.5. Territorial marking

Territories and home ranges are marked by pheromones. Terrestrial vertebrates' territorial behavior is significantly influenced by scent markings. An animal's territory is characterized by constant defense against conspecifics to protect resources, such as food sources or den locations. Territories can be occupied by a single animal, a pair of mated animals, or a group of animals. They are frequently identified by glandular secretions, feces, or urine deposited in noticeable locations. Scent marks are distributed in lines across the territory, toward its center (hinterland marking; e.g., honey badger (*Mellivora capensis*), or at or near the territory's perimeter (boundary marking; numerous carnivores, such as wolf (*Canis lupus*). In general, males tend to mark more than females and dominant males mark more than subordinate individuals.

Animals use scent marking, a type of olfactory communication in which they leave their scent marks in particular locations to communicate with other animals. The primary definition of scent marking is an action used to indicate ownership of a region (Gosling, 1982; Leuchtenberge, 2018). Non-territorial animals, however, can converse with conspecifics by scent marking. Therefore, not all marking is territorial, and scent might differ depending on its social structure and ecological factors (Leuchtenberge, 2018).

The male Bengal tiger, *Panthera tigris*, uses a combination of urine and a small amount of lipid material as its territorial marking fluid. The lipid material may serve as a controlled-release carrier for the fluid's volatile ingredients. In the marking fluid, 98 volatile chemicals and elemental sulfur were found using GC-MS (Burger *et al*., 2008).

6.6. Kinship identification

According to Sherman *et al*. (1997), kin recognition is essential for mate choice, avoiding inbreeding, and infanticide, fostering communal harmony, making use of resources, and preventing sickness in mammals. Mate choice, which has major biological implications for mammalian reproduction and breeding, is directly tied to kin recognition, which is one of the crucial strategies to realize inbreeding avoidance (Zhu *et al*., 2019). *Ailuropoda melanoleuca*, the male panda, excretes urine that contains information related to kin recognition. Only male adults' urine during the mating season contained information about kinship odors; during the non-mating season, this information was lacking. Such kinship scents were absent from adult females and all sub-adults during the mating and non-mating seasons. Thus the panda's kinship odor depended on its age, sex, and season (Liu *et al*., 2008).

According to Baojun and Fadao (2006) and Raymer *et al*. (1984), *Lasiopodomys mandarinus* can create memories of unknown individuals by detecting the volatile compounds generated in their urine. The volatiles or the complex mixture of non-volatiles carry the chemical signal of urine that is used in the individual recognition of each vole. Urine's non-volatile chemicals appeared to be crucial for individual identification as well (Baojun and Fadao, 2006). According to Zhu *et al*. (2019), there were differences in volatile chemical compounds found in the urine of kinship and non-kinship observed in treeshrews (*T. belangeri*). This suggests that urine signals play a significant part in chemical communication, which in turn aids in kin recognition.

6.7. Mother young interaction

Social preferences for their mother are formed via the process of mother-young bonding. Food, warmth, and mother care are provided, which promotes successful reproduction and the survival of young ones. According to Mota-Rojas *et al.* (2022), this mechanism uses a combination of tactile, visual, auditory, and thermal inputs to foster the development of the mother-young relationship. According to Bienboire-Frosini *et al.* (2023), certain species of animals, are immature at birth and need a great deal of care. Their post-natal growth also affects how much time mothers spend with them.

To meet the metabolic demands of nursing, young rats cause their mothers to secrete more prolactin, which increases food intake. After eating a large amount of food, the extra caecotrophe-a material rich in bacteria that emerge from the caecum is excreted. The smell of the microorganisms in this anal excrement create draws the pups to the caecotrophe, their littermates, and the mother. According to Leon (1974), there is a suggestion that this smell could help synchronize the mother-young bond.

The mother-young relationship is developed by chemical stimulation. A highly alluring chemical secreted by the young one attracts the dam. In rats, puppies' preputial gland secretions have been identified as a mechanism that regulates their anogenital licking habit. The vomeronasal organ (VNO) of dams has chemoreceptors that mediate this behavior. The pheromone component is identified as dodecyl propionate. Licking is essential to a pup's life because without it, they are unable to defecate and will die (Brouette-lahlou *et al*., 1999).

7.0. Conclusion

Several studies in mammals are indicating that chemical communication through pheromones is one of the important tool for communication between animals Nowadays sophisticated instrumentation and the development of real time monitoring facilities have made it easy to identify potent pheromones. It facilitates, the construction of pheromone trap, estrus detection kit, regulation of estrus in females, reduction of aggression, appeasing youngones and artificial insemination.

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Effect of Pesticides Toxicity in Aquatic Environment

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Introduction

Rapid industrialization and urbanization in the last few decades, with the concomitant growth in population, had taken a toll on the natural resources. Several anthropogenic activities like pollution by toxic substances through pesticides or heavy metals on regional, or global scale results in climate change. Large-scale mortality of living organisms' most important wildlife such as sea mammals and expanding threat to human health, i.e., chronic respiratory diseases, cancer, damage to several major organs like the brain, lungs, kidneys are being witnessed in the recent years as a result anthropogenic perturbations [1]. Of all the anthropogenic sources of pollution, agricultural, industrial, and domestic activities are the major sources responsible for contaminating natural freshwater resources [2]. For example, about 300 billion kilograms of mixtures used in engineering and farming products reach the freshwater frameworks consistently. 10% of the universally open spillover is utilized, producing a surge of wastewater, which streams into groundwater, waterways, lakes, or the seas [3, 4].

With the increase in world population in recent years, there is a pressure on the existing agricultural system, and nowadays, the prime objective of most of the countries is to increase the food production to meet the demands of a growing population which are expected to grow nearly to 10 billion by the year 2050 [5,6]. The process of increasing cop production utilizes the application of higher quantities of agrochemicals such as herbicides, fungicides, nematicides, and fertilizers. These agrochemicals are used for controlling the pest population and increasing the yield for the production of an ample amount of food for the global population, which is estimated at 6.8 billion in 2009, and it reached 7 billion in 2012 [7, 8]. In underdeveloped countries where 1.02 billion people are undernourished, which accounts for 15% and 1.3 billion people live on an inadequate diet, which accounts for 19% thus, there is a need for sufficient sustenance supply. However, freshwater and

terrestrial ecosystems are highly polluted by a large number of toxic substances, most importantly by the application of pesticides and fertilizers by the agricultural sector [2], which becomes an important issue globally. Pesticides that are used for the eradication of harmful pests are today flattering an essential part of modern life. Ideally, these should only be toxic to the target organisms [7], but several pieces of evidence from the developing research shows that the industrial chemicals, pesticides, heavy metals, and several other toxic substances interfere with the normal functioning of a large number of species including human beings and aquatic organisms [810]. Pesticides are present at higher levels should be removed from drinking water for human safety. There is a need to maintain control on disposal of industrial waste or Agriculture waste in water bodies and to bio-monitor the trace elements in the water and other eatables [11,12].

Pesticides

Pesticides are a mixture of substances that are designed to control or slaughter or control the development of pests (undesirable organisms). These pests usually plant pathogens, nematodes, microorganisms, and insects that compete with human food and are responsible for transmitting diseases and destroying crops. Pesticides are usually categorized into biological or synthetic (Fig. 1). The biological pesticides are derived from natural sources, for example, plant extracts (azadirachtin from neem or pyrethrin from chrysanthemum plants), whereas synthetic pesticides are made through the industrial processes. Another category of pesticides are broad-spectrum (used to control a wide range of species) or narrow-spectrum (used to control a small group of species) and they are also categorized depending on the kind of pest they regulate, i.e., insecticides are used for controlling insects, herbicides for weeds and fungicides are used for controlling fungi.

A: *Insecticides*.

Most of the insecticides affect the nervous system at several target sites; they interfere with the membrane transport system of sodium, potassium, calcium, or chloride ions, which inhibits the selective enzymatic activities involved in the chemical transmission at nerve endings [13] (Table 1).

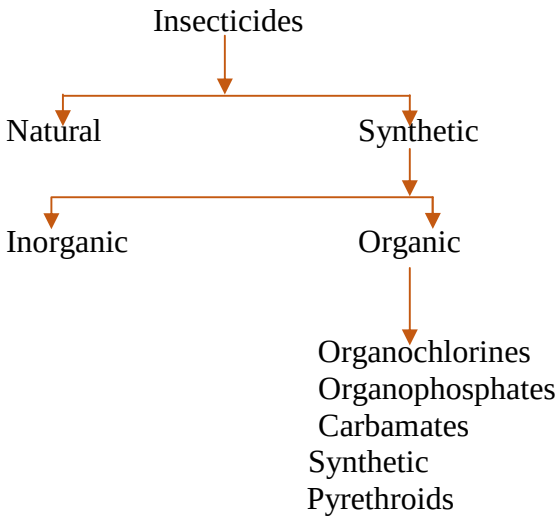

Figure 1. Classification of insecticides.

1: **Organochlorines**.

They are a class of insecticides that affect the nervous system as they are chemically unreactive stable compounds, which leads to long-lasting effects. DDT is the most studied pesticide among all insecticides, which inhibits the release of neurotransmitters. Endrine and lindane are other two organochlorine insecticides, in addition to DDT, which affects the nervous system [11].

2: **Organophosphates**.

In previous years many countries banned some of the organochlorines (DDT), which were replaced with organophosphorus insecticides like malathion and parathion [14]. This group of insecticides is also neurotoxic, i.e., they inhibit the enzyme acetylcholinesterase (AChE), and the signs and symptoms of intoxication are longer and persistent [15, 16].

3: **Carbamates**.

This category of insecticide also inhibits AChE by attaching to the reactive site of the enzyme [10]. It has short and reversible inhibition action of AChE.

4: **Synthetic pyrethroids**.

This is the newest category of insecticide, which shows two different acidic portions chrysanthemic or pyrethric acids resulting in type I and type II

syndrome [13]. Both of these syndromes affect the sodium channels in the nerve membranes, which is responsible for causing the repetitive neuronal discharge; this mechanism is quite similar to the DDT action. Pyrethroid insecticides have several other sites of action. Some of them include inhibition of Ca^{2+}, Mg^{2+}-ATPase, which results in the interference with calcium removal from nerve endings, causing the release of neurotransmitters in the postsynaptic gap.

B: ***Herbicides***.

Herbicides are classified into several categories based on their action. They are produced for killing the harmful plants (weeds) thus. They are associated with affecting various mechanisms that are involved in photosynthesis, respiration, growth, cell and nuclear division, or during the protein or lipid synthesis [13, 17].

1: **Glyphosate**.

The most commonly used herbicide for controlling weeds inhibits the single plant enzyme EPSPS (5- enolpyruvoylshikimate 3-phosphate synthase), which is the key enzyme for catalyzing the amino acid biosynthetic pathway, and the inhibition of this enzymes affects the protein synthesis mechanism [18, 19]. In addition to these, herbicides also inhibit many physicochemical and physiological pathways [20, 21]. The ecotoxicologists are highly concerned about the exposure of a non-target aquatic organism to the formulations of glyphosate because of the extensive use of glyphosate in the shallow water ecosystems, and it also possesses high water solubility [22].

2: **Chlorophenoxy herbicides**.

This category of herbicides mainly includes 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), and MCPA (4-chloro-o-toloxyacetic acid), which are known for mimicking the role of growth hormone, i.e., auxin in plants [13, 23, 24] and it is also responsible for several growth abnormalities at higher concentrations which mainly includes leaf or stem curling, inhibition of shoot and root growth [25] which ultimately results in necrosis and plant death.

C:***Fungicides***.

Fungicides are the group of an insecticide which disturb the energy supply in fungi and inhibits spore germination [26]. For example, dithiocarbamates (e.g., maneb and thiram) and the R-S-CCl3 compounds (e.g., captan and dichlofluanid) have multisite action by inhibiting the enzymes

which are involved in respiratory processes, whereas another group of fungicides, i.e., the phenylpyrroles, which includes fenpiclonil and iprodione inhibits the spore germination and causes several morphological alterations in the germ tubes of plants which means the elongation of germ-tube is inhibited [27, 28]. In addition to this, fungicides are also identified to constrain the electron transport chain in the respiration process [29].

Table 1. Major classes of pesticides and their mode of action.

Pesticide category	Major classes	Purpose	Mode of action	Examples	References
Insecti-cides	Organophospha tes Carbamates Pyrethroids Organochlo-rines Neonicotinoids	Kill or repel insects	Neurotoxic, bioaccumula-tes, and biomagnifies	Malathion, methyl parathion, aldicarb, carbaryl, methomyl	[30]
Herbi-cides	Phosphonates Chlorphenoxy herbicides Dipuridyl herbicides	Kill weeds or unwanted plants	Neurotoxin to specific stages of insect during development	Glyphosat e, 2,4-D, mecoprop, Diquat, Paraquat	[31]
Fungi-cides	Thiocarbamates Triazoles Strobilurins	Kills mouds and other fungi	Prevent fungal spore formation and stop plant diseases	Metarn sodium Flucona-zole, myclobuta nil, triadime-fon	[32]

For agricultural purposes, the pesticide use is enhanced in recent years for increasing the crop yield to meet the needs of the growing human population [33, 34] whereas their use harms the environment and also affects non-targeted organisms along with the targeted pests [26] which are a matter of major concern for decades [35] as it negatively affects several links in the food web. Both the soil and aquatic ecosystem are affected negatively by the pesticide pollution as they move from one ecosystem to another because of their specialized properties such as half-life, solubility, mobility, and degradation (Table 2). The pesticide enters the aquatic ecosystem through

runoff, vapourization to the atmosphere, agricultural returns, groundwater intrusions, or by adsorption or through plant uptake [36-38], which adversely affects the health of aquatic organisms. Most of the pesticides in urban and agricultural settings are negatively affecting the deaths of several aquatic organisms, such as birds, fish, and zooplankton [39].

The following given factors are considered to determine the ecological impacts of pesticides in water:

Table 2. Factors affecting pesticide toxicity in aquatic systems.

Factor	Description	References
Toxicity:	Both in the situation of mammals and non-mammals, the toxicity is expressed in the form of Lethal Dose (LD), which is the concentration of the toxic substance (pesticide) responsible for killing the half test organisms in a certain period of time. The lower the value of LD50, the greater will be the toxicity; values of 0-10 are extremely toxic.	[40, 41]
	Using a risk-based assessment, the drinking water and food guidelines are determined. Generally, Risk = Exposure (amount and/or duration) × Toxicity.	[42]
	Toxic response is observed in two forms: **Acute:** death **Chronic:** in this effect, death does not occur during the test period, but certain observable characteristics such as tumors, reproductive failure, and growth inhibition are noticed in the test organism.	[43]
Persistence:	It is measured in the form of half-life, which is the time required for the diffused concentration to decrease by 50%, and its persistence is determined by the degradational processes, whether it be biotic (biodegradation and metabolism) or abiotic (hydrolysis, photolysis, and oxidation) (Calamari and Barg, 1993).	[44]
Degradates:	The process of degradation leads to the formation of degradates, which may have lesser, equal, or	[45]

	greater toxicity when compared to the parent compound, for example, which DDT degrades it results in DDD and DDE.	
Fate (Environ-mental):	The environmental behavior of the pesticide is mostly affected by the chemical's natural affinity (Calamari and Barg, 1993) for any of the four compartments: solid matter, liquid, gaseous form, and biota.	[46]

3. Pesticides in the Aquatic Ecosystem

To grow high yielding crop varieties farmers, tend to use pesticides to protect the crop from pests as these crops are highly susceptible to the pests and diseases, which may lead to a 40% loss in crop production; thus, these pesticides as used to improve the quality as well as quantity of crop by protecting them from pests [47-51]. Among all the toxic substances that run off into the aquatic ecosystem, pesticides are of major concern as they are known to cause serious threats to the biological organisms, including human beings. Through several different routes such as spillage, industrial effluent, surface runoff, or through pesticide-treated soils, these toxic substances enter into the water sources [52-54]. The toxic effects caused by exposure to these toxic substances can be categorized according to the exposure period, which may be short or long-term, and exposure type, which can be lethal or sub-lethal. The period of short-term exposure does not exceed 96 hours, while long-term exposure is considered to be more than 96 hours (Table 3).

Table 3. Classification of the effect of animals exposed to chemicals [55].

Sr. no	Exposure classification	Classification based on effects	Description
1.	Exposure time	Short-term	≤ 96 h (mortality is measured as endpoint)
2.		Long-term	cellular/molecular/biochemical/physiological level measure as endpoint
3.	Exposure type	Lethal	≥ 96 h (mortality is measured as endpoint)
4.		Sub lethal	cellular/molecular/biochemical/physiological level measure as endpoint

From agricultural fields pesticides generally runoff to reservoirs or drainage systems through rain or by irrigation process [56]. Aquatic organisms are exposed to pesticides primarily by three ways: (i) through the skin: as aquatic organisms are in contact with water thus, through dermal pores, pesticides cause harmful effects, (ii) through breathing: as they respire through gills thus the aquatic organisms directly uptake pesticide through breathing and (iii) orally: aquatic organisms usually get exposed to pesticides by feeding in pesticidecontaminated prey (which is also known as secondary poisoning for example: if fish feeds on pesticide exposed insects then they may get killed if a large amount of toxic compound is consumed by the insects) or by drinking contaminated water.

The aquatic ecosystem consists of various groups of organisms such as invertebrates, plants, microorganisms, fish, or amphibians. Pesticides can affect these organisms directly or indirectly; the direct effect includes physiological changes within an organism [57-59]. For example, the exposure of pesticides to water flea results in their mortality, which can be considered as the direct effect of pesticides, and it may lead to the drastic increase in the biomass of algae because of release from the grazing pressure considered as an indirect effect. Globally, herbicide, mainly glyphosate is used for controlling both the terrestrial and aquatic weeds, and in recent years its use has been tremendously increased, and thus, it is also known to negatively affect the non-target organisms in the aquatic environment [60]. Originally its mode if an action was designed to affect the plants [61] only, but in recent year's several reports have been coming into the picture representing the adverse impact of non-target organisms [17, 62-64], which can be lethal or sub-lethal. The indicators for the exposed organisms at the physical level include a measure of survival, growth, morphological/behavioral changes. The reproductive performance can often be used for the assessment of sub-lethal response, which also includes sexual maturity, time taken to release the first brood, time taken for egg growth, fertility, and modifications in the characteristics of reproduction. In addition to this, several biochemical parameters can also be used to determine the toxicity in exposed animals, which may include disruption in metabolic pathways, steroid metabolism, lipid peroxidation, AChE activity, and activity of cytochrome P450enzymes and levels of blood glucose.

In many studies, two direct measures of growth (body weight and length) have been used for the assessment of sub-lethal effects on arthropods. Simple dry weight is determined by drying organisms, which is sampled at an average temperature of 60º C for 48 hours [6567]. Fishes interact closely with

the physical, biological, and chemical marine ecosystem; thus, they are an important part of the aquatic ecosystem. They are an important food source for other animals such as sea birds and other marine mammals; thus, they are an integral part of the marine food web. Several studies have reported the decline of the fish population to the toxic effect of pesticides [68, 69] as several reports have been mentioned representing the decline in the fish population [12, 70-80].

Conclusion

This review paper deals with the effects of rapid growth in the human population on the aquatic ecosystem, which may be noticed in the form of climate change, nutrient enrichment of aquatic bodies, and pollution by the different types of toxic substances, including pesticides in both regional and global scale. These man-made disturbances within the environment are responsible for adversely affecting the normal functioning of living organisms, which includes developmental abnormalities from invertebrates to higher organisms that are mammals. It is being noticed that in past years the use of pesticides is increasing, and it affects non-target organisms at different biological scales.

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COVID 19, traditional immune boosting drink ‘kadha’and Pharmacovigilance perspective

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History of origin of CoV 19:

Severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002 and Middle East Respiratory Syndrome Corona Virus (MERS-CoV) in 2012 stroked critical threats to humankind. In 2019, coronavirus registered its thirdappearance. On December2019a pneumonia of unknown cause was detected in Wuhan, China and blownout globally. Since the first report on corona was detected, the scientists have been on research trademill. The research on nature of virus, trajectory and evolution, diagnostic and control of the fast spreading disease were being investigated. With the covid toll reaching its highest and worst heights, everyone became curious to know the origin of virus. But, even a year after the outbreak, no stout results, no robust process were established for probing the origin of the virus. Natural host for corona virus is horseshoe bat that inhibits the Yunnan Province. The group of scientists, ShiZengli (et al) from Whuan laboratories and associated countries were working on the virus since years. “The bat lady” Shi Zhengli had visited the Mojiang Copper Mines, the natural niche of the bats a dozen of times and collected the sheet as possible sample to isolate the virus. After the outbreak the laboratory had published report saying that diverse groups of corona virus were discovered. At the same time a case of severe illness in Mojiang miners was published. The miners had cleaned the bat sheet from the mine and suffered deadlier pneumonia like illness. Initially the causative agent for miners pneumonialike disease was declared to be a fungus then as virus from rat urine sampled from the Mojiang mine. The miners illness was very similar to covid-19. Studies across the globe were on its peak to reveal the nature and origin of virus.

In December 2019 the first 41 people who were hospitalized, a week before had passed through the seafood market located in the heart of Wuhan city and hence seafood market in Wuhan was sought as the source for virus and the market was shut down. The question remained unanswered as molecular dating datasets did indicate origin of virus in November. In which animal the viral species did occur? Where did the virus come from, mine, cave or forest? Bats, wild animals, pangolins, wasn't known. However, the genome sequence datasets had showed that the illness is caused by a virus SARS- CoV that belongs to Beta coronaviruses group. The theory accepted was bats from caves are natural reservoirs, passing through some intermediate carnivore reached to man. But how? What is the protein that helps to entre human cells? Simultaneous disclosure of the reports that virus from Malaysian pangolins showed 90% similarities with SARS - CoV but more than 99% similarity for a part of genome that facilitates entry into human cells. Then is that a combination of two viruses? An intentional construct of virus that created a pandemic. Quite a possibility! Was the hybrid escaped the lab un-noticed? Was it a deliberate release? The miner's pneumonia was deadlier disease but was not transmitted further, how is that possible? So many questions remained unanswered. The lab release was thought as a conspiracy theory.

The research (based on Observation, deduction, forensic science, logic and reasoning)by Dr. Rahalkar and Dr Bahulikar presented the paper to journal entitled Frontiers in public health in Feb.2022, until then link between the virus, mine and genomic modified strains was considered as a conspiracy theory.

Corona 2019 Virus: The outbreak traced to be a novel strain of coronavirus and is called 2019-nCoV by WHO, renamed SARS CoV-2 by the ICTV. Corona virus is an enveloped virus with single stranded RNA and crown like appearance when observed under Electron microscope. The virus belongs to Beta CoV genus of *Coronaviridae* family. The members of large family cause respiratory, neurological and hepatic infections in different animals like bat, cattle,etc. Furtherthese viruses are known to cross species barrier and can infect human.SARS-CoV-2 belongs to large family of single stranded RNA viruses. CoV are enveloped, positive stranded RNA viruses along with nucleocapsid. (features, 2020).The CoV19 uses angiotensin enzyme2(ACE2) to infect humans through respiratory system. Several variants of SARS-CoV-2 have been describedand seven variants of CoVs are reported to infect humans (HCoVs). Among the HCoVs, betaCoVs, the novel CoV (severe acute respiratory syndrome SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) are reported as capable of causing epidemics.

SARS CoV variants include Alpha (B.1.1.7), Beta (B.1.351)Gamma (P.1), Delta (B.1.617.2) and Omicron (B.1.1.529), variants of concern (VOCs) with enhanced virulence.Alpha variable of SARS-CoV-2, the variant with seventeen different mutations became 43-82% more transmissible and emerged with mortality hazard ratio of 1.32. The S protein mutation (N501Y) caused increased affinityfor host cell surface receptors and result in enhancing the attachment and ensuing entry into host cells.A variant with multiple spike mutations Beta (B.1.351) resulted in the second wave of COVID19, variant with an increased binding affinity and risk of transmission. Gamma (P.1)(thefirst reported in Brazil in January 2021),Delta (B.1.617.2)in December 2020 and Omicron (B.1.1.529), (first reported in South Africa in November 2021) and subvariants BA.1, BA.2, BA.3, BA.4, and BA.5, have been identified. The incubation period of the SARS-CoV-2 virus is approximately two weeks. Symptoms manifest in 4–5 days after infection and most people become sick by 11–12 days. In rare cases it can be delayed even up to 24 days.

Early Approach to fight CoV19:

Time and again, India's Health Ministry, World Health Organization and many other health agencies advocated measures and urged people to keep immunity strong to stay safe from the virus. Measures like frequent hand washing, cough and sneeze by covering mouth and nose, immediate disposal of used tissue, avoiding hand contact with others, crowds and public gatherings, cleaning all shared surface, wearing of mask. The ministry of Ayush suggested a protocol in the management of COVID 19 based on Ayurveda. Kadha prepared using the selected herbs and spices is reported to provide a boost to improve the natural immunity. It is an ayurvedic herbal drink made of varied herbs. It helps to fight the cold cough by increasing body defence to treat sore throat, improves digestion and prepare body to keep away from the novel coronavirus by boosting immunity. Further, the important findings highlight importance of Kadha to manage blood sugar, the principal ailment known to many diseases. Lopinvir, Ribavirin, Favipiravir, Remdesvir, Oseltamivir, Chloroquine, and Hydroxychloroquine, were prescribed as preliminary drugs with paracetamol and Vitamin C and vitamin.These drugs were associated with various side effects such as insomnia, gastrointestinal adverse effects, immune suppression, and cardiac rhythm disorders. Major drug targets in the virus were identified as the glycoprotein of the surface spike (S), the membrane (M) protein, the glycoprotein of the small envelope (E), and the nucleocapsid(N) (Ludwig and Zarbock, 2020).Spike proteins of Coronaviruses (CoVs) interacts with the angiotensin-converting enzyme 2 receptor present on the surface of

host cell and initiates infection in the upper airway. In the case of immunocompromised host propagation of virus is rapid and hoists the severity of the disease (Walls et al., 2020). Breaking the weak immune control virus enters alveoli and further reaches systemic circulation.Three major approaches were followed to find the theraputic molecules against CoV 19 i.e. 1. use of prevailing broad-spectrum anti-viraldrugs,2. in silico screening of molecular databases to identify potential antivirals, 3.rational drug design based molecular and pathogenic characteristics of COVID-19 (Wu et al., 2020).

With the advent of efforts anti CoV19 vaccines were developed and vaccination drive against SARS-CoV-2 started with great enthusiasm worldwide. Although vaccine is the most accepted tool for COVID-19 prevention, reinfection even to vaccinated individuals were prevalent worldwide. Cough, fever with loss of sense of taste and smell are characteristic symptoms of CoV19. A meta-analysis of 21,682 adults infected with SARS-CoV-2 in nine countries reported that cough was present in 57% patients (Grant et al 2020). Cough is a reflex that occurs due to activation of peripheral sensory nerves, vagus nerves, and afferent signals to brainstem. Amplification of afferent signals triggers cough pathways and causes hypersensitive response. Chung et al (2021) hypothesized that neuroinflammation and neuroimmune interactions (Woo-Jung Song et al 2021) induces a hypersensitivity mechanism and are central to the cough of COVID-19. Further, possibility that sensory nerve infection by CoV19 virus is mediating cough and leading to cough hypersensitivity. Respiratory droplet is the route for community transmission of the viral infection. Measures to control the cough and raise the health defines system was a primary goal as it would help to control the way of community transfer of virus and spread of disease.

Complementary and Alternative Medicines to prevent CoV19 infection:

Since ancient times and even today plant products are known to be useful in controlling various diseases. Folklore, home based medicines, as well many herbal preparations in Ayurveda have been used as medicaments in treatment of viral diseases (Dhawan et al 2012,Khuntia et al 2022).Plants as source of various molecules of therapeutic importance gave a hope that plants can be explored to find and develop anti CoV19 drug.

Ayurveda the ancient vedik medicinal system in India, is a rich source of plant-based formulations revealing antiviral, antibacterial, and anti-protozoal activity of plants.The classical texts of Ayurvedic medicine such as Charaka Samhita and Sushruta Samhita contain descriptions of pandemics of similar

proportions and describe them as Janapadoddhvansa, meaning the destruction of communities, along with their causes and treatment . Its important to know that over last 75 years (that is 1940 to 2014), 49% of all small molecules approved by the U.S. Food and Drug Administration (FDA) were natural products (Newman & Cragg, 2016). Clinical efficacy of herbal Ayurvedic preparations is well proven and has demonstrated safety and usefulness as therapeutic agents in various viral diseases and include Yashtimadhu, Bhunimba, Shunthi, Ghritakumari, Chandana, Dadima, Kulinjana, Pashanabheda, Patranga, Soma, Karaveera,Haridra,etc.(Sharma et al 2022). Haldi (*Curcuma longa*), sunthi (*ZingiberOfficinale Roscoe*), Guduchi (*Tinospora cordifolia)* Dalchini Cinnamomum verum), loang (*Syzygium aromaticum*), Miri (*Piper nigrum*), Kaduneem (*Azadiricta indica*), Tulasi *(Ocimum tenuiflorum)* are the common spices used in kitchen and these coactive herbal combinations have a long tradition of clinical efficacy with safety profiles.Aromatic oils of 5 different herbs like *Eucalyptus globulus, Rosmarinus officinalis, Mentha piperita, Eucalyptus citriodora, Origanum syriacum* are commonly used in the treatment of various health ailments. Further, use of plant essential oils at their specific concentration for the treatment of upper respiratory tract infection like rhino sinusitis, *Salvia officinalis* (containing camphor) for treatment of acute pharyngitis and quick relieve in pain are well documented.(Hubbert et al 2016).

Ayurvedic kadha in weak of COVID 19 crisis.

According to Ayurveda *pitta, kapha and vata* three elements of the human body are considered as prime elements to maintain health or development of a diseased condition (known as *doshas*). The theory of Ayurveda further classifies disease in two ways that is ‘Nija’ (Endogenous) and ‘Agantuja’ (Exogenous) diseases. The cold and cough may be caused due to Abhishanga which means close contact with affected person and Bhutabhishanga that means contact with invisible organisms. Also, according to WHO the covid -19 has two sources of spreading that is contact with the virus and other is close contact with the person affected that is abhishanga and bhutabhishanga respectively. Considering these terms infection by COVID-19 virus can be placed in Agantuja Vyadhi. Pathogenesis by COVID 19 progresses after the virus enters body and vitiates the doshas that leads to development of symptoms. Ayurvedacharya Charaka says, fever ensue when Vataadi dosha either singly or in Sansrista (two dosha) or in Sannipataja (all three dosha) got intensified. further it enters Amashaya and mixed with Rasa Dhatu causing obstruction of Rasavaha and Swedavaha Srotas resulting in the destruction of Agni; Agni then spreads out from its Sthana to whole over the

body causing the febrile condition [21, C.S.Ni.1/20, 23, 26; C.S.Chi.3/129-132].So, in initial stage diseases are in either form (Nija or Agantuja), but shows both the forms, in later stage. Ayurveda believes that even if there is a virus or bacteria, manifestation of disease in human body can be only observed if it causes vitiation of doshas. The acceptance of vitiation of doshas in modern medicine is done in context weakened or dysregulated immune response. In case of communicable diseases certain preventive and protective measures are to be observed, further governance of behaviour and habits may help to keep the balance of the three doshas.

Ayurvedic medicines are derived from herbs, organic matter, minerals whereas medicinal plants are used as source of bioactive component and processed further for preparation of prash, kadha, asawas etc. Kadha or decoction is prepared using dry or less juicy ingredients like spices and herbs.To manage COVID-19 crisis several Indian Ayurvedic herbs, spices and their active phytochemicals have been explored for their possible prophylactic and therapeutic use against COVID-19. Ayurvedic kadha is suggested as prophylactic measure against CoV19. Kadha is decoction of herbals that has Agni promoting, kaphashamak as well as krimghna properties. Thus, it would strengthen natural balance of tridoshas,improve immunity and purify blood, thus alleviate clinical manifestations of CoV19. In early stage of disease herbs with immune boosters and anti-inflammatory property anti thrombotic properties can help to control the disease; e.g Tulsi plant is a known immune modulator, it enhances immune cells, IFN-γ and IL-4, and inhibiting pro-inflammatory NF-κB pathway, COX-2, lipoxygenase-5, and IL-1β. Animal model studies on bioactive compound in Sunthi/ginger have demonstrated anti-inflammatory activity, however therapeutic trials are required.(Pura Ballester 2022, Begona Cerda et al 2022). Proposed mechanism correlates antiinlamatory property of ginger with inhibition of Akt and NF-κB activation, an enhancement in anti-inflammatory cytokines suppression of proinflammatory cytokines. Various phytochemicals of Kadha are found to have selective affinity with vital proteins and restrict process of viral replication and transcription (Mayura et al 2020).Among the various drug targets of CoV 19 the enzymes vital in viral replication viz. 3CLPro (chymotrypsin like protease), MPro (main prortease) and PLPro(papain like protease) are promising drug targets. Phytochemicals from kadha e.g. Sominiferine, Somniferine A, Tinosporide, Tinocordioside, Orientin, Flavonol glucoside, Withanolide, Apigenin, Cyclocurcumin, Withanolide B, Kaempferol, Withanone and Withaferin A etc were found to high affinity for MPro . Phtochemical show very high affinity towards other crucial viral

proteins like nucleo capsid proteins NSP, spike proteins S1 and S2 are druggable targets.

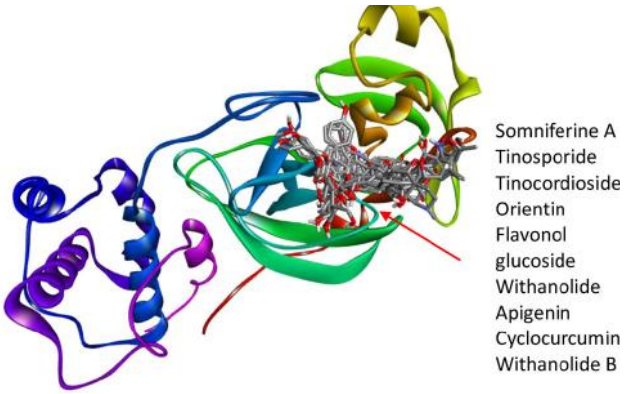


Fig. 1.SARS-CoV-2 Mpro (PDB ID: 6LU7) showing top 12 phytochemicals superimposed on it active site (source: Mayura et al 2020 https://doi.org/10.1080/07391102.2020.1852119)

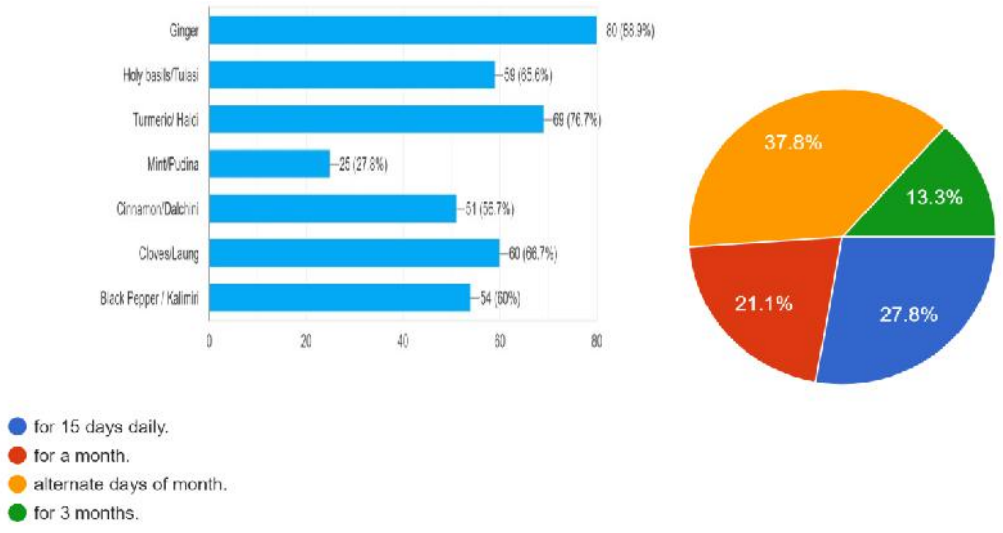
Similarly, phytochemicals high restricting affinity with different macromolecular targets and proteins in host . It is projected to have an effect on the biomarkers like p53, IL-7, TNF, RAS, Camp, MAPK, HIF-1 and boost pathways that can handle pathophysiology of infection caused by the virus(Ranjan et al 2021). As a result, strengthening the immune system (natural body system) may be the most important factor in preventing many pathogenic illnesses and preserving optimal health which can be achieved using immuno booster Kadha

AYUSH (Govt. of India) had recommended various Aayurvedic and herbal preparations like Ayurvedic kadha, turmeric milk, Ayushkwath to be consumed with consultation of a medical practitioner. The age old known home remedies for boosting immunity like kadha, prepared using the ingredients available in a home kitchen appeared as an easy to do approach for the people. Herbal tea is a popular drink in rainy seasons or consuming Kadha at onset of symptoms minor health complaints like soar throat, cough and fever, moreover recipe is passed on generations to generations.

During the initial two global waves of Cov19 there were no medicine , vaccines available and the panic of various recipes of immunity boosting kadha on social media was observed. Ingredient like ginger, turmeric, holy leaves, mint leaves, cinnamon, cloves, black pepper, ashwagandha etc. were added to glass of water, boiled to reduce to 1/4th volume consumed with honey, or ghee and in the form of tea. Kadha and golden milk when administered to

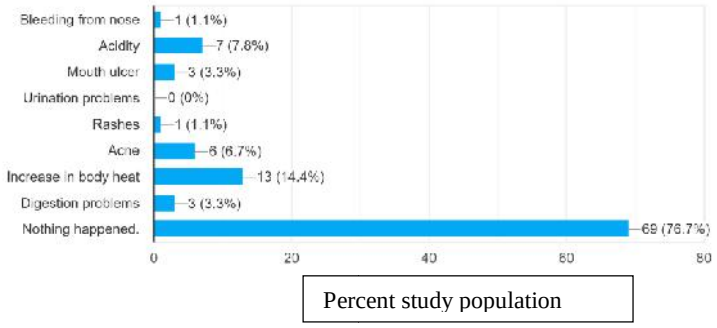
the patients as per guidelines on clinical management of COVID-19, showed early recovery with symptoms lessened earlier. Further, in case of patients with co morbid condition, when given kadha recovered without severity of symptoms. (Chaurssia et al 2020). The home remedy was easily adopted by the general population and everyone accepted their own preparation. Further without seeking medical consultationhomemade remedies with varying proportions of ingredients were consumed (Fig2) over long time periods. Imprudent use of home-made kadha preparations posed various health challenges. A study was conducted to understand the people's perception and find the effect of Ayurvedic homemade Kadha during CoV19 pandemic. Study population was adult Indian citizens (age 18 yearsand above), willing to participate in the study by filling an online questionnaire. A Google form having all the survey questions was circulated through private and institutional networks (e-mail and Whatsapp)Pune, Wapi and Mumbai during the study period. Many similar surveys were conducted countrywide. Observations noted by various researchers highlighted the trust and conviction in Ayurvedic preparations to fight the CoV19battle.Similarly, it was demonstrated that when administered as per AYUSH guidelines and in case of patients when administered as per guidelines on clinical management of COVID-19 helped overcoming the diseased condition. The composition of kadha differed home to home and moreover it was consumed indiscriminately.

Figure 2: Variation in type of ingredients used ,its proportion in homemade kadha and duration of consuming kadha



Various studies report harmful consequences of long term self medication of home made kadha. Though it contradicts the general perception that use of home made remedies do not have any side effects, many reported emergence, aggravation of symptoms that can be adversely correlated with consuming excessive quantity of kadha. However, self-medication of home-made kadha resulted in various health ailments (Fig3) as well as acted as predisposing factor in aggravating various health issues like hyperacidity, urticaria, piles, etc. According to the survey, 4.87 of people are taking kadha (basil, cinnamon, black pepper, ginger, and raisin). According to the survey, 4.87 of people are taking kadha (basil, cinnamon, black pepper, ginger, and raisin) prescribed by Ayush Ministry, India and 92.3% taking homemade kadha. Mostly people (69.1%) think that there isno side effect of kadha, while 13.9% think and experience the side effects of kadha, that is, acidity in thestomach, heartburn, constipation, diarrhea ulcers in mouth, and high blood pressure (especially in senior citizens).Most of the surveys conducted have similar findings, that home made kadha was preferred over Ayush kadha available on market, further different ingredients were used in varying proportion as if every home had its own formula of making it and the dose as decided by the family. Indiscreet use of home made kadha resulted in emergence of side effects. Various studies showed 25-30% population suffered of some or the other health issue.

Fig 3.:Side effects of long term consumption of homemade kadha



Shubhashree et al (2023) reported health issues among the OPD patients at Central Ayurved Research Institute (CARI)Begaluru and found that 43.5% had established information regarding home remedies to fight aginst CoV19 from social media or from their relatives and friends. Further 2.2% were reported who took advice from doctors initially but later continued without further consultation.Appearance of Nettle rash (urticaria) was reported

and varied prevalence among reported OPD cases ranged between 9.7% to 19%, severity and frequency of symptoms were proportional to the amount of kadha consumed. Patients who consumed kadha more frequently or with tea reported faster appearance of nettle rash, as early as within 30mins after consuming kadha (Gurudev et al 2021). Overall findings show that people had perception as use of ayurvedic home made remedies do not have any side effects. Further, study revealed that a majority of the participants involved (>85) were not aware of the side effects and consequences of long term consuming a homeremedy. Distressedduring the pandemic, to avoid the infection, people had consumed immunity boosting homemade kadha and other used varied remedies from 1- 6 months without knowing probable side effects. It is essential to raise the public's awareness about the appropriate uses of home remedies and encourage people to procure clinical advice. Additionally, in case of comorbid ailments, it is essential to ensure the safety of consuming these preparations in combination with ongoing medication.Unexpected adverse effects may develop due to unknown interaction between herbal molecules and drugs,further the CoV 19 itself is known to cause liver and kidney disfunction. In the long run the cumulative effect of unknown e drug-herb interaction in presence of stress instigated through viral infection may worsen functionality of vital organs.

Excess is harmful, that holds true in case of home made recipe of kadha. Many people thought that more quantity of ingradients is to more immunity, more boiling is to more extraction and more benefit, more they drink kadha better will be the effect and it lead to excessive consumption of the kadha. Further every body responds differently to same kadha as each individual has its unique Prakriti. The concept of Prakriti is exclusive to Ayurveda, and is used for deciding the preventive and curative strategy to be adopted in the treatment of patients. Every individual has a unique combination of these constituent elements, which is known as the individual's Prakruti or unique mind-body constitution (Ashtanga Hridaya, Sharira Sthana, Chapter 3, Verse 83).It is the total of anatomical, physiological, and psychological domains of an individual. The diseases often manifest by susceptibility that depends upon Prakriti of individuals. COVID 19 is a new disease, where the status of the susceptibility of its victim in terms of Prakriti is not known. It was crucial to consume the kadha and other phytochemical based remedial preparations under the guidance of a Ayurvedic practitioner. Though the remedial preparation i e kadha, its ingredients and preparation method are same it may have different effects in long term use as prakruti of each person is an unique combination of kafa-vata-pitta. There are no reports showing side

effects of Kadha when consumed under a proper vigilance of ayurveda practitioner. The outcome of this study highlights that a significant proportion of the population opted using Ayurvedic preparation but a very few chose AYUSH 64 the recommended medicine. Community approach to use home made preparations raised the question of safety and toxicity that may arise when these herbs and spices are used as concentrated extracts and in high doses. The adverse effects can be observed if the plant based medicinal preparations are used with larger dose or long duration. The concern is about the irrationality that devastated the belief in age old herbal medicines.

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Neurodegenerative Disorders

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Introduction

Neurodegenerative disorders are a group of diseases that cause progressive damage and degeneration of the nervous system, including the brain and spinal cord. This damage can lead to a variety of symptoms, including cognitive decline, physical disability, and death.

There are over 600 known neurodegenerative disorders, and the exact cause of most of them is unknown. However, researchers believe that a combination of genetic and environmental factors play a role.

The most common neurodegenerative disorders include :

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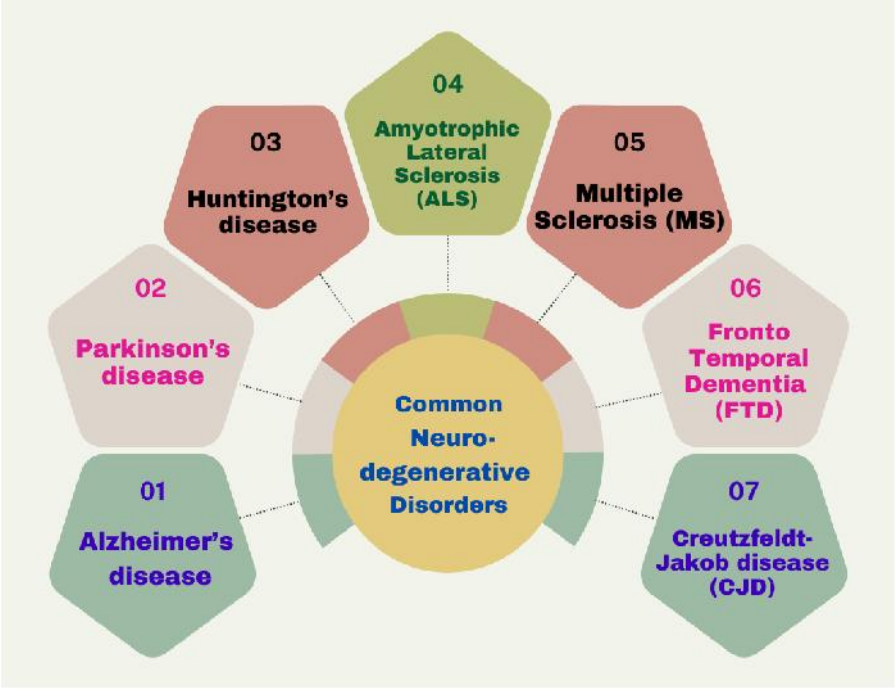


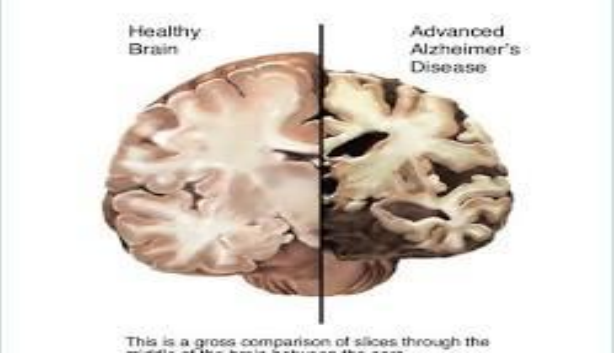
There is no cure for anyneurodegenerative disorder, but there are treatments that can help manage symptoms and slow the progression of the disease. Treatment options vary depending on the specific disorder, but may include medications, therapy and lifestyle changes.

Some tips for managing neurodegenerative disorders:

- Get regular medical checkups and follow the doctor's treatment plan.
- Eat a healthy diet and exercise regularly.
- Get enough sleep.
- Avoid smoking and excessive alcohol consumption.
- Manage stress levels.
- Stay connected with loved ones and social activities.

Alzheimer's disease

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder that is the most common cause of dementia. It is a chronic disease that gradually destroys memory and thinking skills and eventually leads to death.



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Alzheimer’s disease affects the hippocampus**,** a part of the brain that is responsible for memory formation and storage. **As** this progresses, the hippocampus shrinks and nerve cells die**.** This leads to memory loss, difficulty learning new information, and confusion. Progression of Alzheimer’s disease leads to synapse loss, neural network dysfunction and cognitive failure. Accumulation of protein aggregates and brain immune activation have triggering roles in in synaptic failure (Stoner et al., 2023).

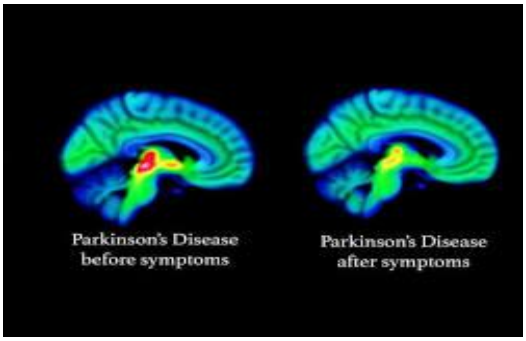
- Alzheimer's disease is the most common form of dementia. It accounts for 60-70% of all dementia cases.
- Alzheimer's disease is a progressive brain disorder. It slowly destroys memory and thinking skills, and eventually, the ability to carry out the simplest tasks.
- Alzheimer's disease is the seventh leading cause of death in the United States. It is the fifth leading cause of death for people over the age of 65.
- More than 6 million Americans are living with Alzheimer's disease. This number is projected to reach nearly 13 million by 2050.
- The risk of Alzheimer's disease increases with age. Most people with Alzheimer's are age 65 or older. However, it is also possible for people to develop Alzheimer's at a younger age.
- There is no cure for Alzheimer's disease. Early diagnosis and treatment are important for people with Alzheimer's disease which can help people maintain their independence and quality of life for longer.

Alzheimer’s disease begins in older adults, but it can also affect younger people. The average age of diagnosis is 65, but about 5% of people with Alzheimer’s disease are diagnosed before the age of 65. The exact cause of Alzheimer’s disease is unknown, but researchers believe that a combination of genetic and environmental factors play a role. Some of the known risk factors for Alzheimer’s disease include :

- Age. Family history of Alzheimer disease.
- Head injuries. Down Syndrome.
- Certain medical conditions, such as diabetes and heart disease.
- Mild Cognitive impairment.

Parkinson’s Disease (PD)

Parkinson’s disease is a progressive neurodegenerative disorder that affects movement. It is caused by the death of dopamine producing cells in the brain. Dopamine is a neurotransmitter that helps to control movement, coordination and balance. Degeneration of dopaminergic neurons leads to Parkinson’s disease.The hallmark symptoms of Parkinson’s disease are impairment in posture, balance and gait. As the disease progresses they become increasingly prevalent (Kliger & Ganguly, 2023).



www.genengnews.com

The most common symptoms of Parkinson's disease include:

- Tremor: a shaking of the hands, feet, or head, especially when at rest
- Slowness of movement (bradykinesia): difficulty moving or starting to move
- Muscle stiffness (rigidity): muscle tension that can make it difficult to move or to maintain a comfortable posture
- Impaired balance and coordination: difficulty with walking, turning, and standing up

Other symptoms of Parkinson's disease may include:

- Fatigue. Soft voice.
- Difficulty swallowing. Constipation.
- Sleep problems.
- Cognitive problems, such as memory loss and difficulty thinking clearly.
- Mood changes, such as depression and anxiety.

Parkinson's disease typically begins in people over the age of 60, but it can also occur in younger people. The average age of onset is 60. Parkinson's disease is a progressive disease, but the rate of progression varies from person to person. Some people may experience a rapid decline in symptoms, while others may have a more gradual decline.

Some of the known risk factors for Parkinson's disease include:

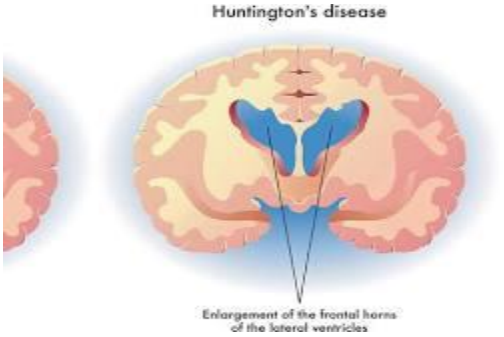
- Age.Family history of Parkinson's disease. Head injuries.

- Exposure to certain toxins, such as pesticides and herbicides.

Huntington’s Disease (HD)

Huntington's disease (HD) is a progressive neurodegenerative disorder that affects movement, cognition, and behavior. It is caused by a mutation in the huntingtin gene, which leads to the production of a defective huntingtin protein. This protein damages nerve cells in the brain, leading to the symptoms of Huntington’s disease. It is an autosomal dominant, neurodegenerative disorder with complete penetrance caused by a cytosine-adenine-guanine (CAG) trinucleotide repeat expansion in the huntingtin (HTT) gene on chromosome 4. The expanded CAG results in a mutant protein (huntingtin) rich in glutamine amino acids (polyQ), with toxic properties to the cell (Rojas et al.,2022).

The expanded CAG results in a mutant protein (huntingtin (HTT)) rich in glutamine amino acids (polyQ), with toxic properties to the cell.



The most common symptoms of Huntington's disease include:

- Involuntary movements (chorea): These movements can be jerky, twitchy, and uncontrollable. They may start in the face and hands, and eventually spread to the rest of the body.
- Cognitive decline: This includes memory loss, difficulty concentrating, and problems with judgment and reasoning.
- Personality changes: People with Huntington's disease may become irritable, aggressive, or withdrawn. They may also have difficulty controlling their emotions.

Other symptoms of Huntington's disease may include:

- Slurred speechDifficulty swallowing
- Problems with balance and coordinationFatigue

- Depression Anxiety

Huntington's disease typically begins in people between the ages of 30 and 50, but it can also occur in younger or older people. The average age of onset is 40.

Amyotrophic lateral sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a progressive neurodegenerative disorder that affects nerve cells in the brain and spinal cord. These nerve cells are responsible for controlling voluntary muscle movement, including talking, swallowing, breathing, and moving the arms and legs.

As the nerve cells in ALS degenerate, they stop sending messages to the muscles. This leads to muscle weakness, atrophy (wasting away), and paralysis. Eventually, people with ALS lose the ability to walk, talk, swallow, and breathe. About 50% of patients will suffer from extra-motor manifestations to some degree in addition to their motor problems. In 10-15% of cases, an additional diagnosis of frontotemporal dementia (FTD) can be made (Phukan et al., 2007), whilst 35-40% of patients will have mild behavioral and/or cognitive changes.

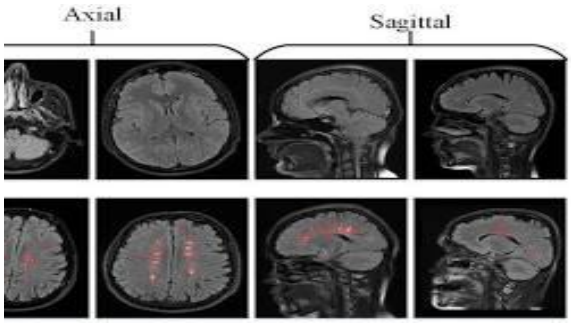
Some of the known risk factors for ALS include:

- Age: ALS is most common in people between the ages of 40 and 70.
- Family history: About 10% of people with ALS have a family history of the disease.
- Military service: Veterans have a slightly higher risk of ALS than the general population.
- Exposure to certain chemicals: Exposure to certain chemicals, such as pesticides and herbicides, may increase the risk of ALS.
- Head injuries: People with a history of head injuries may have a slightly higher risk of ALS.

Multiple Sclerosis (MS)

Multiple sclerosis (MS) is a chronic autoimmune disease that affects the central nervous system (CNS), which is made up of the brain, spinal cord, and optic nerves. In Multiple Sclerosis, the immune system mistakenly attacks the myelin sheath, the protective covering that insulates nerve cells. This damage to the myelin sheath disrupts the transmission of signals between the brain and

the rest of the body. Immunological and neurological disease processes can impact the composition of circulating body fluids. As a result, changes in protein levels in blood and Cerebrospinal Fluid can be used as biomarkers for disease recognition and disease activity (Huang J et al., 2020).



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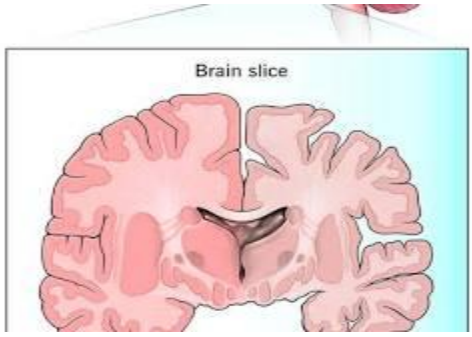
The symptoms of Multiple Sclerosis vary depending on the location and severity of the nerve damage. Some common symptoms include:

- Fatigue Blurred vision Double vision
- Numbness or tingling Muscle weakness Spasticity
- Coordination problems Balance problems Difficulty walking
- Difficulty thinking and concentrating Memory problems
- Cognitive impairment Bladder and bowel problems

Multiple Sclerosis can be a very unpredictable disease. Some people have mild symptoms that come and go, while others experience more severe symptoms that progress over time. There is no cure for Multiple Sclerosis, but there are treatments that can help manage symptoms and slow the progression of the disease.

Frontotemporal dementia (FTD)

Frontotemporal dementia (FTD) is a group of neurodegenerative disorders that affect the frontal and temporal lobes of the brain. These lobes are responsible for a variety of functions, including personality, behavior, language, and memory.



Frontotemporal dementia is a relatively rare condition, but it is the second most common type of dementia after Alzheimer's disease. It typically affects people between the ages of 45 and 65, but it can also occur in younger and older people. Frontotemporal dementia is a highly heritable disorder despite varying heritability among different clinical syndromes and subtypes due to a range of gene mutations (Rohrer et al., 2009). Up to half of Frontotemporal dementia cases with autosomal-dominant inheritance report a family history of Frontotemporal dementia (Rademakers et al., 2012)

The exact cause of Frontotemporal dementia is unknown, but researchers believe that a combination of genetic and environmental factors play a role. Some of the known risk factors for Frontotemporal dementia include:

- Family history: About 40% of people with FTD have a family history of the disease.
- Certain genetic mutations: Mutations in certain genes have been linked to FTD, including the C9orf72 gene, MAPT gene, and GRN gene.
- Head injuries: People with a history of head injuries may have a slightly higher risk of FTD.
- Exposure to certain chemicals: Exposure to certain chemicals, such as pesticides and herbicides, may increase the risk of FTD.

The symptoms of FTD vary depending on the specific area of the brain that is affected. However, some common symptoms include:

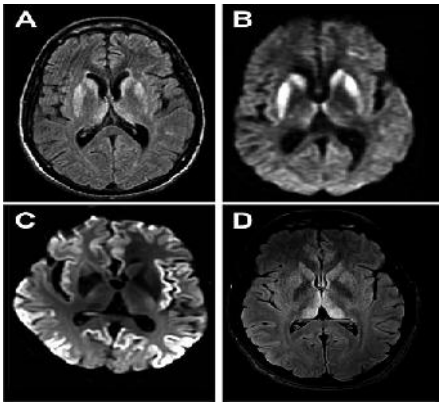
- People with FTD may become more withdrawn, apathetic, or disinhibited. They may also have difficulty controlling their emotions.
- People with FTD may exhibit repetitive behaviors, such as pacing or hand washing. They may also have difficulty with social interactions and may become aggressive or violent.

- People with FTD may have difficulty understanding and expressing language. They may also have difficulty with naming objects and finding words.
- People with FTD may experience memory loss, but it is not as severe as the memory loss that occurs in Alzheimer's disease.

FTD is a progressive disease, meaning that symptoms get worse over time. Eventually, people with FTD will need assistance with all activities of daily living.

Creutzfeldt-Jakob disease (CJD)

Creutzfeldt-Jakob disease (CJD) is a rare, rapidly progressive, and fatal neurodegenerative disorder. It is caused by prions, which are misfolded proteins that can damage and kill nerve cells. CJD can affect people of any age, but it is most common in adults over the age of 60. There are four different forms of Creutzfeldt-Jakob disease that have been identified so far: familial, iatrogenic, variant and sporadic. The sporadic form of the disease is the most frequent and accounts for 85% of Creutzfeldt-Jakob disease cases (Rus et al., 2018 & Ladogana et al., 2005).



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Symptoms of Creutzfeldt-Jakob disease

The symptoms of Creutzfeldt-Jakob disease vary depending on the part of the brain that is affected. Some common symptoms include:

- Rapidly progressive dementia Personality changes
- Behavioral changes Vision problems

- Movement problems, such as tremors, muscle weakness, and coordination problems
- Speech problemsDifficulty swallowing
- Insomnia

There are several different types of Creutzfeldt-Jakob disease, each with its own cause:

- Sporadic CJD: This is the most common type of CJD and occurs in people with no known risk factors.
- Familial CJD: This type of CJD is caused by a mutation in the prion protein gene.
- Iatrogenic CJD: This type of CJD is caused by exposure to prions from contaminated medical instruments or tissue grafts.
- Variant CJD (vCJD): This type of CJD is caused by eating beef from cattle that were infected with bovine spongiform encephalopathy (BSE), also known as mad cow disease.

Diagnosis of Creutzfeldt-Jakob disease

CJD can be difficult to diagnose, especially in the early stages. Doctors may perform a variety of tests to rule out other possible causes of the symptoms, such as Alzheimer's disease, Parkinson's disease, and stroke.

Some of the tests that may be performed to diagnose Creutzfeldt-Jakob disease include:

- Neurological exam: This exam assesses the patient's mental function, coordination, and movement.
- MRI: This imaging test creates detailed pictures of the brain.
- EEG: This test measures the electrical activity in the brain.
- Spinal tap: This procedure involves removing a small sample of cerebrospinal fluid for analysis.
- Brain biopsy: This procedure involves removing a small sample of brain tissue for analysis.

Conclusion

Neurodegenerative diseases are a group of chronic conditions that affect the nervous system, leading to a progressive loss of neurons. This loss of

neurons can result in a variety of symptoms, including cognitive decline, physical disability, and death. There is no cure for neurodegenerative diseases, but there are treatments that can help manage symptoms and slow down the progression of the disease. Neurodegenerative diseases are a serious group of conditions that can have a devastating impact on people's lives. However, there is hope for the future. Researchers are making progress in understanding the causes of these diseases and developing new treatments. With continued research, we may one day be able to prevent or cure neurodegenerative diseases.

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Orphan drugs- High priced miracle medicines

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Introduction

Orphan drugs are medicines developed to help treat rare diseases, which are conditions that affect a small number of people, which makes it difficult and expensive for pharmaceutical companies to develop and market treatments for them. As a result, many people with rare diseases do not have access for an effective treatments. [1]

Orphan drugs are important because they can provide life-saving or life-changing treatment for people with rare diseases.These are most often congenital or genetic disorders, but malignant and autoimmune diseases, poisonings, and rare infections are also members of this group of medical conditions.

There are a number of challenges associated with developing and marketing orphan drugs. One challenge is the small number of people who are affected by rare diseases. This makes it difficult for pharmaceutical companies to recoup the costs of developing and marketing orphan drugs. Another challenge is the lack of awareness of rare diseases. This can make it difficult for people with rare diseases to get the treatment they need. [2]

Despite the challenges, there has been progress in the development of orphan drugs. In recent years, there have been a number of new orphan drugs approved by the U.S. Food and Drug Administration (FDA). This progress is due in part to the Orphan Drug Act, which was passed by the U.S. in 1983.[3] Some of the famous orphan drugs, actions and their side effects are discussed below.

1 .SPINRAZA:

Spinraza is a type of medication called an antisense oligonucleotide. It is used to treat children and adults with spinal muscular atrophy (SMA), a type

of motor neuron disease. SMA is an inherited disorder that causes skeletal muscle weakness and wasting, which worsens with age. SMA is most commonly caused by mutations in a gene called SMN1 (survival motor neuron 1), which leaves the body without enough functional SMN protein to keep motor neurons healthy and functioning.

Side effects : Given by intrathecal route produce thrombocytopenia and coagulation abnormalities, Renal toxicity , lower respiratory infection and post-lumbar puncture syndrome[4]

2.REVLIMID

Revlimid is used to treat multiple myelomas (bone marrow cancer), either in combination with another medicine or after stem cell transplant. Revlimid is also used to treat anaemia in patients with myelodysplastic syndrome caused by an abnormal chromosome. This disorder is also called deletion 5q MDS, because part of chromosome 5 is missing. In people with this disorder, the bone marrow does not produce enough healthy blood cells. It is also used to treat mantle cell lymphoma (a rare cancer of the lymph nodes)[5]. Promotes immune responses to help slow tumour growth.

Side effects include :Anaemia , Blood dyscrasias, Muscle pain, Peripheral neuropathy (nerve damage),Rash , Sleep problems, Sore throat, Swelling of the hands, feet, ankles, or face and Vision problems. Revlimidcan alsocause birth defect.[6]

3.TRIKAFTA:

It is used in children and adults with cystic fibrosis who have at least one copy of the F508del mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene or another mutation that is responsive to treatment with Trikafta. Trikafta must be taken with a meal for a better absorption of the medicine.

Side effect includes: Allergic reaction, difficulty breathing, swelling of face, lips, tongue, or throat.[7]vision changes , liver problems - loss of appetite, stomach pain, dark urine, jaundice .[8]

4. IMBRUVICA:

It is used to treat chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), and Waldenströmmacroglobulinemia (WM). It is also used to treat rheumatoid arthritis (RA), polyarticular juvenile idiopathic arthritis (PJIA), and systemic lupus erythematosus (SLE).Imbruvica works by directly inhibiting an enzyme, called Bruton’s tyrosine kinase (BTK). It forms a

covalent bond with a cysteine residue on the enzyme, preventing its activity. BTK works as a signaling molecule for the B-cell antigen receptor (BCR) and cytokine receptor pathways. Activation of these pathways causes the proliferation of B cells and other interactions, and inhibition has been shown to reduce the growth and spread of malignant B cells. It belongs to the class of medicines known as BTK inhibitors.[9]Imbruvica is a cancer medication .[10]

Side effects include ;Allergic reaction to Imbruvica like hives, difficulty breathing, swelling of face, lips, tongue, or throat.Imbruvica may cause a brain infection that can lead to disability or death, easy bruising, unusual bleeding, or any bleeding that will not stop inside the body. Other than that, low blood cell counts ,muscle spasms, bruising, rash ,muscle, joint, bone pain are also seen.

5. ALGLUCERASE:

Mainly preferred in rare genetic disorder called Gaucher disease. Gaucher disease is a disorder in which the body does not produce enough of an enzyme called glucocerebrosidase. This enzyme is needed to break down a type of fat called glucocerebroside. When glucocerebroside builds up in the body, it can damage organs and tissues. Alglucerase is made from human placenta tissue that is collected after baby is born.[11]

Side effects include :possibility of diseases caused by viruses could be passed on, like hepatitis and HIV and severe allergic reaction like , difficulty breathing, swelling of the face, lips, can occur. Chest pain, Shortness of breath, Swelling of the hands, feet, ankles, or face, Severe headache, Seizures, Confusion, Loss of consciousness are other effects[12]

6. LONAFARNIB:

Lonafarnib is for use in people with certain rare genetic conditions that cause premature and rapid aging- Hutchinson-Gilford Progeria Syndrome (HGPS).Lowers the risk of death in people with Hutchinson-Gilford Progeria Syndrome (HGPS).It can also used to treat a rare genetic disorder called neurofibromatosis type 1 (NF1). NF1 is a condition that causes tumours to grow on nerves throughout the body. Lonafarnib works by slowing the growth of these tumours. It is also used to treat a rare type of cancer called juvenile xanthogranuloma.[13]

Side effects include : electrolyte imbalance infection, increased blood pressure, stomach pain, nausea, WBC and RBC count, decreased platelet count. Problems related to eye, skin ,Kidney, liver, heart, nervous system occur. [13]

7. RUCAPRIB:

Rucaparib is used to treat ovarian cancer, fallopian tube cancer,primarily peritoneal and prostatecancer .Rucaparib is a PARP inhibitor. PARP inhibitors work by blocking the action of PARP enzymes, which are involved in DNA repair. This can lead to the death of cancer cells that have damaged DNA.[14]

Side effects are : Blood clots, Bone marrow suppression, Decreased white blood cell count, Decreased platelet count, Dizziness, Headache, Hair loss, High blood pressure , Itching, Joint pain, Vaginal bleeding, Vision problems. Liver damage, Lung problems, Pancreatitis, Stevens-Johnson syndrome, Toxic epidermal necrolysis.[14]are rare.

8. TOCILIZUMAB:

Tocilizumab is a monoclonal antibody that works by blocking the action of interleukin-6, a protein that is involved in inflammation.[15]. Used to treat rheumatoid arthritis, juvenile Idiopathic arthritis, polyarticular juvenile idiopathic arthritis, systemic juvenile idiopathic arthritis, giant cell arteritis, Takayasu arteritis, and Castleman disease. It is also used to treat severe COVID-19.

Side Effects include: stomach cramps, bloating, unusual bleeding, runny or stuffy nose, sinus pain, sore throat, headache, increased blood pressure, abnormal liver function tests, pain, swelling, burning, or irritation where an injection was given.[15]

9. NITISINONE:

Nitisinone is used to treat a rare genetic condition called hereditary tyrosinemia type 1 (HT-1). HT-1 is a metabolic disorder that occurs when the body does not produce enough of an enzyme that breaks down proteins from certain foods. This condition occurs most often in young babies.Nitisinone works by preventing the body from breaking down an amino acid called tyrosine and by keeping other toxic substances from building up and causing harm to liver or kidneys.[16]

Side Effects include: Vision changes, eye pain, Nosebleeds, rash or itching, dry or peeling skin, thinning hair, headache, diarrhoea[16]

Few examples of Orphan drugs in various systems.

System	Orphan drugs used
Cardio vascular system	Tafamidis, PCSK9, Evolocumab ,Ambrisentan ,Bonsentan, EpoprostenolIvacaftor, Migalastat
Central nervous system	Lamotrigine,Riluzole, Fingolimod,Cannabidiol (CBD), Pitolisant , Tetrabenazine
Oncology	Imatinib (Gleevec), Pembrolizumab (Keytruda), Larotrectinib, Rucaparib, Olaparib, Acalabrutinib, Blinatumomab, Vemurafenib
Immunology	Rilonacept, Emapalumab, Tocilizumab, Icatibant, Migalastat, Eculizumab, Adalimumab, Belimumab
Respiratory System	Iloprost, Alpha-1 Proteinase Inhibitor, Orkambi, Arikayce
Digestive System	Teduglutide, Icatibant , C1 Esterase Inhibitor.
Endocrine System:	Lanreotide, Cinacalcet
Reproductive system	Ganirelix

CHALLENGES AND COMPLICATIONS IN DEVELOPING ORPHAN DRUGS :

- High Costs: Developing orphan drugs can be expensive due to the limited patient population, leading to higher production costs. Consequently, these costs may translate into high prices for these medications, which can pose challenges for accessibility and affordability.
- Limited Market: The small patient population for rare diseases means a limited market for these drugs. This factor can make it challenging for pharmaceutical companies to recover the costs of research, development, and production, leading to higher prices.
- Regulatory Hurdles: Despite incentives provided by regulatory bodies to encourage development, navigating the regulatory landscape for orphan drugs can be complex. This includes challenges in obtaining orphan drug designation, conducting clinical trials with limited patient numbers, and meeting stringent regulatory requirements.
- Access and Availability: Limited production and high costs can lead to issues of accessibility, where patients in certain regions or countries might face challenges in accessing these medications.
- Challenges in Research: Researching rare diseases can be difficult due to the scarcity of patients and limited data available for study. This can slow down the research process and development of effective treatments.
- Shortage of Data: The limited number of patients can result in insufficient data available for monitoring long-term effects, potential side effects, and the overall safety and efficacy of these drugs.
- Variability in Disease Presentation: Rare diseases often have a wide range of symptoms and disease progression, making it challenging to design a one-size-fits-all treatment.[17]

IMPORTANCE OF ORPHAN DRUGS :

- Addressing Rare Diseases: They fill critical gaps in healthcare by treating conditions affecting a small number of people.
- Improved Quality of Life: Orphan drugs significantly enhance the lives of those with rare diseases, offering relief and treatment options.
- Incentivizing Research: Their development encourages innovation and drives research in treating less common conditions.
- Regulatory Benefits: Designations provide regulatory support and financial incentives for pharmaceutical companies, facilitating development.

- Economic and Social Impact: By addressing rare diseases, they reduce economic burden and enhance overall healthcare.[1,2,17,18]

Conclusion

Orphan drugs are critical for addressing rare diseases where few to no treatments exist. They significantly impact the quality of life for those affected, often being the only hope for these conditions. Their development encourages innovation and research in medical science, benefiting not only those with rare diseases but also contributing to broader healthcare advancements. Regulatory support and incentives enable pharmaceutical companies to invest in treatments for these less common diseases, reducing the economic burden on healthcare systems. In essence, these drugs play a fundamental role in offering care, hope, and progress for individuals dealing with rare diseases.

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Efficient and reliable healthcare with - Artificial intelligence (AI)

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Abstract

Artificial intelligence (AI) is making significant strides in the field of healthcare, transforming the way medical professionals diagnose, treat and manage diseases. AI in health care is a broad and diverse field that uses various devices, algorithms and software to enhance health care delivery. AI also plays a crucial role in predictive imaging, to enable healthcare providers to predict disease progression and to identify high-risk patients. Its powered systems are revolutionizing personalized medicine, by analyzing a patient's genetic information, medical history and lifestyle factors helps in accelerating accurate diagnosis. By analyzing vast amounts of biomedical data, AI algorithms can identify potential drug targets, design novel molecules and optimize drug formulation leading to development of more effectivedrug discovery and development. Currently, AI holds immense potential in transforming healthcare by improving diagnostic accuracy, enhancing patient monitoring and drug discovery, ultimately leading to better patient outcomes and a more efficient healthcare system

Keywords: Artificial intelligence, novel molecules, patient monitoring and drug discovery

Introduction

Artificial intelligence is a field of science concerned with building computers and machines that can reason, learn and act that would normally require human intelligence.

In healthcare, artificial intelligence works by analyzing large amounts of data such as medical records, research papers and patient information. AI algorithms can identify patterns, make prediction and assist in decision making, depending on the application. AI devices can be placed in different locations, such as inside the body, on the skin, in the hospital, in the laboratory or in the cloud.AI does not physically enter the human body; instead it processes and analyzes data to provide insights and support decision-making.

Artificial intelligence methods include Machine learning (ML)and Deep learning (DL) Algorithms are widely used in the prediction and diagnosis of several diseases. Machine learning is based on learning methods and can be divided into three categories:-

MACHINE LEARNING MODELS

1. supervised learning ----------> classification and regression

2. unsupervised learning---------> clustering

3. reinforcement learning---------> decision making

Deep learning is a method for designing the machine learning algorithm

HOW ML PLAYS A ROLE IN AI?

Machine learning algorithms are used to train AI systems to identify objects and faces in images. This ability to learn and adapt is essential for AI Systems to be able to perform complex tasks in the real world.A more complex form of machine learning is the NEURAL NETWORK- a technology that has been well established in healthcare research for several decades and has been used for categorization- applications like determining whether a patient will acquire a particular disease.Its key application is to examine and diagnose medical images like MRI, X-Rays and C.T. Scans and to make accurate detection.

1. MRI, X-RAY,C.T SCAN:-

Artificial intelligence (AI) is rapidly transforming the field of medical imaging, including MRI, X-ray, and CT scans. AI algorithms can be trained to identify patterns and anomalies in medical images that may be difficult or time-consuming for human radiologists to detect. This can help to improve the accuracy and efficiency of diagnosis and treatment. (1)

MRI

Image reconstruction: AI can be used to develop new image reconstruction algorithms that can produce higher quality MRI images with shorter scan times. In addition AI can be used to develop algorithms that can automatically detect and segment lesions in MRI images, such as tumors, strokes, and multiple sclerosis plaques. This can help radiologists to identify and diagnose diseases more quickly and accurately. More than that by Radiomics, AI can be used to extract quantitative features from MRI images, such as texture, shape, and intensity. This information can then be used to develop predictive models that can help to predict the risk of disease progression that fail to be appreciated by naked eye, response to treatment, and patient outcomes.

X-ray

AI algorithms can be trained to identify and diagnose common chest abnormalities, such as pneumonia, tuberculosis, and heart failure. This can help radiologists to read chest X-rays more quickly and accurately, especially in high-volume settings. Also AI algorithms help to detect fractures in X-ray images of bones. This can help to improve the diagnosis and treatment of fractures, especially in complex cases.To screen Cancer at the earlier stage, AI algorithms are found to be much beneficial.

CT scan

A high quality CT scan images with low radiation doses can be developed with AI and reconstruction of images can also be done. Lesion detection and segmentation made easy with AI. An automatic detection and segment lesions in CT scan images, such as tumors, metastases, and blood clots can be developed by AI algorithms. This can help radiologists to identify and diagnose diseases more quickly and accurately. Virtual colonoscopy images from CT scan data can be developed from AI to screen for colon cancer, a replacement for a traditional colonoscopy.

In addition to these specific examples, AI is also being used to develop new and innovative applications in medical imaging. For example, AI is being used to develop algorithms that can fuse images from different imaging modalities, such as MRI and CT scans, to provide more comprehensive information about a patient's anatomy and pathology. It is also being used to develop algorithms that can generate synthetic medical images, which can be used for training AI algorithms or for creating personalized treatment plans. (2)

AI is a powerful tool that has the potential to revolutionize the field of medical imaging. By automating tasks and providing insights that are difficult or impossible for human radiologists to detect, AI can help to improve the accuracy, efficiency, and accessibility of medical imaging

2. BIOPSY :

This is a procedure that involves taking a small sample of tissue from a part of the body and examining it under a microscope to diagnose diseases, such as cancer. AI can help to improve the accuracy and efficiency of biopsy by guiding the needle to the target area, classifying the tissue type, or predicting the prognosis. AI devices can be placed on the needle, on the microscope, or in the cloud.

Artificial intelligence algorithms are trained using large datasets of biopsy images. These images are carefully annotated by experts to indicate the presence or absence of specific diseases or conditions. The AI algorithms learn to recognize patterns and features in the images that are associated with different diseases. During the analysis process, the biopsy images are fed into the AI system, which then uses its learned knowledge to identify potential abnormalities or diseases. The AI algorithm can detect subtle patterns or variations that may be difficult for human pathologists to spot.The AI system generates a report or highlights areas of concern in the biopsy images, providing valuable insights to pathologists. This can help them make more accurate diagnoses and treatment decisions.

It's important to note that AI is not meant to replace human pathologists, but rather to assist and enhance their capabilities. The combination of AI and human expertise can lead to more precise and efficient biopsy analysis, ultimately improving patient care. (3)

3. MEDICAL ASSISTANT :

The first step towards building an artificially intelligent system (after problem selection and development of solutions strategy) is data collection. The creation of well performing models relies on the availability of large quantities of high quality data. The issue of data collection is shrouded in controversy due to patient privacy and due to recent incidents of data breaches by major corporations. Advances in technology have resulted in increased computational and analytic power as well as the ability to store vast amounts of data.

Technology such as facial recognition and gene analysis provides a path for an individual to be identified from a pool of people. Patients and the public in general have a right to privacy and the right to choose what data, if any, they would like to share. Data breaches now make it possible for patient data to fall into the hands of the insurance companies resulting in a denial of medical insurance because a patient is deemed more expensive by the insurance provider due to their genetic composition. (4)

AI can help augment or replace some of these tasks by using voice recognition, natural language processing, or robotic process automation. AI devices can be placed on the phone, on the tablet or in the cloud

4. PREDICTIVE ANALYTICS:

This is a branch of data analytics that uses statistic methods and machine learning to forecast future outcomes based on historical data. AI can help improve predictive analytics by using deep learning, neural networks, or reinforcement learning to handle complex and dynamic data sets(5)

First, predictions should discriminate between individuals with and without the disease (i.e, higher predictions in those with the disease compared to those without the disease). risk predictions should be accurate. (6).Private companies may invest in the development of an algorithm that uses predictors for which the company offers measurement tools (example: kits, biomarkers) . AI devices can be placed on the server, on the laptop, or in the cloud.

5. CHATBOTS:

AI chatbots in healthcare are virtual assistants that use artificial intelligence to interact with users and provide healthcare-related information and support. These chatbots utilize natural language processing and machine learning algorithms to understand user queries and respond in a human-like manner.

They can assist with a range of tasks, such as answering medical questions, scheduling appointments, providing medication reminders, and offering personalized health advice. AI chatbots can also offer mental health support by giving counselling.

By leveraging large amounts of medical data, AI chatbots can analyze symptoms and medical histories to make preliminary diagnoses and recommend appropriate next steps. They can also provide educational resources, helping users understand various health conditions and treatment

options. These chatbots are accessible through various platforms, including websites, mobile apps. (7,8)

AI chatbots in healthcare have several benefits. They offer 24/7 availability, allowing users to seek assistance at any time. They can reduce the burden on healthcare providers by handling routine inquiries and freeing up their time for more complex cases. Chatbots can also improve patient engagement and satisfaction by providing personalized and timely support.

AI chatbots represent a powerful supplement to healthcare, but they should not be seen as a complete substitute for the irreplaceable role of medical professionals.

6. AUTOMATED ADMINISTRATIVE TASKS:

These are tasks that involve managing and organizing information and documents in a health care setting, such as coding diagnoses and procedures, filling claims or updating records. AI can help automate these tasks by using character recognition, natural language understanding, or robotic process automation. AI devices can be placed on the scanner, on the computer, or in the cloud.

AI plays a crucial role in automating administrative tasks in healthcare. It can streamline processes like appointment scheduling, billing, and record-keeping. AI powered systems can analyze and extract relevant information from medical documents, reducing the need for manual data entry. This helps save time, minimize errors and improve overall efficiency in healthcare administration. (9)

It is OUTLINED in a FIVE STEP PROCESS:

1. Identify events, tasks and overflows
2. Standardize system events
3. Uncover hidden network structure
4. Measure administrative burden
5. Optimize network flows and redefine roles

7. DIAGNOSIS AND TREATMENT:

These are processes that involve identifying and resolving health problems based on symptoms, tests and evidence. AI can help improve diagnosis and treatment by using machine learning, computer vision, or natural language processing to analyze data from various sources and provide

recommendations or decisions. AI devices can be placed on the device such as a stethoscope, on the monitor such as an electrocardiogram or in the cloud.

AI is used in various diseases and medical conditions. It can assist in diagnosing diseases like cancer, heart disease and neurological disorders. It also helps in predicting disease progression and developing personalized treatment plans.

AI's advanced algorithms also greatly assist in detecting and predicting BREAST CANCER in the early stages. The WISCONSIN BREAST CANCER DATASET (WBCD) is a widely used dataset for researchers investigating ML methods to diagnose breast cancer at early stage (11)

Machine Learning methods, include neural networks, random forests and support vector machines have been used to predict and categorize genetic disorders from different amounts of genetic data. AI especially ML and DL methods could enhance the accuracy of predicting genetic disorders.

8. DRUG DISCOVERY:

This is a process that involves finding new compounds that can treat diseases by testing their effects on biological targets. AI can help to accelerate drug discovery by using deep learning, generative models, or reinforcement learning to design novel molecules, predict their properties, or optimize their synthesis. By accelerating the drug discovery process, AI has the potential to bring new treatments to patients faster. AI can be used effectively in different parts of drug discovery such as (13).

AI in drug design: To Predict 3D structures of target protein, drug protein interactions,

determining drug activity, and de novo drug design.

AI in polypharmacology: To Designbio specific drug molecules, multitarget drug molecules.

AI in chemical synthesis: To predict reaction yield, retrosynthesis pathways, to develop insights into reaction mechanism and to design synthetic route.

AI in drug repurposing: To Identify therapeutic target, and to predict new therapeutic use.

AI in drug screening: To Predict the toxicity of the drug, its bioactivity, physiochemical property and to identify and classify the target cells.

AI devices can be placed on the laboratory equipment such as spectrometer, on the workstation such as molecularmodelling software or in the cloud. (15)

9. DIABETES :

This is a chronic condition that affects how the body regulates blood sugar levels. AI can help manage diabetes by using machine learning, computer vision or natural language processing to monitor blood glucose levels, insulin doses or dietary intake.(16)

Efforts towards the clinical application of AI in the diagnosis and treatment of diabetes are mainly categorized into four areas:

(1) Automatic retinal screening, (2) clinical diagnosis support, (3) patient self-management tools, and (4) risk stratification.

1. Automatic retinal screening, that automatically interprets the presence or absence of diabetic retinopathy- from fundus images. An example of this technology is the IDx-DR device manufactured by Digital Diagnostics Inc., (17).This device facilitates the screening and diagnosis of diabetic retinopathy, especially in rural communities where patients have difficulties accessing an ophthalmologist.

2. Clinical diagnostic support, that mimic the “hidden tips of treatments by a specialist,” such as fine-tuning insulin dose, are being developed rather than just a support system for diabetes diagnosis itself.Advisor pro, manufactured by DreaMed Diabetes, Ltd., which the FDA approved in 2018. This system sends information obtained by continuous glucose monitoring (CGM) and self-monitoring of blood glucose (SMBG) to a cloud server and uses AI to determine and propose the necessity for insulin dose adjustments remotely. (18)

3. Patient self management tool, is familiar with some diabetes patients because they have already self-checked various biometric data such as actively measuring blood glucose levels through SMBG(self monitoring blood glucose) .With the patient self-management tools, the AI technology interprets their biometric data and alert like a diabetologist to improve the patient’s blood glucose control. The Guardian Connect System, manufactured by Medtronic, is an example of an AI system with this functionality.

4. Prediction and risk satisfaction, could be a part of preemptive medicine, accurately identifying individuals that are highly likely to develop a specific disease from the general population at the pre-illness stage. Thus, this technology would eventually eliminate the incidence of diabetes by implementing medical intervention for these people at a very early stage. (19).

10. ALZHEIMER DISEASE:-

This is a degenerative brain disorder that causes problems with memory, thinking and behaviour. AI help detect, diagnose, or treat Alzheimer disease by using machine learning, computer vision or natural language processing to analyze brain images, cognitive tests or speech patterns. In MRI, AI can be used to detect shrinkage of the hippocampus, a brain region that is involved in memory and learning, which is a hallmark of Alzheimer's disease. In biopsy, AI can be used to analyze biopsy samples from the brain to identify biomarkers of Alzheimer's disease, such as amyloid plaques and tau tangles.

AI devices can be placed on the head (such as electroencephalogram), on the tablet Artificial Intelligence in Current Diabetes Management and Predictiondevelops for diagnosing neuropsychiatric disorders, manage the disease and to identify patients who are at risk of developing a stroke. The most widely used algorithm for disease detection is DL-based CNN models. (20)

11. SURGICAL PROCEDURES - ROBOTICS:-

Robotic surgery, also known as robot-assisted surgery, is a minimally invasive surgical procedure that utilizes robotic technology to perform complex operations. AI is playing an increasingly important role in robotic surgery, providing surgeons with enhanced precision, control, and decision-making capabilities.

The operative procedure, using robotic technology, is connected to hardware calibrated with a handpiece. Surgeons perform surgery through minimally invasive means. This mode of surgery limits blood loss to a minimum. The active robotic sensors map the location of joint to be replaced and proceed autonomous resection with utmost precision. Also the machine alerts the surgeon of any sudden movement and stops automatically to maintain safety of procedure.(21)

Image based robotic joint replacement system use 3D CT scan of patient's bone and the same is used by surgeon in preplanning for the surgery as per the patient's anatomy. Tension - Sensors on the robotic arms and the integration of augmented reality methods can help enhance the surgical experience and monitor organ movements. (22)

12. ANESTHESIA:-

A Computer can be used in anesthesia to design a servo system, for instance to maintain Bi - spectral index(BIS) within a specified range by continuous assessment and adjustment of the infusion rate of the anesthetic agents.

Target controlled infusion (TCI) systems can be considered the first-generation open-loop pharmacological robots. It possess in-built pharmacokinetic models of different drugs, to achieve the actual concentrations of plasma drug levels, especially in patients who display extreme anthropomorphic features or in patients with different racial characteristics. (23, 24)

In closed-loop systems, a “goal” for a measured variable is set by the operator and drug delivery is adjusted to make the gap between the set goal and actual variable as small as possible. The initial models were single input single output (SISO) systems; recent advances have led to multiple inputs multiple output (MIMO) robots, addressing hypnosis, analgesia, and muscle relaxation simultaneously. Anesthesia parameters targeted by the SISO systems initially included hypnosis (BIS-guided) or muscle relaxation (nerve-stimulator guided) as feedback for drug delivery. (25,26,27,28)

The application of AI in the field of anesthesia is also thriving, including in airway management, ultrasonic-assisted diagnosis, intelligent drug infusion systems, accurate intraoperative monitoring and early warning, perioperative complications, and fatality prediction and intensive care treatment; this can change clinical anesthesia practice, optimize treatment processes, and improve patient prognosis.Thus, mastering AI technology is crucial to providing safe, efficient, and cost-effective clinical anesthesia. (29)

ARTIFICIAL INTELLIGENCE (AI) IN DENTISTRY

1. PERIODONTITIS:-

Periodontitis is the sixth most prevalent disease worldwide. It is characterized by microbially associated, host-mediated inflammation that results in loss of alveolar bone and periodontal attachment, which can lead to tooth loss (30). AI can be preferred in automated segmentation of gingivitis disease from oral images, diagnosis and prediction of periodontal compromised teeth, bone lose using deep learning, olfaction in halitosis etc., (31). Periodontitis is the base for oral diseases that affect the teeth and the gums, causing pain, infection or tooth loss. AI can help to prevent, detect, or treat dental decay by using machine learning, computer vision, or natural language

processing to analyze dental images, oral hygiene habits, or patient feedback.AI devices can be placed on the toothbrush (such as a smart toothbrush), on the scanner (such as a dental scanner), or in the cloud.

2. ORAL CANCER:-

This occurs in the mouth or in the throat, causing symptoms such as ulcers, lumps, or bleeding. Machine learning (ML), a subset of AI that enables computers to learn from training data, has been highly effective at predicting various types of cancer, including breast, brain, lung, liver, and prostate cancer. In fact, AI and ML have demonstrated greater accuracy in predicting cancer in the clinicians. This innovative system capitalizes on the power of convolutional neural networks (CNNs), strengthened by the synergy of transfer learning (TL), and further fine-tuned using the novel Aquila Optimizer (AO) and Gorilla Troops Optimizer (GTO), two cutting-edge metaheuristic optimization algorithms. (32)

ML can help in cancer prediction and diagnosis by analyzing pathology profiles, imaging studies, and its ability to convert pictures to mathematical sequences. (33)AI can help diagnose or treat oral cancer by using machine learning, computer vision, or natural language processing to analyze oral images, biopsy samples, or patient records. AI devices can be placed on the tongue such as oral cancer screening device, on the microscope such as digital pathology system or in the cloud .

3. ENDODONTICS:-

This is a branch of dentistry that deals with the diagnosis and treatment of diseases of the pulp and the root of the teeth, such as root canal therapy.

AI works in endodontics by analyzing large amounts of data, such as dental images, patient records, and treatment outcomes, to identify patterns and trends. This information can then be used to improve the diagnosis, treatment, and prognosis of endodontic procedures. AI-powered diagnostic tool for endodontics is the DexisCariVu caries detection system. This system uses trans illumination technology to create a real-time image of the tooth, which can be used to identify areas of decay that may not be visible on traditional dental radiographs.

AI can be used to predict the prognosis of endodontic procedures. For example, AI algorithms can be trained to identify factors that are associated with a higher risk of treatment failure. AI-powered prognostic tool for endodontics is the EndoPredict system. This system uses a variety of

factors, including the patient's age, gender, medical history, and tooth type, to predict the probability of success of an endodontic procedure. (34)

AI devices can be placed on the tooth such as an endodontic sensor, on the hand piece such as endodontic motor or in the cloud.

4. ORTHODONTICS:-

This is a process that involves designing and applying braces or aligners to correct the alignment and occlusion of the teeth.

* AI can be used to identify and assess the patient's facial structure, including the jaw bones, teeth, and lips, to predict the movement of teeth during orthodontic treatment, to develop personalized treatment plans for patients with complex orthodontic problems, such as cleft palate and craniofacial anomalies.

AI can help optimize orthodontic treatment planning by using machine learning, computer vision, or natural language processing to analyze dental impressions, facial scans, or patient references. AI devices can be placed on the teeth such as orthodontic scanner, on the computer such as orthodontic software or in the cloud.The aim of this was to investigate the use of advanced AI software in orthodontics, particularly for the purposes of CBCT diagnosis and assessment, treatment assessment and outcome stability in the follow up case. (35)

Conclusion

AI play an important role in healthcare in future. AI system will not replace human clinicians, but rather it will augment their efforts to care for patients.

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Piper longum and its hepatoprotective role – A Review

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Introduction

Liver plays an important role in metabolism and disposition of many drugs and chemicals, when exposed to xenobiotics. Drugs administered for various therapeutic purposes found to precipitateacute and chronicliver toxicity, expressed as inflammatory changes later developed to hepatic necrosis, steatosis, steatohepatitis, cholestasis, fibrosis, cirrhosis and hepatocellular carcinoma. The toxic manifestations of many drugs might be reversible or irreversible and it is dependent upon the dose, duration of exposure, the susceptibility of individuals and several other factors (1). In the traditional system of Ayurvedic medicine practice, plant products are considered to be lesstoxic and free from side effects when compared to synthetic drugs(2). This review discusses the characteristic features, of one such product *Piper longum*, and itsrolein hepatoprotective effect and probable mechanismslike overcoming oxidative stress and membrane stabilizing properties.

Source and chemistry of Piper longum

Piperine was discovered in 1819 by Hans Christian Orsted, who isolated it from the fruits of *Piper nigrum* and *Piper longum*. It has got a pungent taste and first extracted as a yellow crystalline material having molecular formula C17H19NO3 with a melting point of 128-130°C. Later, the chemical structure was elucidated, and its IUPAC name is (2E, 4E)-5-(benzo[d] [1,3]dioxol-5-yl)1-(piperidin-1-yl)penta-2,4-dien-1- one. It is weakly basic in nature and sparingly soluble in water(3). Its medicinal property was first written by Hippocrates and it was Theophrastus who distinguished between the two types of pepper, black and long peppers (4). It is a small shrub with a large woody root and numerous creeping, jointed stems that are thickened at the nodes. The fruits, which grow in fleshy spikes 2.5 – 3.5 cm

long and 5mm thick, are oblong, blunt and blackish green. The mature spikes are collected and dried as the commercial form of pippali, and the root radix is known as pippalimula.(4). The plants grows in evergreen forests of India, Nepal, Indonesia, Malaysia, Srilanka, Timor and Phillippines. In India, the plant is grown in high humidity regions like in Assam, Tamil Nadu and Andhra Pradesh, on a large scale in limestone soil.(4,5)

Piperine is the major and active constituent of long pepper (*Piper longum*). The piperine content is 3-5% (on dry weight basis) in *P. longum*. The fruit of *P. longum* contains a large number of alkaloids and related compounds, the most abundant of which is piperine, together with methyl piperine, iperonaline, piperettine, asarinine, pellitorine, piperundecalidine, piperlongumine, piperlonguminine, refractomide A, pregumidiene, brachystamide, brachystamide-A, brachystine, pipercide, piperderidine, longamide and tetrahydropiperine, terahydropiperlongumine, dehydropipernonalinepiperidine, piperine, terahydropiperlongumine and trimethoxycinnamoyl-piperidine and piperlongumine have been found in the root of *P. longum*.(4) .

Piperine as an effective bioenhancer in drug absorption

Piperine enhances the bioavailability of various drugs and chemicals by inducing alterations in membrane dynamics and permeation characteristics, along with the induction in the synthesis of proteins associated with cytoskeletal function, thereby increases the absorptive surface in small intestine. It shows high lipophilicity, is weakly basic, and exhibits non-saturable passive absorption kinetics (3,4,5). The intestinal absorption might be due to enhancement in the secretion of bile acids and causes inhibition of bile acid metabolism thereby increasing the formation of micelle, this enhances solubility and absorption. Piperine interacts with intestinal epithelial cells and stimulates gamma-glutamyltranspeptidase activity and increases the uptake of aminoacid by epithelial cells (6). In 1979, Indian scientists at the Regional Research Laboratory (Indian Institute of Integrative Medicine) in Jammu discovered piperine as the first bioenhancer in the world and also coined the term bioavailability enhancer. Piperine when used in combination with Indomethacin, Diclofenac sodium, Rifampicin, Phenytoin, Losartan etc., reduces dose, side effects and increases bioavailability (5,6).

Piperine as an anti-oxidant

Oxidative stress and lipid peroxidation mediated liberation of ROS has been implicated as the common cause for induction of drugs-induced liver diseases. Due to various metabolic reactions in the liver leads to a higher

generation of ROS. Hepatocytes are vulnerable for ROS formation through the binding of toxic products to the membrane phospholipids and leads to alteration in integrity of cell membrane resulting in various pathogenicity of liver. Malondialdehyde (MDA) is a product of LPO and its elevation is the most common indicator of oxidative stress. Generation of ROS in the liver cause the loss of oxidant-antioxidant homeostasis, resulting in cellular damage (7,8).

Theoccurrence of phenols, flavonoids, and terpenes in plants make them better anti-oxidant agents,which are not only economic but also diverse and produces action without side effects (9,10). On the contrary, before translating the health benefits of antioxidants for biomedical sciences, detail analysis of plant will be required, as they might also leadto toxicity due to pro-oxidative activities (11).

The action of *P. longum*extracthave been confirmed using isoproterenol induced oxidative stress (12),adriamycin induced oxidative stress (13), and in monosodium glutamateinduced stress (14). *P. longum*extract also produces synergistic anti-oxidantaction when administered with other herbs (15). The above-reportedexperiments have confirmed the use of *P. longum*in pathology associated with oxidative stress,although limited information on dose-dependent effect is available. Moreover, the anti-oxidantpotential of the extract is postulated to be due to the presence of phenolic compounds andflavonoids. Also, the action has been evaluated through various chemical assays like DPPH andfree radical scavenging assays, which do not provide any pharmacological relevance and limitsthe validation of established biological action. Scientists can further adopt chromatographictechniques, apply some *in vitro* biological test(s) exploiting cell lines and simulated digestionand *in vivo* assessments, which will reveal the dose and time-dependent action of *P. longum*extract and can be utilized for the development of pharmaceutical and/or nutraceutical products.

Additionally, protection offered against oxidative injury paves path to employ this plant aschemopreventive and in neurodegenerative disorders.

Piperine as an hepatoprotectant

The alkaloid piperine has been shown to protect liver against various drugs and chemicals induced hepatotoxicity, oxidative stress, hepatic fibrosis in experimental animals. Studies have reported that piperine possess anti-inflammatory, anticancer, anti-hyperlipidaemic, antimicrobial properties etc., Liver fibrosis is the exorbitant gathering ofextracellular matrix proteins incorporating collagen that happens in many sorts of continual liverillnesses.

Liver fibrosis in advanced stage results in liver failure and may require liver transplantation. Being a severe threat to humans, anti fibrotic agents are in developmental phase and this hepatoprotective herbs are useful in clinical conditions (16).

The protective action of *P. longum*is documented by its anti-oxidant property in increasing the intra-cellular content of reduced glutathione and by enhancing the hepatocellular membrane permeability and its stability by scavenging free radicals. Several studies have reported that *Piper longum* is an excellent hepatoprotectantagainst CCl4induced liver fibrosis model (17). CCl4 has been found to induce hepatictoxicity by metabolic activation. It is being converted to CCl3**.** free radical by cytochrome P450in endoplasmic reticulum. This free radical generation causes lipid peroxidation, demolition ofCa2+ homeostasis and cell death. Thus, it selectively serves as good model to study liver toxicity(18). Gurumurthy*et al.*, experimented with aqueous extract of *Piper longum* and piperine for its hepatoprotective effect on antitubercular drugs, there was a decrease in lipid peroxidation with an increase in reduced glutathione levels. (19).An *in vivo* hepatoprotective action of *P. longum*has also been validated byproducing AlCl3 induced liver toxicity. This metal induced toxicity was reversed by *P. longum*extract and the action was ought to be due to anti-oxidant effect (20). Theflavonoids present in the *P. longum*seem to be responsible for hepatoprotective action, althoughsome studies have reported the action with single dose and in acute regimen, which was amendedin other report.

Piperine as an anti-hyperlipidemic agent

Alcoholism and several agents have been reported to disrupt lipid homeostasis by inducing hyperlipidemia, micro and macro-vesicular steatotis and steato hepatitis. The increased deposition of triglycerides and their decreased secretion from the liver was attributed as the cause for several drugs and chemicals induced hyperlipidemia and hepatic steatosis(21). Currently, statins are preferred to reduce the cholesterol levels (22). However,the statins have been known to be associated withundesirable side effects including severe myopathy andmemory loss (23).Thus, there iscontinued quest to develop new molecules which show prominent action of lipid levels with noside effects (24). The potential herbs with hypolipidemic action havecommon target of lowering the serum lipid levels, although physiologically, herbs like *Ocimum basilicum* produces changes in lipid oxidation, *Vaccinium angustifolium* induce inhibited lipidaccumulation, *Taraxacum officinale* inhibit adipocyte differentiation and lipogenesis, and *Nigella sativa* decreases hepatic β-Hydroxyβ-methylglutaryl-

CoA (HMG-CoA) reductaseactivity(25). Similarly, an ethanol extract of Piper longum significantly lowered the serum total cholesterol and serum triglyceride in experimental animals.(26)

Conclusion

In this review, an attempt has been made to know more about the hepatoprotective effect of *Piper longum*, a medicinal herb used in Indian system of medicine. Research on its active principle, piperine has gained a special attention in recent times for its antioxidant, anti hyperlipidaemic and as a bioenhancer. In view of this, progress can be made to elucidate the cellular/molecular mechanisms involved and further steps may be taken for clinical validation ontowards phytopharmaceutical development.

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Nutritional and therapeutic benefits of *Hibiscus sabdariffa*

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Introduction

Throughout history, humans have utilized various elements of the environment for sustenance and medicinal purposes. Particularly plants have been a great source for traditional medicine, treating a wide range of illnesses. Initially, individuals experimented with these plants through trial and error. Subsequently, knowledge about the medicinal properties of these plants was transmitted across generations. *Hibiscus sabdariffa* is one of the recognized medicinal plants employed in Ayurveda, Siddha, and Unani medicinal traditions.[(2)]

Hibiscus sabdariffa, commonly known as roselle or red sorrel represents the family Malvaceae. The plant is called as Roselle in English, Gongura in Hindi, Pulichchaikerai in Tamil, Jamaica in Mexico and Spain.[(12)]Roselle is an annual or perennial, simple or branched herb that can grow up to a height of 2.4 meters with Leaves transition from green to red, and the plant boasts large, short-peduncled flowers that range from red to yellow, featuring a distinctive dark center.[(1)] The whole plant represents commercial and medicinal benefits. Thefresh or dried calyces, seeds, and leaves of Hibiscus Sabdariffa are consumed either in their raw state or incorporated into herbal medicines, beverages, and fermented drinks.

Therapeutic uses

Scientific studies have unveiled the notable efficacy of *Hibiscus sabdariffa,* commonly referred to as sour tea, in lowering blood pressure, particularly among individuals diagnosed with stage 1 hypertension. These findings are corroborated by comparable investigations that emphasize the benefits of incorporating sour tea into patients' diets, coupled with lifestyle modifications, as a promising approach for effectively managing high blood pressure.[(6)] *Hibiscus sabdariffa* (Hs) extends its therapeutic potential beyond

cardiovascular health, demonstrating hypolipidemic and antidiabetic effects. In a study involving individuals with hypercholesterolemia, the administration of Roselle extract, specifically two capsules (1g each) three times a day, totaling 3g/day, resulted in a significant reduction in serum cholesterol levels. This effect was further substantiated by another scientific investigation, highlighting the hypolipidemic properties of the ethanolic extract derived from Roselle leaves. Additionally, a study involving participants with metabolic syndrome revealed that those who received the ethanolic extract of Roselle experienced substantial reductions in glucose, total cholesterol, and low-density lipoprotein, accompanied by an elevation in high-density lipoprotein.[3,4,7] Furthermore, in a study utilizing a type 2 diabetic model characterized by high glucose and hyperinsulinemia, the administration of Hibiscus sabdariffa polyphenol extract (HPE) demonstrated a noteworthy impact in lowering blood glucose levels and ameliorating insulin resistance.[10] These collective findings underscore the multifaceted therapeutic potential of Hibiscus sabdariffa in cardiovascular health and metabolic disorders.

Nutritional uses

The *Hibiscus sabdariffa* calyx, seed, and leaf components exhibit varying Nutrient compositions among studies, likely because of genetic variations, environmental factors, plant ecology, and harvesting conditions. The roselle Calyces contain 68.7g of Carbohydrate, with an excellent source of fiber of 14.6g, protein 4.7g and 12.2% of ash respectively[8] As the calyx is high in citric acid and pectin, it can be used to make a variety of foods, including soups, jellies, puddings, pickles and jams. The juice adds a vibrant color so it is used in beverages and confections. The leaves and tender stems of Roselle are suitable for raw consumption in salads or can be cooked as greens either independently or in conjunction with other vegetables or meat. Additionally, they are added in curries as a seasoning agent due to their high content of organic acids.

Hs exhibits elevated concentrations of niacin, ascorbic acid, and pyridoxine, alongside substantial quantities of thiamin, riboflavin, pantothenic acid, and folic acid.[8] Furthermore, they are abundant in bioactive compounds, including anthocyanins, flavonoids, organic acids, polysaccharides, triterpenoids, steroids, and alkaloids. These constituents contribute to the beverage's antioxidant, antibacterial, anti-inflammatory, hepatoprotective, and anti-cholesterol and antimicrobial effects.[12]

Given its active nutritional and functional attributes, *Hibiscus sabdariffa* (Hs) has the potential to contribute to the prevention of chronic and degenerative diseases, as well as to address challenges related to micronutrient deficiency.

Nutritional Composition of *Hibiscus Sabdariffa*

Constituents	Calyces (per1000ml)	Leaves	Seeds
Moisture	9.2	86.2%	9.9%
Protein	1.14	1.7-3.2%	33.5g
Fat	2.16	1.1%	22.1 g
Fiber	12	10%	18.3 g
Calcium	12.63 mg	0.18%	-
Phosphorus	273.2 mg	0.04%	-
Iron	8.98 mg	0.0054%	-
Ascorbic Acid	6.7 mg	-	-
Niacin	3.765 mg	-	-
Riboflavin	0.277 mg	-	-
Thiamine	0.117 mg	-	-

Source: Nmahadevan and Pradeep, 2009 & Hainida E, Amin I, Normah H, 2008

Conclusion

Hibiscus sabdariffa contains substantial quantities of nutritionally significant components, comprising moisture, crude fiber, lipid, protein, and essential mineral elements, which are pivotal for the body's growth, disease prevention and developmental processes. Roselle exhibits activities such as anti-hypertension, anti-cancer, antibacterial, antifungal, antipyretic, anti-parasitic, antioxidant, anti-cholesterol, and antimicrobial effects which

In conclusion, *Hibiscus sabdariffa* emerges as a globally recognized plant possessing various nutritional and therapeutic benefits. Its rich composition, encompassing essential nutrients such as vitamins, minerals, and bioactive compounds, positions it as a valuable dietary addition. The plant's antioxidant, anti-inflammatory, and hypocholesterolemic properties contribute to its potential role in promoting overall health and mitigating various health conditions. *Hibiscus sabdariffa* not only serves as a source of essential nutrients but also exhibits promise in the realm of traditional and modern medicine. From its application in herbal teas for promoting cardiovascular health to its potential in managing metabolic disorders, the plant's therapeutic versatility continues to attract scientific interest.

The exploration of its potential benefits serves as an invitation for continued research, fostering a deeper understanding of the mechanisms underlying its effects and opening avenues for its integration into holistic approaches to health and wellness. In the ever-evolving landscape of nutritional science and medicine, *Hibiscus sabdariffa* stands as a promising ally, offering a blend of ancient wisdom and contemporary insights for the benefit of human health.

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Novel Drug Discovery and Development from Medicinal Plants – Steps from Concept to Commercialization

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Abstract

In many traditional medical systems around the world, medicinal plants have been utilized for ages to heal human illnesses. The chemical components of these plants' therapeutic effects and the pharmaceutical combinations made from them are known as phytochemicals. In order to find innovative and biologically significant compounds or medications, a great deal of study has been focused on the identification of phytochemicals of medicinal plants. Synthesis, characterisation, validation, optimization, screening, isolation, and assessment of the medicinal efficacy of identified plant compounds are all steps in the process of developing novel pharmaceuticals. This chapter provides an overview of the key processes in the drug discovery process. Furthermore covered is the progression from research laboratories to preclinical trials and ultimately into clinical trials upon identification of a novel therapeutic target, promising chemical, bioactive molecule, or secondary metabolite. From initial conception to commercialization, the complete pharmaceutical development process can take up to ten years and exceed seventeen billion dollars. The budgets for clinical trials, research, and development are the most expensive steps in the drug discovery process.

Keywords: Phytochemicals, Computational methods, Drug Discovery & Development, Clinical Trials

1.0 Introduction

Compared to other scientific disciplines, drug development has made the greatest advancements in medicine during the last century. Although chemistry is still the main force behind it, pharmacology and the clinical sciences are becoming more and more influential. The advancement of genomic sciences and molecular biology in particular is having a significant impact on drug development. Finding a drug molecule with therapeutic potential for use in the treatment and management of diseases at different stages is the first step in the drug development process [1]. The method is costly, labor-intensive, and intricate. The process is divided into two main phases: the first is a comprehensive assessment, and the second is the optimization of the selected chemical component [2, 3]. Drug development and discovery are conducted using two different approaches: target-based drug discovery and structure - based drug discovery. In the process of developing structure-based drugs, the biological profile is further refined and optimized after it has been established. In contrast, target-based drug discovery finds the drug target [4, 5]. The advancement of genomic sciences and molecular biology in particular is having a significant impact on drug discovery. Therapeutics is now much more widely available to the utilization of recombinant proteins and monoclonal antibodies. Utilizing genome sciences and bioinformatics techniques to examine the genetic basis of complicated illnesses and choose the optimal targets for upcoming drugs expands the pool of available therapy options. Because of the dramatic increase in complexity in drug development, the administrative underpinnings of this multidisciplinary activity are evolving. The research and development arm of the pharmaceutical industry is increasingly referred to as the "biotech" sector. In order to bridge the knowledge gap between large pharmaceutical businesses and academic institutions, biotech enterprises have proven to be effective accelerators for technology transfer [1].

2.0 Various phases of the drug development process

1. Identification of the drug target
2. Validation of the target
3. Lead compound identification and optimization
4. Product development and characterization

5. Formulation of drug
6. Preclinical research projects
7. The novel drug application under evaluation
8. Human Trials (Clinical Research and Reviews)

The overall steps involved in drug discovery process are explained in Fig 1.

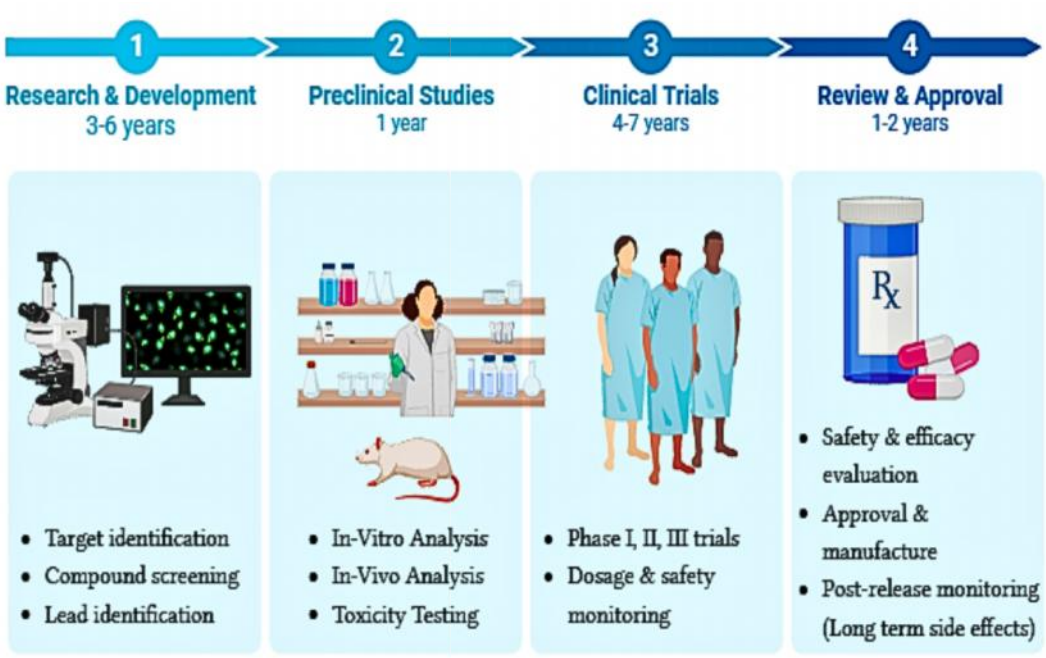

Fig 1: Steps involved in drug discovery process.

2.1 Target Identification

The first step in developing a new medicine is identifying the target. One way to describe it is as a disease phenotype and a possible etiology of a certain ailment. A naturally occurring cellular or molecular structure that is involved in the pathogenesis of the illness may be this target [4].

2.2 Target Validation

Target validation can be carried out by doing experiments to confirm the repeatability via a variety of techniques, including expression cloning, microarray DNA, affinity chromatography, biochemical suppression, and study of presently marketed drugs. Chemical genomics must also be used to attack

the protein-encoding genome by chemical means in order to induce variation into the Ligand target environment.

2.3 Lead compound identification and optimization

One phase in the lead identification procedure that is essential for locating the lead molecule that has drug-like properties. On the other hand, lead optimization entails maximizing the lead molecule's compatibility with the target receptor in order to enable drug development [6]. The following table lists many of the computational techniques that are used to investigate the different stages of the drug development process:

S.No.	In-Silico/ Computational Analysis in Drug Discovery	Software/ Tools Used
1.	Target Identification & Validation	**Genomics-** ⁾ Microarray Equipment. Microarray Scanners. ⁾ PCR Thermocyclers. **Proteomics-** ⁾ Blast - NCBI Basic Local Alignment Search Tool. ⁾ ChemCalc - molecular mass calculator. ⁾ Comet - an open source tandem mass spectrometry (MS/MS) sequence database search tool. ⁾ Delta Mass - database of protein post-translational modifications. ⁾ Entrez - NCBI database search and retrieval system.
2.	Protein data retrieval	**NCBI resources** include ⁾ Entrez ⁾ PubMed & PubMed Central ⁾ NCBI Taxonomy Browser
3.	Chemical Structure retrieval	⁾ **ChemSpide**r is a free chemical structure database providing fast text and structure search access to over 100 million structures from hundreds of data sources.

		⁾ **Chem draw/ Chem Sketch -** Free software for structure drawing. ⁾ **MolView**, as an advanced chemical drawing tool, brings together functionality for creating both 2D and 3D molecular structures in a user-friendly format. ⁾ **PubChem** is a database of chemical molecules and their activities against biological assays.
4.	Medicinal Phytocompounds retrieval	⁾ **Indian Medicinal Plants, Phytochemistry And Therapeutics 2.0 (IMPPAT 2.0)** It is a manually built database which has been constructed via digitalization of information from more than 100 books on traditional Indian medicine, 7000+ published research articles and other existing resources.
5.	Target and Ligand / Drug Interaction	⁾ **Docking** can be used to predict the binding modes of small molecules to the protein and generate a homology model of the protein based on the binding mode prediction. ⁾ **PyRx** is a Virtual Screening software for Computational Drug Discovery that can be used to screen libraries of compounds against potential drug targets.
6.	Visualization of Target - Drug Interaction	⁾ **UCSF Chimera** is an extensible program for interactive visualization and analysis of molecular structures and related data, including density maps, supramolecular assemblies, sequence alignments, docking results and conformational ensembles. ⁾ **The BIOVIA Discovery Studio Visualizer** is a free, feature-rich molecular modeling application for viewing, sharing and analyzing protein and small molecule data.
7.	ADMET & Drug likeliness Prediction	⁾ **SwissADME** a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules.

		⁾ **pkCSM**, a novel method for predicting and optimizing small-molecule pharmacokinetic and toxicity properties which relies on distance-based graph signatures.

2.4 Ethnopharmacology

Ethnopharmacology is defined as "the interdisciplinary scientific exploration of the biologically active agents that are traditionally employed". It covers botany, chemistry, and pharmacology. This includes field observations, reports on the use and bioactivities of traditional remedies, phytochemical and pharmacological research, and botanical identification of the plant material. Many researchers have been curious about the possible outcomes of looking into local remedies for a very long time.

In light of ethnopharmacological research, the development of natural drugs has been crucial to the advancement of modern treatment approaches. For centuries, the natural compounds found in creatures of all kinds and minerals have served as the foundation for the medicinal treatment of various ailments. Pharmacognostical, phytochemicals, and pharmacological investigations of traditional medicinal plants have received a lot of interest lately. Furthermore, several preclinical and clinical investigations have examined the biological activity potential of natural medicines, demonstrating a variety of biological effects of a broad spectrum of chemicals derived from plants in different classes of chemical groups. As a result, a lot of pharmaceutical companies have recently updated their approaches to the study of natural products in an effort to find new compounds and possible sources for medication development. Because of its method that is potentially backed by an experimental foundation, ethnopharmacological knowledge can be helpful for the discovery and development of new, safe, and reasonably priced medications [7].

2.5 Pre-clinical research

Pre-clinical research is a step in the drug development process that involves evaluating a drug's safety and efficacy in animal models that may have implications for human health. The respective regulatory bodies must also approve the preclinical trials. Regulatory agencies, who also have a duty to supervise the ethical and safe conduct of trials, will only approve pharmaceuticals that have been demonstrated to be both safe and efficacious. A foundational set of guidelines for the technical requirements of appropriate preclinical drug development has been established by ICH [8].

There are two methods for carrying out the pre-clinical trials. They are the fields of

1. Toxicology
2. General pharmacy.

2.6 Pharmacokinetics

It tries to investigate chemical metabolism and find out what happens to the drug from the moment it is administered until it is completely eliminated from the body.

2.7 Pharmacodynamics

The study of a drug's physiological and biochemical effects particularly pharmaceutical medicines, the consequences may appear in microbes, animals (including humans), or mixtures of organisms.

Pharmacodynamics specifically examines how a medicine affects an organism, whereas pharmacokinetics explores how the drug affects the organism itself. Pharmacology is the study of the pharmacokinetic and pharmacodynamic properties of pharmaceuticals. They influence side effects, benefits, and dosage collectively. It is important to monitor any undesirable pharmacological effects in toxicological research and to investigate them in suitable animal models.

Pharmacokinetic investigations are essential to ascertain the safety and efficacy parameters of distribution, metabolism, excretion, and absorption. The rate of absorption for different routes of administration is provided by these studies, which helps with the selection of dosage form, distribution, rate of metabolism, and rate of elimination—all of which affect the drug's half-life. The medication's half-life provides information about its safety profile, which is necessary for regulatory bodies to approve a medication. The drug's distribution method is determined by its bioavailability and affinity, which elucidates its therapeutic efficacy. Drug metabolism provides information on the probability of moving through various phases of the biotransformation process and generating drug metabolites. Learning about the enzymes and procedures involved in biotransformation is also aided by it [9].

Toxicological study on the medicine can be done using both *in vitro* and *in vivo* studies that evaluate its effects. Through *in vitro* studies, the direct effects on cell proliferation and phenotype can be investigated. Through in-

vivo study, toxicological effects can be ascertained both qualitatively and statistically. Since many drugs are species specific, selecting the appropriate animal species for a toxicity research is essential. To evaluate a substance's pharmacological and toxicological characteristics, including its mechanism of action, and to further support their recommended use, clinical studies typically rely on in-vivo examinations [10].

Drug developers are required to file an Investigational New Drug application to the FDA prior to initiating clinical trials. Before beginning any clinical studies, drug developers must file an application for an investigational new drug (IND) to the FDA [11]. Developers are required to provide the following information with their IND application:

- Clinical research procedures for the studies that will be carried out in Clinical Trials.
- Data from preclinical and toxicology studies
- Prior clinical research data (if any available).
- Details about the researcher or developer[12]

In Fig. 2, the experimental work plan for preclinical research is described in detail. This work protocol supports effective preclinical research projects, drug formulation, product development and characterization, and novel drug application evaluation.

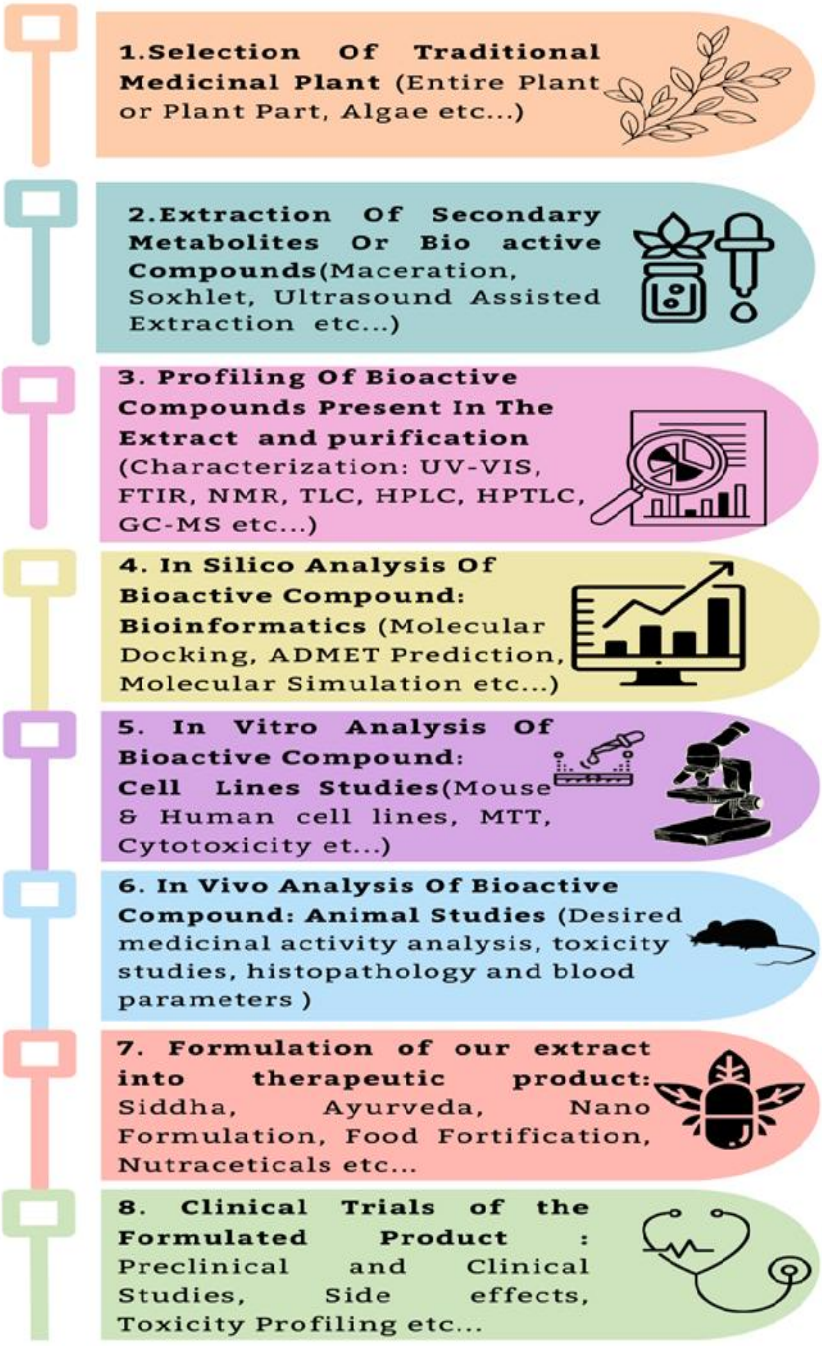

Fig 2: Experimental work flow for pre clinical research

2.8 Clinical trials

Figure 3 provides a clear explanation of the complete work process that clinical trials undertake. The various stages of clinical trials are as follows:

i. Phase 0 Trials:

Phase 0 trials (sometimes called human micro-dosing studies) are designed to accelerate the development of drugs with significant potential. Phase 0 investigations involve administering a single sub-therapeutic dose of the study medication to a limited number of participants (10 to 15) in order to gather preliminary data on the pharmacokinetics of the agent (the way the body responds to medications). A Phase 0 study doesn't offer safety or efficacy information. Clinical trials typically bypassed this stage.

ii. Phase 1:

Phase I trials, formerly referred to as "first-in-man studies," Usually, a small sample of two to one hundred healthy individuals will be recruited. Clinical trial facilities are often used for these research, enabling full-time professionals to keep an eye on the subject.

iii. Phase 2:

The next goal is to determine whether the medicine has any biological effect or activity. Phase II studies are carried out on bigger cohorts, which are groups of individuals sharing common features and typically consist of 100–300 participants, in order to assess the medication's efficacy. Furthermore, to conduct Phase I safety assessments including a larger sample of volunteers and patients.

iv. Phase 3:

Phase III studies are randomized controlled multi-center trials with a maximum of 300–3,000 subjects per phase. They stand for the ultimate assessment of the medication's effectiveness in respect to the "gold standard treatment" as it is currently defined. Phase III studies are the most expensive, time-consuming, and challenging to organize and carry out, especially when it comes to long-term sickness illnesses.

Marketing and approval of drugs: The developed formulation is submitted to regulatory bodies like the CDSCO in India and the USFDA in the US for approval for marketing. Phase IV clinical trials are initiated for the authorized medication alone.

v. Phase 4 and Post-Marketing Surveillance:

A Phase IV trial is often known as a post-marketing monitoring trial or a confirmatory trial informally. Phase IV trials include pharmacovigilance, sometimes known as safety surveillance. Its objective is to identify uncommon or chronic side effects over an extended duration and a larger patient group [13, 14, and 15].

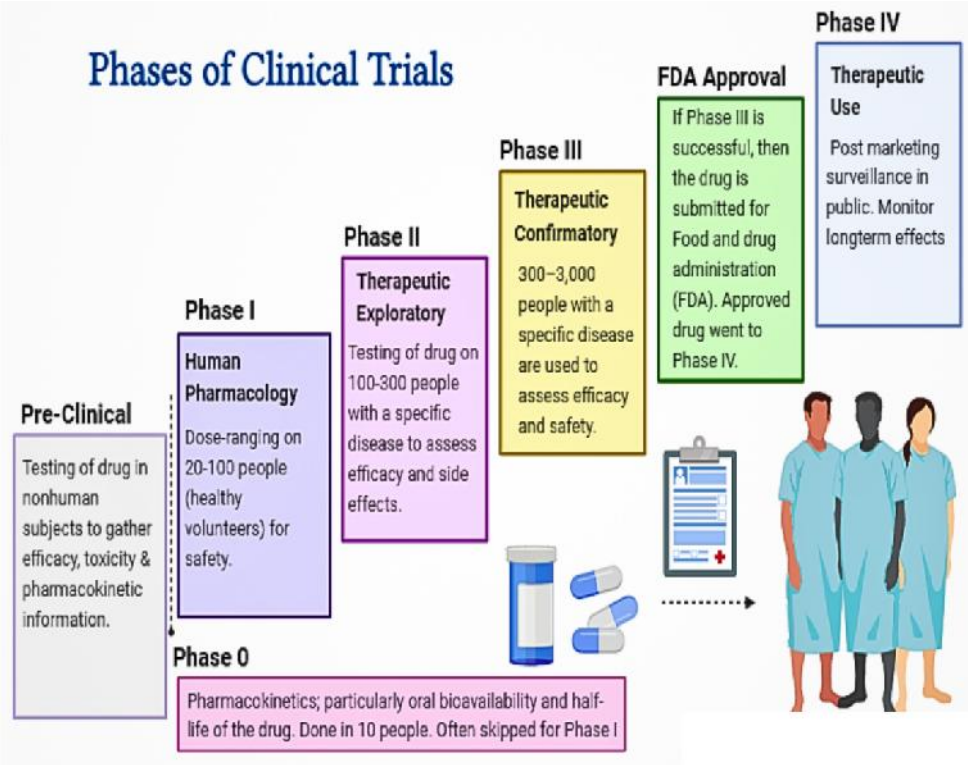

Fig 3: Phases of clinical trials.

3.0. Conclusion and Future Direction

There has been a lot of debate on the best strategy to boost the industry's success rate by "failing fast and cheap". Since the study is now public knowledge and stopping it could harm the company's reputation and shareholder value, it can become more difficult to terminate a project after a candidate approaches the clinical stage. Before going into clinical development, further research needs to be done to build predictive translational models based on a thorough understanding of the disease, identify biomarkers, and enhance toxicity screenings (which use failed drugs as a basis for these assays). In particular in these latter two domains, cooperation between

academia and industry have the potential to greatly improve preclinical value and ultimately enable the delivery of more effective pharmaceuticals to patients [16].

The process of researching and developing a new drug is extremely time-consuming, costly, challenging, and inefficient; it frequently takes ten to fifteen years. Failure is a major financial setback that occurs often. Over the past 20 years, despite advances in technology and a deeper comprehension of biological systems, the pharmaceutical sector has seen a deterioration of research and development productivity due to increased prices. increased regulatory obstacles and the increased challenge of discovering the next big blockbuster medication (either in a new disease area or a far superior therapy to what is currently on the market) have also contributed to a constant decline in the number of newly approved pharmaceuticals.

The process of developing new drugs may be significantly impacted by the creation of increasingly complex machine learning (ML) algorithms, among other recent developments in artificial intelligence (AI). AI technology can assist in addressing some of the main problems associated with drug development, including reducing costs, time, and human resource requirements in the early stages of the process, by utilizing in silico methodologies for de novo drug design, synthesis prediction, and bioactivity prediction [17]. The best physicists and scientists, cutting edge laboratories and equipment, a plenty of resources, and meticulous project management are all essential for success. Persistence and good fortune are also necessary. [18] The process of medication research ultimately leads to the healing, hope, and faith of millions of patients [19 and 20].

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Mesenchymal Stem Cells: Unveiling the Regenerative Promise

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Abstract

Mesenchymal stem cells (MSCs) are versatile adult stem cells that can transform into diverse cell types. MSCs have become a beacon of hope in the field of regenerative medicine and hold great promise for treating many diseases and restoring tissue function. Their amazing flexibility, immunomodulatory properties, and ability to secrete large amounts of bioactive factors make them ideal candidates for cell therapy. However, despite significant progress in MSC research, many challenges remain in translating preliminary findings into effective clinical applications. This chapter briefly describes basic features, types, regulation and therapeutic applications of MSCs.

Keywords: Mesenchymal stem cells, multipotency, cell differentiation, stem cell therapy, regenerative medicine

Introduction

Stem cells are the body's building blocks, master cells from which all other cells with specialized functions are generated. They have the ability to differentiate into multi lineage cells and self- renewal [1]. One of the most renowned and interesting types from the lot is the Mesenchymal stem cell commonly abbreviated as (MSCs). MSCs are versatile adult stem cells present in various locations, such as the bone marrow, adipose tissue, umbilical cord blood, and other bodily tissues. Having a remarkable capacity of self-renewal, they can transform into a wide range of cell types, including osteoblasts (cells responsible for bone formation), chondrocytes (cells involved in cartilage formation), adipocytes (fat cells), myocytes (muscle cells), and neurons (nerve cells) [2].

The journey of MSCs commences in the early stages of embryonic development, emerging from the mesenchyme, a tissue layer responsible for generating a variety of connective tissues. As the embryo progresses, MSCs travel to various tissues and organs, where they remain in a dormant state until specific signals activate them. [3-4]. Activation of MSCs is prompted by a range of elements, encompassing inflammation, tissue injury, and growth factors. Once activated, MSCs have the capacity to multiply and transform into various cell categories, influenced by the specific conditions and signaling cues within their immediate surroundings [5-6]. The process of guiding MSCs to differentiate into distinct cell types is intricate, involving a series of molecular and cellular processes [6]. Transcription factors, signaling molecules, and interactions among neighboring cells all hold pivotal significance in directing MSCs toward becoming osteoblasts, chondrocytes, adipocytes, or other types of mesenchymal cells (Figure 1) [1,2,7].

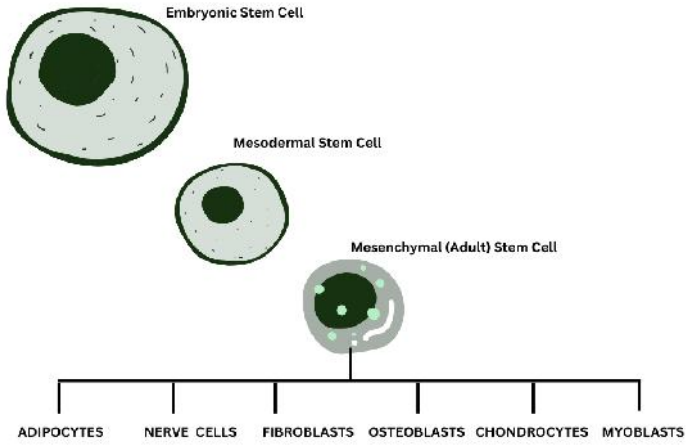

Figure 1: Origin and potency of Mesenchymal stem cells

MSCs are known for some special characteristics and abilities that they possess, mainly:

1. Self-renewal: MSCs possess the remarkable capability to undergo cell division, creating exact replicas of themselves. This characteristic enables them to proliferate in laboratory cultures, ensuring a stable and abundant supply for various research and therapeutic purposes [7, 8].

2. Multipotency: MSCs exhibit multipotency, meaning they can transform into a diverse array of mesenchymal cell types. This versatility provides MSCs with the remarkable potential to participate in tissue repair and regeneration, as they can differentiate into cell lineages such as osteoblasts (bone-forming cells),

chondrocytes (cartilage-forming cells), adipocytes (fat cells), and more [7]. This capacity allows addressing a wide range of tissue injuries and disorders.

3. Immunomodulatory properties: MSCs possess immunomodulatory properties that grant them the ability to regulate the activity of immune cells. They can effectively dampen the responses of immune cells, leading to a reduction in inflammation. This immunosuppressive feature helps to alleviate inflammatory conditions and promotes the healing of damaged tissues [9].

4. Homing ability: MSCs exhibit a unique homing ability, enabling them to migrate at sites within the body where tissue damage or inflammation has occurred. This migratory capacity makes MSCs well-suited for targeted therapeutic interventions [5, 9]. By naturally homing to areas in need of repair, they can be harnessed to deliver therapeutic benefits precisely where they are required, enhancing their potential in regenerative medicine and other clinical applications.

5. A Treasure Trove of Therapeutic Potential:

a) Bone Repair and Regeneration: MSCs have demonstrated their potential in facilitating the repair and regeneration of bone tissue. They achieve this by differentiating into osteoblasts, the cells responsible for bone formation, and by producing the bone matrix. This ability makes them valuable in the treatment of a wide range of bone disorders, including fractures, osteoporosis, and conditions like osteogenesis imperfecta [10].

b) Cartilage Repair and Regeneration: MSCs exhibit the capacity to transform into chondrocytes, the cells responsible for cartilage formation. Consequently, they have promise in the treatment of cartilage damage associated with conditions such as osteoarthritis and rheumatoid arthritis, where the production of a healthy cartilage matrix is crucial for recovery[11].

c) Heart Muscle Repair and Regeneration: MSCs have shown their potential in improving heart function following a myocardial infarction (heart attack). They achieve this by promoting angiogenesis, the formation of new blood vessels, which helps restore blood supply to damaged heart tissue[10, 11].

d) Treatment of Autoimmune Diseases: MSCs possess immunomodulatory properties, enabling them to suppress the activity of immune cells. This characteristic makes them a potential treatment option for autoimmune diseases like lupus, rheumatoid arthritis, and Crohn's disease, where an overactive immune response is a key factor in the disease process [12].

e) Treatment of Neurological Disorders: MSCs have demonstrated the ability to promote neurogenesis, the formation of new neurons, and to reduce inflammation within the central nervous system. These features make them subjects of investigation for the treatment of various neurological disorders, including stroke, Alzheimer's disease, and Parkinson's disease, where neural cell regeneration and inflammation control are essential therapeutic goals [11,13].

f) Graft-versus-Host Disease (GVHD) Therapy: MSCs have been found to mitigate the risk of GVHD, a potentially severe complication that can occur following bone marrow transplantation. Their use is being explored as a potential therapy to reduce the impact of GVHD and other graft-versus-host diseases, enhancing the safety and effectiveness of transplant procedures[12].

g) Cancer Therapy: MSCs possess anti-cancer properties, including their ability to deliver drugs to cancer cells and promote the death of tumor cells (apoptosis). Consequently, they are under investigation for their potential role in treating various cancers, such as leukemia, lymphoma, and solid tumors. Their unique properties in the context of cancer therapy offer new avenues for more targeted and effective treatments [14, 15].

The different type of MSCs and their sources are as follows:

1. Bone Marrow MSCs (BM-MSCs):

BM-MSCs are the most extensively researched and well-defined MSC type. They are typically isolated from the iliac crest, which is the back part of the ilium, a flat bone located in the pelvis. BM-MSCs have been extensively explored in clinical trials for their potential to address a range of medical conditions, such as bone fractures, cartilage injuries, heart muscle damage, and autoimmune diseases [1, 7].

2. Adipose Tissue-Derived MSCs (AD-MSCs):

AD-MSCs are derived from adipose tissue, commonly known as fat tissue, which is abundant in the body. They are less invasive to collect compared to BM-MSCs and have shown similar therapeutic potential in various clinical trials [7].

3. Umbilical Cord Blood-Derived MSCs (UCB-MSCs):

UCB-MSCs are sourced from umbilical cord blood, a rich reservoir of stem cells. They are considered a valuable MSC source due to their ease of isolation and distinctive attributes, including faster proliferation rates and immunomodulatory properties [9, 16].

4. Placenta-Derived MSCs (PL-MSCs):

PL-MSCs are obtained from the placenta, an organ that sustains the developing fetus during pregnancy. These are regarded as a promising source of MSCs due to their abundance, ease of extraction, and capacity to differentiate into multiple cell types [16].

5. Dental Pulp-Derived MSCs (DP-MSCs):

DP-MSCs are acquired from the dental pulp, the soft tissue within a tooth. They are viewed as a source of MSCs due to their accessibility and their capability to differentiate into various mesenchymal cell types, such as bone, cartilage, and fat tissue [7].

6. Menstrual Blood-Derived MSCs (Mn-MSCs):

Mn-MSCs are collected from menstrual blood, a non-invasive source of stem cells. These are considered a promising MSC source due to their abundance, easy collection, and ability to differentiate into various mesenchymal cell types [7, 16].

Each type of MSC possesses distinct properties and advantages, rendering them suitable for diverse therapeutic applications (Figure 2). The selection of an MSC source depends on various factors, including the specific clinical context, the desired therapeutic outcomes, and the individual requirements of the patient.

The Embryonic Origin of Mesenchymal Stem Cells (MSCs)

The origin story of mesenchymal stem cells (MSCs) commences during the early stages of embryonic development. As the embryo takes shape, it differentiates into three fundamental germ layers: the ectoderm, mesoderm, and endoderm [4]. Positioned between the ectoderm and endoderm, the mesoderm is responsible for giving rise to a diverse spectrum of tissues, encompassing bone, cartilage, muscle, and various connective tissues. The precursors of MSCs reside within these developing mesodermal tissues, referred to as mesenchymal progenitor cells (MPCs) [3, 4].

Mesodermal Progenitor Cells (MPCs): The Pioneers of the MSC Lineage

MPCs are characterized by their inherent capacity for self-renewal and their remarkable potential to transform into a variety of mesenchymal cell types. These early progenitor cells assume a pivotal role in the morphogenesis and patterning of tissues, actively contributing to the development and organization of various organs and anatomical structures[17].

Migration and Niche Establishment: MSCs Finding Their Residences

As the embryo matures, MPCs embark on an extensive journey, dispersing throughout the evolving organism [4]. They eventually take up residence in specialized niches within different tissues, positioning themselves in proximity to blood vessels. These niches offer a distinct microenvironment that sustains the quiescent, self-renewing, and differentiating capabilities of MSCs [18].The constitution of this niche microenvironment is of paramount importance in upholding the stem cell characteristics of MSCs. It involves a complex interplay of various niche factors, including growth factors, cytokines, and interactions between neighboring cells [6, 19]. These elements work in concert to govern the behavior of MSCs and prevent their premature differentiation.

Factors that regulate the development and differentiation of MSCs

The orchestration of mesenchymal stem cell (MSC) development and differentiation involves a rich tapestry of factors, both intrinsic and extrinsic, operating in harmony to determine their destiny. These multifaceted factors, working collectively, guide MSCs through a multifaceted journey, transforming them from quiescent progenitor cells into mature mesenchymal cell types, thereby contributing to the processes of tissue repair, regeneration, and maintenance of homeostasis [5, 16].

1. Intrinsic Factors: The Genetic Compositions

The genetic underpinnings of MSCs play a foundational role in shaping their developmental and differentiation potential. Within this genetic framework, specific genes, known as transcription factors, emerge as the conductors of this symphony, overseeing the expression of other genes involved in the intricate dance of cell fate determination [20, 21].

- Runx2: This transcription factor assumes a pivotal role in orchestrating osteogenic differentiation, steering MSCs towards their destiny as bone-forming cells. It acts as a maestro in the ballet of bone formation [5, 6].
- Sox9: As another virtuoso among transcription factors, Sox9 takes the lead in the intricate performance of chondrogenesis, guiding MSCs in their transformation into cartilage-forming cells, contributing to the harmony of joint and tissue development [6].
- PPARγ: Playing a leading role in the theater of adipogenesis, PPARγ directs MSCs towards their roles as fat cell performers. This

transcription factor choreographs the storied transformation of MSCs into adipocytes [20].

- MyoD: In the realm of myogenesis, MyoD acts as the conductor, directing MSCs into the transformation that leads them to become muscle cells. This transcription factor harmonizes the development of muscle tissue with precision [6, 20].

2. Extrinsic Factors: The Guiding Signals

Surrounding the MSCs is a dynamic environment filled with a multitude of signals that influence their actions. These extrinsic factors, including growth factors, cytokines, and components of the extracellular matrix (ECM), provide cues that direct MSCs to grow, differentiate, or remain in a state of dormancy [5, 21].

- **Bone morphogenetic proteins (BMPs):** BMPs emerge as powerful composers, inducing the crescendo of osteogenic differentiation, thereby inspiring MSCs to embrace their roles as bone-forming cells. They craft the harmony of bone development with striking resonance [21].
- **Transforming growth factor-β (TGF-β):** As a versatile conductor, TGF-β can either raise or lower the tempo of MSC differentiation, depending on the context. It can evoke the crescendo of chondrogenesis while also extinguishing the flames of adipogenesis, illustrating its adaptability in concert of cellular events [5, 21].
- **Fibroblast growth factors (FGFs):** FGFs function as virtuosos, not only promoting the proliferation of MSCs but also directing their paths towards various mesenchymal cell lineages. They play a multifaceted role in the symphony of cell fate [9, 21].
- **Extracellular matrix (ECM):** The composition and rigidity of the ECM encompassing MSCs contribute to the texture and timbre of their differentiation. A stiffer ECM sets the stage for osteogenesis, while a softer backdrop encourages the performance of adipogenesis. The ECM acts as a skilled set designer, shaping the scenery for cellular transformation with precision [21].

Immunomodulatory Properties of Mesenchymal Stem cells

Mesenchymal stem cells exhibit immunomodulatory properties, this allows them to surpass immune rejection, increase anti-inflammatory activity and environment.

Avoiding Rejection

When put into the body, MSCs are able to avoid rejection. The key to their success is in their properties. These properties give them the ability to hide from the immune system, calm its activity and create an anti-inflammation environment [9].

Hiding from Detection

The first step to avoiding immune detection is by limiting the levels of major histocompatibility complex (MHC) class II molecules. These are what T cells use to find foreign entities. On top of this, they're also able to produce a molecule called the human leukocyte antigen-G5 (HLA-G5). It binds with immune cells, stopping them from being activated [16].

Calming Immune Activity

Immune cells such as T cells, B cells and natural killer (NK) cells can all be over active. MSCs have the ability to suppress them. They do this with a number of mechanisms including:

1. Producing immunomodulatory molecules: By releasing immunomodulatory molecules like interleukin-10 (IL-10), transforming growth factor-beta (TGF-β) and prostaglandin E2 (PGE2), it hinders both growth and activation of immune cells [9, 22].
2. Interaction with immune cells: MSCs interact with various cells and particularly immune cells via cell to cell interactions and secretion of important transcription and soluble factors, these interactions can cause anergy of immune cells and reduce their ability to attack the transplanted cells[9].

The challenges and future directions of MSC-based therapies

While mesenchymal stem cell (MSC) treatments hold great promise, several hurdles must be overcome before they can be widely used in clinical settings. These obstacles include:

Sourcing and Characterization:

- Obtaining enough high-quality MSCs for clinical use is challenging.
- MSCs vary in their properties and therapeutic potential, making it difficult to standardize their isolation, expansion, and characterization [17, 23].

Delivery and Engraftment:

- Effective delivery methods are needed to ensure MSCs reach the target site and integrate into the desired tissues.
- The long-term survival and persistence of transplanted MSCs in the body are not always consistent [17, 23].

Immunogenicity and Safety:

- While MSCs are generally considered immune-privileged, there is a risk of immunogenicity in some patients, leading to rejection or adverse effects.
- The potential for MSCs to promote tumor growth or other adverse consequences requires careful evaluation and long-term monitoring [5].

Mechanisms of Action:

- The precise mechanisms of MSC-mediated therapeutic effects are not fully understood, limiting the ability to optimize treatment strategies[24].
- The potential for MSCs to exert paracrine effects through the release of soluble factors or other mechanisms needs further investigation.

Clinical Trial Design and Standardization:

- There is a need for more standardized clinical trial designs and outcome measures to evaluate the efficacy and safety of MSC-based therapies [22, 24].
- The optimal dosage, administration route, and timing of MSC administration require further optimization [23, 24].

Future Directions of MSC-Based Therapies

Despite these challenges, MSC-based therapies hold immense promise for treating a wide range of diseases.Future research directions in this field include:

Improving MSC Sourcing and Characterization:

- Develop new methods for isolating and expanding MSCs with consistent and desirable therapeutic properties [23].

- Identify and utilize markers to distinguish between MSC subpopulations with different therapeutic potentials.

Enhancing MSC Delivery and Engraftment:

- Develop biocompatible and targeted delivery vehicles to improve MSC homing and engraftment at the desired site.
- Utilize genetic engineering or pharmacological approaches to enhance MSC survival and function *in vivo* [23, 24].

Elucidating Mechanisms of Action:

- Identify the specific paracrine factors, cell-cell interactions, or other mechanisms by which MSCs exert their therapeutic effects.
- Understand the role of MSCs in modulating the immune system and promoting tissue repair and regeneration [8, 24].

Optimizing Clinical Trial Design and Standardization:

- Establish consensus guidelines for MSC-based clinical trial design, including patient selection, treatment protocols, and outcome measures.
- Utilize biomarkers to predict patient response and optimize treatment strategies [23, 24].

Developing Personalized Medicine Approaches:

- Tailor MSC-based therapies to individual patient characteristics, such as genetic background, disease stage, and immune status.
- Combine MSC therapy with other modalities, such as gene therapy or pharmacotherapy, for synergistic effects [11].

One of the biggest challenges is ensuring consistency and reproducibility of MSC isolation, characterization and expansion. The heterogeneity of mesenchymal stem cells from different sources and their sensitivity to changes during *ex vivo* manipulation pose major challenges in modeling mesenchymal stem cell-based therapy. In addition, optimizing the target delivery and integration of MSCs is an important issue because their survival and recovery *in vivo* are often not good.

Elucidating the exact mechanisms by which MSCs exert their therapeutic effects is another important area of research. Although paracrine signaling through soluble factors and exosome release is a well-established

mechanism, identification of specific molecules and their downstream signaling pathways is important for the development of mesenchymal stem cell-based therapy. Additionally, understanding the role of MSCs in modulating the immune system and promoting tissue repair and regeneration is important to realize their therapeutic potential.

Despite these challenges, the future of MSC-based therapy is promising. Ongoing research focuses on developing new MSC procurement strategies, improving delivery methods, and improving MSC survival and function *in vivo*. Additionally, the possibility of combining mesenchymal stem cell therapy with other treatments such as gene therapy or chemotherapy is being investigated in a synergistic way. As we delve deeper into the intricacies of mesenchymal stem cell biology and improve our understanding of its therapeutic mechanisms, we may see significant advances in mesenchymal stem cell therapy, paving the way for regenerative medicine breakthroughs that will revolutionize health.

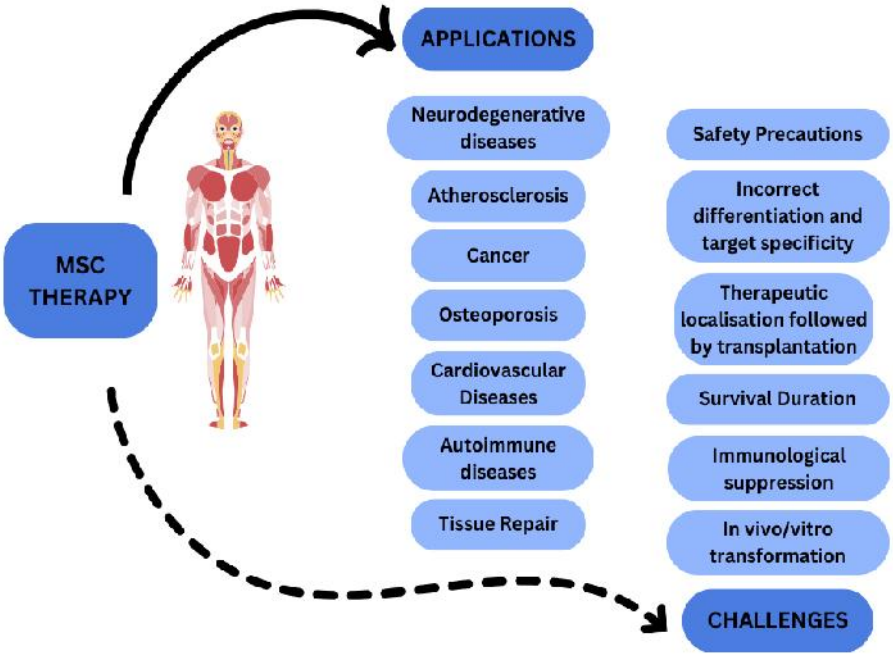

Figure 2: Different therapeutic applications of MSCs and the challenges faced

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Lamiaceae- A reservoir family for therapeutic applications

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Introduction

Throughout the ages, humans have relied on plants as a source of food, flavours, fragrances, and medicines. Even today a large number of people use traditional medicinal plants containing mixtures of various compounds acting individually, additively or in synergy to improve health. There are an increasing number of studies that highlight the applicability of medicinal plants and herbs as sources of more potent or even innovative ingredients. The mint family (Lamiaceae) is an important medicinal plant family

The Lamiaceae, previously called as Labiatae is a family of flowering plants commonly known as the mint or ocimum or sage family. It is one of the most important herbal families and the often with an aromatic smell. Members are with a cosmopolitan distribution containing about 236 genera and have been stated to contain 6900–7200 species. They incorporate a wide variety of plants with biological and medical applications. Many within *Lamiaceae* hold great economic and cultural significance – sage (which also form the edible chia seeds), rosemary, lavender, peppermint (and all other mints), thyme, basil, oregano, marjoram, lemon balm, bergamot, coleus, beauty berry shrubs, and even teak are all members of the mint family. [1]

Systematic position:

Division	: Spermatophyta
Sub- division	: Angiospermae
Class	: Dicotyledone
Sub-class	: Gamopetalae

Series	: Bicarpellate
Order	: Lamiales
Family	: Labiatae

Morphological Characteristics

The Labiatae are annual or perennial herbaceous plants or shrubs, often densely glandular and aromatic. Their stems are usually four-angled. The leaves are usually simple and always opposite. The flowers are hermaphrodite or male-sterile, functionally female and form whorls , verticillasters that are often arranged in spikes, heads, racemes, or cymes. The calyces are usually five-lobed and two-lipped; rarely, the calyx is one-lipped or actinomorphic. The corollas are sympetalous and two-lipped; rarely, they are one-lipped or more or less actinomorphic. They usually have four stamens, rarely reduced to two; the upper, posterior pair is usually shorter than the anterior. The ovary is superior, doubly carpellate but appearing equally four-lobed when mature. The style is single, usually gynobasic, shortly bifid above. The fruit consists of four dry one-seeded nutlets. Besides the non-glandular or clothing hairs, the leaves and other aerial parts of the Labiatae plants bear a great number of glandular trichomes. Two main types of these secretory structures occur in the Labiatae: the glandular hairs (capitate hairs) and the glandular scales (peltate hairs). The former type is very variable in stalk length and head shape, whereas the latter is more or less stable, consisting of a uni- or bicellular foot, a uni- or bicellular stalk, and an 18-celled head. The bulk of the essential oil is thought to be produced and accumulated in the glandular scales. Because of their size and number, they can be seen as bright yellowish dots when the lower leaf surface is examined with a hand lens.[2]

Let us learn few of the medicinally used plants of Lamiaceae in this chapter.

Mint

Mentha piperita L. (peppermint)

Mentha spicata L. (spearmint)

This perennial herb smells "minty", grows from creeping rhizomes, contains a square erect or creeping stem with hairs that are 30-100cm tall. The leaves are opposite, attaching directly to the stem, 2-8cm long, 6-40mm wide, narrow-oval to elliptic-oval, gradually tapering to a pointed tip and sharply toothed along the margins. The flowers are 3-5mm long, lavender, containing a 5-lobed, tube-shaped calyx and a 4-lobed, tube-shaped corolla. The four stamens are not longer than the petals. Inflorescence are broad, tapering terminal spikes 3-12cm long, that are originating in leaf axils. Fruits are lacking.[3]

Science has proven that mint has plenty of health benefits for your body. Here are some of the best ways that you can use mint to help your body stay healthy.Mint leaves are known as an amazing appetizer. It helps to promote the digestive system by stimulating digestive enzymes. Mint is a calming herb that people have used for thousands of years to help soothe an upset stomach or indigestion. Indigestion may occur when food sits in the stomach for too long before passing into the rest of the digestive tract. Multiple studies have shown that food passes through the stomach quicker when people take peppermint oil with meals, which could relieve symptoms from this type of indigestion. Studies support the use of peppermint oil as a remedy for a range of gastrointestinal conditions including stomach pain in children, and feelings of sickness after surgery. Irritable Bowel Syndrome is a common disorder of the digestive system. It can cause stomach pain, constipation, diarrhoea, bloating, and indigestion. The main and important treatment for irritable bowel syndrome is a change of diet but some studies have shown that mint oil can be helpful. Mint works against harmful microbes, regulates muscle relaxation, and helps control inflammation. In addition to ingesting mint, there are claims that inhaling the aroma of essential oils from the plant could provide health benefits, including improved brain function. Study found that smelling these oils while driving increased alertness and decreased levels of frustration, anxiety and fatigue. Mint contains menthol. This aromatic decongestant that

might help to break up phlegm and mucus, making it easier to expel. Applying menthol ointments or vapor rubs may be a safe and effective treatment for children who have a common cold. Many people believe menthol is an effective nasal decongestant that can get rid of congestion and improve airflow and breathing. Mint leaves are highly recommended for asthma patients, as it acts as a good relaxant and relieves chest congestion. Consumption of mint leaves daily can give a soothing effect for asthmatic patients. Mint is an essential part of aromatherapy. It's strong and refreshing smell could help beat stress and rejuvenate the mind. By breathing in the aroma of mint, your mind is instantly calmed. Mint leaves play an essential role in losing weight in a healthy way. Mint leaves promote digestion and boost metabolism to help in losing weight. Mint tea is a great refreshing calorie-free beverage to promote weight loss. Mint-flavored chewing gum and breath mints are some of the first things people reach for when trying to prevent or get rid of bad breath.Experts agree that most of these products can mask foul-smelling breath for a few hours. Drinking peppermint tea and chewing on fresh leaves may be able to both mask bad breath and kill bacteria.

Mint is a particularly good source of vitamin A, a fat- soluble vitamin that is critical for eye health and night vision. It is also a potent source of antioxidants, which help protect our body from oxidative stress, a type of damage to cells caused by free radicals. Breastfeeding mothers commonly experience sore and cracked nipples, which can make breastfeeding painful and difficult. Studies have shown that applying mint to the skin can help relieve pain associated with breastfeeding.

The powerful antibacterial, antifungal, and anti-inflammatory properties of mint leaves are effective in treating acne and lowers the inflammation and redness associated with acne outbursts. Mint leaves contain high salicylic acid and vitamin A which controls the secretion of sebum oil in the skin and helps to cure acne. Mint leaves extract is useful to treat and prevent acne. The richness of menthol and natural antioxidants in mint leaves are used as an amazing cleanser, toner, astringent and moisturizer for the skin. Mint leaves tones the skin, softens dry and itchy skin.

Mint leaves extract is a great source of carotene and antioxidants that promotes hair growth and prevents hair fall. The potent antimicrobial and antifungal properties of mint leaves use to ward off dandruff, head lice, etc. You can apply mint leaves paste mixed with lemon juice on the hair scalp and allow it to stay for 30-40 minutes and rinse the hair well. [4]

Holy Basil

Ocimum tenuiflorum

The holy basil plant is a small annual or short-lived perennial shrub, up to 1 metre (3.3 feet) in height. The stems are hairy and bear simple toothed or entire leaves oppositely along the stem. The fragrant leaves are green or purple, depending on the variety. The small purple or white tubular flowers have green or purple sepals and are borne in terminal spikes. The fruits are nutlets and produce numerous seeds.[5]

Tulsi has a special place in Ayurveda as well as the home of Hindus in India. It is considered sacred by Hindus and worshipped by them. Three main types of Tulsi are seen growing in India:

- Bright green leaves called Ram Tulsi
- Purplish green leaves called Krishna Tulsi
- Common wild Vana Tulsi.

Tulsi has proved to be highly effective in protecting our body from various infections and diseases of the liver, skin, kidney, etc. It contains powerful oxidants that can help in keeping your blood pressure levels and cholesterol levels under control, making it one of the best heart-healthy foods. It is also good for diabetes as it possesses hypoglycaemic properties which are known to help lower blood sugar levels. It is recommended to include tulsi in your diabetic diet plan. Owing to its numerous health benefits, tulsi is rightly called the ‘Queen of Herbs’.

Tulsi is rich in Vitamin C and zinc. It thus acts as a natural immunity booster and keeps infections at bay. It has immense anti-bacterial, anti-viral and anti-fungal properties which protect us from a variety of infections. Tulsi has anti-bacterial and anti-viral properties which help to fight infections, thus reducing fever. The fresh juice of Tulsi taken with black pepper powder cures periodic fevers. Tulsi leaves boiled with powdered cardamom in half a litre of water and mixed with sugar and milk, are also effective in reducing temperature.

Camphene, cineole and eugenol present in Tulsi help reduce cold and congestion in the chest. Juice of Tulsi leaves mixed with honey and ginger is effective in bronchitis, asthma, influenza, cough and cold. Tulsi contains

compounds Ocimumosides A and B. These compounds reduce stress and balance the neurotransmitters serotonin and dopamine in the brain. The anti-inflammatory properties of Tulsi reduce inflammation and blood pressure. Phytochemicals present in Tulsi have strong antioxidant properties. Thus, they help in protecting us from skin, liver, oral and lung cancers.

Tulsi has a profound effect on the treatment and prevention of cardiovascular diseases by lowering blood lipid content, suppressing ischemia and stroke, reducing hypertension and also due to its higher antioxidant properties. Extract of Tulsi leaves has shown to lower blood glucose levels in patients with type 2 diabetes. Tulsi detoxifies the body and has diuretic properties. It decreases the level of uric acid in the body, which is the main reason why kidney stones are formed. Reduction in uric acid levels also provides relief to patients suffering from Gout. Tulsi is known to strengthen the kidney. The juice of Tulsi leaves, when taken with honey, helps in expelling kidney stones through urine. Tulsi leaves help to cure indigestion and loss of appetite. They are also used for the treatment of flatulence and bloating. Tulsi helps clear out the skin of blemishes and acne. It is rich in antioxidants and that helps it to prevent premature ageing. Tulsi also strengthens our hair roots, thus preventing hair loss. Tulsi has often been used in herbal toothpaste and that is simply because of its amazing teeth and gum strengthening properties. Moreover, it can act on mouth ulcers and therefore provide comprehensive oral health care. It is also available commercially in the form of ingestible pills and topical ointments. These can be used for the treatment of skin conditions like eczema. They also provide long-lasting relief from itching and irritation. Consuming a tulsi drink after a tiring day at work can be revitalising and help to relieve stress and fatigue. Similarly, a tulsi drink during prolonged hours of study can also help to enhance concentration for students. The leaves of Tulsi are used to manage fevers. Tulsi leaves boiled with tea can be used to prevent malaria and dengue. A decoction of Tulsi leaves can be boiled with powdered cardamom in water and mixed with sugar and milk to manage acute fevers. The juice obtained from Tulsi leaves helps bring down the fever. It is also effective in reducing fever in children. Tulsi is helpful in the management of disorders related to the respiratory system. A decoction obtained from the leaves of Tulsi is used with ginger and honey for dealing with asthma, cough, influenza, cold, and bronchitis. A decoction of the leaves, common salt, and cloves also provides quick relief in cases of influenza. The leaves of Tulsi have expectorant properties and help in expelling mucous from the bronchial tube.

Tulsi is known to be effective in the management and prevention of heart diseases by lowering blood cholesterol levels and also preventing ischemia and stroke. Apart from this, Tulsi also plays an essential role in platelet aggregation and preventing the risk of pulmonary hypertension. Tulsi is proven to be the safest skin cream that can be used and the benefits are massive. Tulsi reflects on your skin when you consume it as well as applied. This wonder herb is used to treat acne, skin infections, lighten dark spots and improve skin texture. Tulsi is a natural ingredient that aids weight loss. If you're wondering how to burn fat in a quick way without any side effects, then you need to opt for drinking tulsi tea. Two cups a day will make a difference. Also, you need to keep in mind that drinking tulsi tea will act more efficiently only if you work out. Of course without exercising tulsi tea can slim you down, but exercising will make the process faster. Your eyes are prone to a lot of dust and pollution every day. Thus most people develop eye-related problems and Tulsi acts as an immediate cure for eye-related problems such as: Tulsi soothes the eyes. Tulsi leaves left in boiled water overnight can be used to wash your eyes. Tulsi eyewash can also reduce strain on your eyes. Vitamin C & A and phytonutrients are essential oils that are found in Tulsi, which are used as excellent antioxidants that protect the body from premature ageing. Consuming 2 cups of Tulsi tea can help you look younger and prevent premature ageing.[6]

Sweet Basil

Ocimum basilicum

A small herb, much branched with square stem, purple, hairy. Leaves Opposite, ovate acute gland-dotted. Inflorescence is Verticillaster with flowers in 6 flower whorls, bracteates, bracts stalked, ovate, hairy. Calyx bilabiate, hairy, Corolla bilabiate, pink or purple with long white hairs. Stamens 4, didynamous. Ovary bicarpellary, 4-celled, deeply 4-lobed, style gynobasic, stigma bifid. Fruit - Nutlets 4 with persistent calyx.[7]

It acts principally on the digestive and nervous systems, easing flatulence, stomach cramps, colic and indigestion. The leaves and flowering tops are antispasmodic, aromatic, carminative, digestive, galactogogue, stomachic and tonic. Externally, they are used to treat acne, loss of smell, insect stings, snake bites and skin infections. The mucilaginous seed is given in infusion in the treatment of gonorrhoea, dysentery and chronic diarrhoea. It is

said to remove film and opacity from the eyes. The essential oil is used in aromatherapy. Ocimum basilicum (L.) has been used to cure ailments like pyrexia, infections, infections from insects, stomach pains, coughs, migraines, and constipation. It also has antispasmodic and anti-diabetic effects and helps regulate and lower blood sugar. Basil has been used as a folk remedy for an enormous number of ailments, including boredom, cancer, convulsion, deafness, diarrhea, epilepsy, gout, hiccup, impotency, insanity, nausea, sore throat, toothaches, and whooping cough[8,9]

Indian Borage

Plectranthus amboinicus

It is an aromatic succulent. Their leaves possess aroma and a refreshing odour. The plant may reach up to a height of 1 metre. The stem has long hairs. Leaves are simple and hairy giving them a frosted appearance. The flowers are bell shaped with purplish corolla. They are fast growing and are usually propagated with stem cuttings. [10]

The leaves of Indian borage may be chewed on or prepared as tea from them to cure cough, sore throat, or stuffy nose. The herb's constituents operate as a strong expectorant, cleaning your sinuses and eliminating mucus and phlegm from your respiratory tracts. The immune system may benefit from this as well.Infections of the urinary system can be treated using the extract made from the leaves, which also helps to cure vaginal discharges and lessen infections. It is a typical treatment in India because it helps the kidneys function properly. Indian borage leaf's omega-6 fatty acids are known to be beneficial for arthritis. Eat this leaf, athletes are frequently told, to prevent osteoporosis.The use of Indian borage as a skin treatment is one of its most well-liked uses. Insect bites and stings, eczema, and psoriasis can all benefit from the anti-inflammatory properties of Indian borage, which can quickly reduce redness, swelling, itching, and inflammation. Recent research has also shown the herb's effectiveness in maintaining cardiovascular health. Additionally, it helps lower cholesterol and blood pressure. Folk medicine has utilised it as a herbal treatment for those with excessive anxiety or chronic stress, particularly in the form of tea, to promote relaxation, serenity, and sound sleep. It has been demonstrated that this plant's extract possesses antiplatelet aggregation and anti-cancer cell growth capabilities. It is also used to treat fever, one of the most typical signs of the flu or a cold. As a sudorific herb,

Indian Borage causes sweating, which helps to remove impurities from the skin and hastens the healing process? Additionally, it is applied to a range of sensory problems.[11]

Thumbai

Leucas aspera

It is an erect and diffusely branched annual herb. Leaves are linear or oblong, 2.5 to 7.5 cm long with blunt tips and scalloped margins. Whorls are large, terminal and axillary, about 2.5 cm in diameter and crowded with white bell shaped flowers. Calyx is variable, with an upper lip and short, triangular teeth.[12,13]

Leucas aspera is beneficial for respiratory health, providing relief from cough, cold, and bronchial conditions. The herb supports digestive health, alleviating indigestion, bloating, and gastrointestinal discomfort. *Leucas aspera* enhances the immune system's function, promoting the body's natural defense mechanisms.

The plant's antimicrobial and anti-inflammatory properties contribute to faster wound healing and skin regeneration. It is used to address menstrual irregularities and discomfort, providing relief to women's health issues. The herb helps in reducing fever by promoting sweating and cooling the body. **It** supports heart health by regulating blood pressure and cholesterol levels. Some studies suggest that certain compounds in *Leucas aspera* may have anti-cancer effects, making it a subject of ongoing research. It is known for its calming effects, reducing stress and anxiety. The plant exhibits hepatoprotective properties, supporting liver health and detoxification processes. Preliminary studies suggest that *Leucas aspera* may help regulate blood sugar levels, benefiting individuals with diabetes. It can alleviate allergic reactions and provide relief from allergies. The herb's anti-inflammatory properties are beneficial for managing arthritic conditions and joint pain. The plant's extracts can be used topically to improve skin health, reduce acne, and enhance complexion. **It** is effective in reducing fever, providing relief during febrile conditions. It may support kidney health and function, particularly in cases of kidney stones.

Paste made from leaves and stems of the plant after adding some water, two tea spoons taken twice a day for a week to treat asthma. Paste made with

leaves and stems of the plant, then plaster is made on the fractured bone with that paste and kept for two days to treat bone fracture. Pea-sized pills are made from leaves and stems of the plant are taken thrice a day, two pills each time for 30 days to treat cirrhosis. Fresh juice extracted from the leaves and stems of the plant is taken twice for five days to treat fever. Plant extract is given in jaundice. Paste made from leaves and stems of the plant after warming it is applied on the infected throat twice a day for three days to treat tonsillitis. [14]

Teak

Tectona grandis

Tectona grandis is a large, deciduous tree reaching over 30 m in height. Crown open with many small branches; bark is brown, distinctly fibrous with shallow, longitudinal fissures. The root system is superficial, often no deeper than 50 cm. The very large, 4-sided leaves are shiny above, hairy below, vein network clear. Flowers small, about 8 mm across, mauve to white and arranged in large, flowering heads, about 45 cm long; found. Fruit is a drupe with 4 chambers; round, hard and woody, enclosed in an inflated, bladder-like covering; pale green at first, then brown at maturity [15]

Teak contains anthelmintic properties and as such it is effective for destroying parasitic worms. Teak leaves are cooling in nature thus can act as an anti-inflammatory agent for reducing the inflammation of the skin. The leaves can be decocted or squeezed and used for preparing herbal medicines for treating skin diseases. The leaves can also help to tackle pruritus, which is a skin condition marked by severe itching of the skin. The wood can be used for treating leucoderma, which is a cutaneous condition with localised loss of skin pigmentation that may occur after a series of inflammatory skin conditions, post-dermabrasion, burns or intralesional steroid injections have taken place. Leucoderma skin condition is mainly a cosmetic problem thus it is neither contagious nor infectious. The oil extracted from teak flowers can be used for treating scabies. Scabies is a contagious skin disease symptomized by the itching of skin with small raised red spots, caused by the itch mite. The bark can be used for treating leprosy. It is worthy to note that leprosy is a contagious disease that affects the skin, nerves and mucous membranes by causing discoloration and lumps on the skin. In severe cases, leprosy can lead to

permanent disfigurement and deformities. Teak contains diuretic properties and as such, it is useful for preparing herbal remedies for increasing the passage of urine. Oil extracted from teak flowers can be applied on hair for promoting hair growth. Teak wood can be used for preparing herbal tonic that can be taken as a laxative. Due to the laxative properties of teak wood, it tends to stimulate and facilitate the evacuation of faecal matters from the bowel. Teak leaves contain haemostatic properties thus can be squeezed and applied on a cut skin to stop bleeding. Studies reveal that the bark of the teak wood can be decocted and used for curing diabetes due to its antidiabetic properties and also used for treating gastrointestinal disorders such as dysentery, stomach ache, piles and constipation. The decoction may also be used for used for treating anuria, a health condition marked by the failure of the kidneys to produce urine. Studies reveal that the oil extracted from teak wood can be applied on the forehead for relieving headache. This is attributed to the analgesic properties of this plant. [16]

Horehound, mint weed, pignut

Hyptis suaveolens

It grows up to about 1.5 m tall, with hairy stems that are square in cross-section. The leaves have a strong smell when crushed. They are ovate to obovate, the margins serrulate, the lower surface densely hairy, and the petioles up to 3 cm long. The flowers are in small.The corolla is bluish. The fruits are nutlets about 1.2–1.5 mm long, slightly notched at the ends. The seeds are protected in a spined burr and this helps greatly in their dispersal. [17]

It is used medicinally as a treatment for indigestion, stomach pain, nausea, flatulence, colds, and infections of the gall bladder. The essential oil the plant contains has antibacterial and antifungal properties. Besides all this, the plant has insecticidal properties, and is said to be a mosquito-repellant. Traditionally, it is taken to treat ailments, the leaves in the form of infusions, decoctions, teas, and syrups are used to treat ulcers, inflammation, respiratory diseases (asthma, bronchitis, colds, flu, and sinusitis), diseases related to the gastrointestinal tract, pain, dizziness, nausea, nervousness, and constipation The leaves are also used to treat headaches, malaria , fever and used to reduce labor time and labor pain. The leaves, stems, inflorescence and roots are used to treat urinary calculi, stomach pain, healing, itching, boil, eczema, diabetes, pneumonia and fever. Besides that, the seeds are used to treat gynecological

disorders such as menorrhagia, leucorrhea, and rheumatism. The fresh poultice of the leaves is applied to snake bites, wounds, and mycoses, while the paste of the fresh leaves is also indicated for skin diseases.[18]

Sage

Salvia officinalis

Sage is a perennial plant that grows about 60 cm (2 feet) tall. The oval leaves are rough or wrinkled and usually downy; the colour ranges from gray-green to whitish green, and some varieties are variegated. The flowers are borne in spikes and feature tubular two-lipped corollas that are attractive to a variety of pollinators, including bees, butterflies ,and humming birds. The flowers can be purple, pink, white, or red and produce nutlet fruits.[19]

Sage is very high in vitamin K, and it also contains vital minerals like magnesium, zinc, and copper. Sage leaves have been used in traditional medicine as a treatment for diabetes, and some studies do back this up. Sage has been shown to have antimicrobial effects that aid in killing plaque. It has been used to treat mild dyspepsia such as heartburn and bloating, excessive sweating, age-related cognitive disorders, and inflammations in the throat and skin. The body naturally experiences a decline in estrogen levels during menopause. That decline causes side effects like hot flashes, excessive sweating, vaginal dryness, and irritability. Sage has been used as a traditional medicine to reduce these symptoms. Taking common sage extract by mouth for 4 weeks improves some symptoms of menopause, especially hot flashes, night sweats, and sleep problems. Memory and thinking skills, cognitive function. Taking sage by mouth seems to improve memory and thinking skills in healthy adults. But it's not clear if sage aromatherapy helps. High levels of cholesterol or other fats (lipids) in the blood (hyperlipidemia). Taking common sage three times daily for 2 or 3 months reduces low-density lipoprotein, LDL or bad cholesterol and triglyceride levels. It also increases high-density lipoprotein, HDL or good cholesterol, in people with high cholesterol. For a long time, sage species have been used in traditional medicine for the relief of pain, protecting the body against oxidative stress, free radical damages, angiogenesis, inflammation, bacterial and virus infection. SALVIA species, in addition to treating minor common illnesses, might potentially provide novel natural treatments for the relief or cure of many

serious and life-threatening diseases such as depression, dementia, obesity, diabetes, lupus, heart disease, and cancer. [20]

Chiya Beej

Salvia hispanica

Chia is an annual herbaceous plant that can reach nearly 1 metre (3 feet) in height. Its lime-green leaves are oppositely arranged and have serrated (toothed) margins. The plant bears spikes of small blue, purple, or white flowers that have a high rate of self-pollination. The small oval seeds are about 1 mm (0.04 inch) in diametre and feature a shiny, mottled, or speckled seed coat that ranges in colour from dark brown to gray-white. The seeds produce a mucilaginous gel when soaked in water [21]

It is used in prophylaxis of several non-infectious diseases such as obesity, hypertension, cardiovascular diseases (CVDs), cancer and diabetes. Inflammatory diseases are linked with redness, pain, and swelling, which might lead to the loss of essential functions. Chronic inflammation can lead to health conditions like heart disease and cancer. Caffeic acid, an antioxidant found in chia seeds, can help to fight inflammation in the body. Eating chia seeds regularly may also help to reduce inflammatory markers, which often indicate the presence of an inflammatory disease. A chia seed oil in diet may have been shown to lower the risk of pro-inflammatory agents, involved in inflammation. Therefore, it is suggested that chia seed oil might benefit the management of inflammatory diseases. High levels of high-density lipoprotein (HDL) cholesterol,bad cholesterol, in the blood may be directly linked to the development of heart-related diseases in humans. Consuming chia seeds may have been shown to lower serum cholesterol levels, as it contains a high amount of unsaturated omega-3 fatty acids and dietary fibre. As chia seeds are low in carbohydrates and high in dietary fibre, they may reduce the desire to eat and increase satiety, a state of being fed beyond capacity. Chia seeds are high in fiber. Studies show that fiber may help to reduce insulin resistance and improve blood sugar levels, reducing your risk of metabolic syndrome and type 2 diabetes . Chia seeds have several nutrients that are vital for bone health, including magnesium and phosphorus. A single ounce of the seeds also contains 18% of your recommended daily allowance of calcium, which is vital for healthy bone, muscle, and nerve functioning. [22]

Lemon balm

Melissa officinalis

Lemon balm is a bushy herbaceous perennial that grows to about 0.6 metre (2 feet) tall. The wrinkled toothed leaves are roughly heart-shaped or oval and are borne in pairs along the square stems. The leaves can be smooth or somewhat hairy. The tiny white to pale yellow or pale purple flowers are inconspicuous and appear in whorled clusters in the axils of the leaves; they are a good nectar source for honeybees and other insect pollinators.[23]

Lemon balm is known by many other names, such as bee balm, cure-all, dropsy plant, honey plant, garden balm, heart's delight, sweet balm, and sweet Mary. It is considered a calming herb. It was used as far back as the Middle Ages to reduce stress and anxiety, promote sleep, improve appetite, and ease pain and discomfort from indigestion including gas and bloating, as well as colic. If you need to relieve stress, lemon balm can help lower anxiety and nervousness. Research has shown that when combined with herbs like valerian, lemon balm helps improve sleep. Also, drinking lemon balm tea can help with insomnia. Lemon balm ointments have been found to help heal cold sores caused by the herpes simplex virus (HSV). The compound rosmarinic acid in lemon balm may help minimize the severity of menstrual symptoms like cramps and fatigue. Taking lemon balm by mouth might help to improve symptoms of depression in adults with depression and/or anxiety. Applying a lotion containing a lemon balm extract (LomaHerpan by Infectopharm) to cold sores right after they appear seems to shorten healing time and reduce symptoms. [24]

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This is a member captured database. informations have been taken from various sources like websites, journals and books. consequently, some information may resemble works already published by others. the authenticity or originality of the database is not claimed.

The traditional medical system has recently come into focus as a viable resource to address the rising incidence of chronic, degenerative, environmental, lifestyle, and stress-related disorders. Always seek advice from a professional before using a plant medicinally.

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Biological synthesis, characterization and applications of silver nanoparticles

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Abstract

Bionanotechnology has emerged up as an integration between biotechnology and nanotechnology for developing biosynthetic and environmental-friendly technology to synthesize of nanomaterials / nanostructures. Both top-down and bottom-up methods are generally employed to synthesize nanostructures, especially nanoparticles. It is further separated into three categories: chemical, biological, and physical approaches. This chapter discusses the biological method, which uses plants and microorganisms. Fungi are having good features for large-scale synthesis, but they are labor-intensive. These days, plant-mediated nanoparticle synthesis is very common with amazing qualities and a wide range of uses. The biological mode of synthesis is simple, economical, and environmentally benign. UV-visible spectroscopy, XRD, AFM, SEM & TEM, DLS, EDAX, DSC, LEIS and AES, and other instrumentation techniques are used to characterize the synthesized nanoparticles. The wide range of uses of silver nanoparticals has also been discussed .

Key words: Nanobiotechnology, Silver nanoparticles, biological methods, Characterization.

Introduction

Manipulation of matter at the atomic and molecular level is known as nanotechnology, or simply "nanotech". It is also described as the art and science of working with matter at the atomic or molecular level. It has the potential to significantly advance the environmental protection technologies and offers a viable treatment option for a wide range of diseases.

The father of nanotechnology, physicist Richard Feynman, introduced the field's theories and concepts on December 29, 1959, at a meeting of the American Physical Society held at the California Institute of Technology. His address was titled "There's Plenty of Room at the Bottom." In industries like engineering, agriculture, construction, microelectronics, and healthcare, nanotechnology is becoming more and more significant. As a result, numerous researchers in a variety of academic domains, including engineering and the basic sciences, are currently delving into the fundamental aspects of nanotechnology (Kreuter, 2007).

Nanotechnology was divided into three types : 1. Wet nanotechnology works with biological systems, including membranes, cells, and Enzymes; 2. Dry nanotechnology that focuses on the creation of structures out of carbon, silicon, and other inorganic materials. It also deals with surface science and physical chemistry; 3. Computational nanotechnology, which models and simulates the intricate structure at the nanoscale (Sinha *et al*., 2009). These three fields are interdependent to each other (Fazal*et al.*, 2019).

1.0 NANOBIOTECHNOLOGY

Within the biological sciences, nanoscience has emerged as a new multidisciplinary field. It has the potential to enter into a variety of applied science fields, including engineering, chemistry, physics and life sciences (such as botany, zoology, biochemistry, microbiology, and biotechnology). As depicted in table 1, it is not the same as nanotechnology in the following respects. (Khillar, 2020); https://www.brainkart.com/article/Short-answers_41599/).

Table 1. Differences between nanoscience and nanotechnology

S. No.	Nanoscience	Nanotechnology
1.	It is the study of materials and structures at the nanoscale ($1nm = 10^{-9}$ m).	This technology deals with the design, manufacturing, testing, and use of materials with nanostructures.
2.	It involves understanding the fundamental interaction of systems confined to nanoscale dimensions and their properties	It is a multidisciplinary field that encompasses understanding and control of matter at about 1-100 nm.

Bionanotechnology has emerged up as an integration between biotechnology and nanotechnology for developing biosynthetic and environmental-friendly technology to synthesize nanomaterials (Rai *et al.,* 2009; Krithiga *et al*., 2015).Nanobiotechnology is also a new frontier of biology with important applications in medicine. In many ways, including imaging, sensing, targeted drug delivery, gene delivery systems, and artificial implants, it is a tool to investigate the darkest corners of the medical sciences (Shrivastava *et al*., 2007).

2.0 NANOPARTICLES

Nanoparticles, which are clusters of atoms with sizes ranging from 1 to 100 nm, are thought to be the basic building blocks of nanotechnology. It is believed that their exploitable biomedical activities are due to their smaller particle size combined with special chemical and physical properties (Yildirimer *et al*., 2011).

Because they can bridge the gap between bulk materials and atomic or molecular structures, these particles are of great scientific interest. The physical, chemical, electronic, electrical, mechanical, magnetic, thermal, dielectric, optical, and biological properties of nanoparticles are distinct from those of bulk materials (Table 2). (Narayanan and Sakthivel, 2010; https://www.brainkart.com/article/Short-answers_41599/;https://pediaa.com/what-is-the-difference-between-nanomaterials-and-bulk-materials/ (Hasini, 2017); https://www.differencebetween.com/difference-between-nanomaterials-and-bulk-materials/; Madhu, 2018)).

Table 2. Difference between nanomaterials and bulk materials

S.No.	Nanomaterials	Bulk materials
1.	Extremely smaller materials with unique properties	Larger materials without any unique properties
2.	Size of the material is lesser than 100 nm.	Size of the material is greater than 100 nm.
3.	Larger surface area per unit mass	Smaller surface area per unit mass compared to nanomaterials
4.	Higher surface to volume ratio	Do not have higher surface to volume ratio
5.	Invisible to naked eye	Most of the particles are visible to naked eye
6.	Useful in nanoelectronics, nanomedicine and sensors	Useful in traditional manufacturing, construction and everyday items
7.	Examples: nanotubes, nanoparticles	Examples: wood, steel

Furthermore, when compared to their macroscale counterparts, these particles exhibit distinct and significantly altered physical, chemical, and biological properties. This is due to the following reasons:

i. Surface Plasmon Resonance (SPR)
ii. Enhanced Rayleigh scattering
iii. High surface area - to-volume ratio
iv. Surface enhanced Raman scattering (SERS) in metal nanoparticles
v. Quantum size effect in semiconductors and
vi. Super magnetism in magnetic materials.

2.1 Metallic nanoparticles

Metallic nanoparticles have wider applications in both conventional technology and experimental medicine in recent years. The two main categories of nanoparticles are organic and inorganic. Carbon nanoparticles are one type of organic nanoparticles. Magnetic nanoparticles, noble metal nanoparticles such as silver, gold, iron, copper, palladium and platinum, and semiconductor nanoparticles such as titanium as lead (II) sulfide, silica (II), zinc (II), copper oxides, indium (III), and titanium (IV) oxide) are examples of inorganic nanoparticles.

2.2 Silver nanoparticles and their advantages over other metals

Silver nanoparticles are highly commercial due to their inherent properties such as good conductivity, chemical stability, catalytic activity, and antimicrobial activity. Due to these properties, they are commonly used in medical and electrical applications (Chanda, 2013).

Silver is a precious metal. Due to its lack of adverse effects, it was first employed as disinfectant and efficient antimicrobial agent. Silver nanoparticles exhibit strong antibacterial properties, which make them useful in the food industry, clothing, sunscreen, cosmetics, and a variety of home appliances (Chanda, 2013- https://m.moam.info/silver-nanoparticles-formatex-research-center_5c2a041e097c4709308b4592.html). The distinctive optical, electrical, and biological characteristics of silver nanoparticles have garnered substantial interest owing to their potential utility in numerous applications, including drug delivery, biosensing, catalysis, and the creation of nanodevices. In contrast to other metals, silver is more harmful to microorganisms but less harmful to mammalian cells (Wijnhoven *et al*., 2009). A more recent development in metal nanoparticle research reveals that silver nanoparticles possess many applications in health care products, including burn dressings, scaffolds, water purification systems, antimicrobial applications, and medical devices (Sahayaraj and Rajesh, 2014- https://www.researchgate.net/publication/216807433_Bionanoparticles_Synthesis_and_antimicrobial_applications). Wong *et al*. (2009) noted that silver nanoparticles also possessed anti-inflammatory properties.

3.0 SYNTHESIS OF METALLIC NANOPARTICLES

There are two alternative approaches for the synthesis of metallic nanoparticles such as "bottom up" approach and "top down" approach (Fazal *et al*., 2019) (Table 3).

Table 3. Differences between bottom-up and top-down approaches of nanoparticle synthesis

S.No.	Bottom-up approach	Top down approach
1.	Assembling process:Single atoms and molecules into larger nanostructures	Subtractive process: Breaking down of large pieces of material to generate the required nanostructures
2.	Constructive method	Destructive method
3.	It is based on the principle of molecular recognition (i.e., self assembly)	It is based on grinding of material
4.	Growth process is faster	Growth process is slow
5.	The starting material is either in liquid state or gaseous state.	Only possible in hard and brittle materials. Not possible for soft samples.
6.	Less chances for contamination	Possible for contamination with milling tools and atmosphere
7.	Controlled particle size is possible	All the precursor materials may not breakdown to the required particle size.
8.	Chemical purification is required	Chemical purification is not required
9.	High production rate	Low production rate
10.	Large quantity of nanoparticles can be synthesized in short time	It is not possible to synthesize large quantity of nanoparticles in short time.
11.	Less expensive and comparatively expeditions	Expensive and time consuming techniques
12.	Eg Green synthesis, spray pyrolysis etc	Eg. Ball milling, Sol-gel, lithography etc.

3.1 Biological or greener synthesis of metallic nanoparticles

In order to produce low-cost, energy-efficient, and nontoxic metallic nanoparticles, three main biological methods are followed: 1. Microorganisms such as fungi, yeasts (eukaryotes), or bacteria, actinomycetes (prokaryotes); 2.

Plant extracts or enzymes; and 3. Templates such as DNA, membranes, viruses, and diatoms (Li *et al*., 2012).

Among biological synthesis, plants and plant products mediated nanoparticle synthesis is more advantageous since it is very easy to perform with low maintenance. The criteria of good nanoparticles are fascinating biological properties, bioavailability, bioreactivity, and biocompatibility and no toxicity. However, compared to chemical and physical methods of nanoparticle synthesis, biological methods may be less stable (Barhoum *et al*., 2022).

3.1.1 Synthesis of nanoparticles by microorganisms

Many microorganisms like bacteria, fungi and yeast produce inorganic nanomaterials either intra or extra-cellularly (Table 4). Bacterial cells play an important role in nanoparticle synthesis. Bacteria has the ability of sustainable synthesis of nanoparticles at a large scale (Fariq *et al*., 2017; Lahiri *et al*., 2021). It is also reported that fungi plays a predominant role in the synthesis of nanoparticles both extra-cellularly and intra-cellularly.

Table 4. Extracellular and intracellular synthesis of nanoparticles

S.No	Intracellular synthesis	Extracellular synthesis
1	The intracellular method involves transporting ions into the microbial cell to form nanoparticles in the presence of enzymes (Li *et al*., 2011).	The extracellular mode involves trapping the metal ions on the cell surface and reducing ions in the presence of enzymes (Li *et al*., 2011).
2	Intracellular production of nanoparticles uses enzymes to move ions into the cells of microbes and produces smaller sized nanoparticles in the organism (Nkele and Ezema, 2020).	Extracellular synthesis does not involve cell components but it yields nanoparticles outside the cell, uses fungi with large secretory organs (Nkele and Ezema, 2020).

3.1.1.1 BacteriagenicSNPs synthesis

Bacteria are a very good source for fabricating silver nanoparticle. The first report on bacteriagenic silver nanoparticle came in 1999, when the bacteria, *Pseudomonas stutzeri* AG259, was isolated from a silver mine, an extreme silver rich environment (Klaus *et al*., 1999; Singh *et al*., 2015). Several reports proved the applications of bacteria in silver nanoparticle synthesis (Table 5).

Table 5. Bacteria mediated silver nanoparticle synthesis

S.No.	Bacterial species	Size (nm)	Shape	Activity	Reference
1.	*Pseudomonas indica*	2.4 to 53.5	Spherical	Antioxidant and Antifungal	Salem *et al*., 2022
2.	*Bacillus licheniformis*	38	Spherical	Antimicrobials	Tufail *et al*., 2022
3.	*Cytobacillus firmus*	55.8	Spherical	Dye degradation and antimicrobial	Saied *et al*., 2022
4.	*Lactobacillus plantarum*	40-50	Spherical	Antioxidant and antibacterial	Prema *et al*., 2022
5.	*Streptomyces catenulae*	33 ± 2.2	Spherical	Anti-inflammatory	Khalil *et al*, 2022
6.	*Paenarthrobacter nicotinovorans*	13 to 27	Crystal	Antimicrobial	Huq and Akter, 2021
7.	*Serratia nematodiphila*	10–31	Crystal	Bactericidal	Malarkodi *et al*., 2013
8.	*Staphylococcus aureus*	10-60	Spherical	Antibacterial	Saleem *et al*., 2020
9.	*Pseudomonas aeruginosa*	40-60	Spherical	Antimycotic	Deshmukh *et al*., 2012
10.	*Pseudomonas deceptionensis*	10-30	Spherical	Antimicrobial	Jo *et al*., 2016

Drawbacks

i. Culture contamination
ii. Lengthy procedure
iii. Less control over the size of nanoparticles

3.1.1.2 Synthesis of nanoparticles by the fungal system

The synthesis of silver nanoparticles can also be carried out involving fungi (Table 6). The synthesis of silver nanoparticles in fungi is attributed to the enzyme nitrate reductase. According to Tikariha *et al*. (2012), biomass used in the synthesis of nanoparticles is easier to handle, as it deposits easily in the environment, and can be processed more easily afterwards. According to several studies (Balaji *et al*., 2009; Du *et al*., 2015; Netala *et al*., 2016; Velusamy *et al*., 2016; Zielonka and Klimek-Ochab, 2017; Khandel *et al.*, 2018; Guilger-Casagrande and Lima, 2019; Moradi *et al*., 2021; Remya *et al*., 2022) the use of fungi is superior to bacteria and plants when synthesizing nanoparticles. It is due to the following reasons:

i. Excellent resistance to metals and ease of handling
ii. Secrete large quantities of extracellular proteins, enzymes and reducing components
iii. Because fungi have higher biomass contents than bacteria, they typically produce more nanoparticles.
iv. Presence of mycelia provides greater surface area for interactions.
v. Fungi produce a greater quantity of enzyme than bacteria do.
vi. The absorption mechanism and the reduction of metal ions for the formation of nanoparticles are significantly influenced by the fungal cell wall.
vii. The mycelial mass of fungi is more resistant to agitation and pressure than that of plants, making it a better option for large-scale syntheses.
viii. Their capacity to break down or mineralize the substances that pose a risk to environment.
ix. By modifying certain culture parameters such as time, temperature, pH, and biomass amount, one can control fungal metabolism and produce nanoparticles with desired properties like precise size and shape.

Table 6. Fungi mediated silver nanoparticle synthesis

S.No.	Fungal species	Size (nm)	Shape	Activity	Reference
1.	*Aspergillus terreus*	7-23	Crystalline	Antimicrobial& Anticancer	Lotfy*et al.,* 2021
2.	*Agaricus bisporus*	8-20	Spherical	Antitumour	El-Sonbaty, 2013

3.	*Chaetomium thermophilum*	8.93	Spherical	Cytotoxicity	Alves *et al*., 2022
4.	*Fusarium oxysporum*	50-100	Globular	Antimicrobial	Abdel-Hadi *et al*., 2023
5.	*Penicillium cinnamopurpureum*	30	Spherical and polydispersed	Bactericidal	Dinesh *et al*., 2022
6.	*Penicillium brasilianum*	10-60	Crystal	Anticancer	Rudrappa *et al*., 2023
7.	*Trichoderma harzianum*	31.13	Spherical	Antifungal	El-Ashmony *et al*., 2022
8.	*Emericella dentata*	10-80	Spherical	Anticancer	Alqaraleh *et al*., 2022

3.1.1.3 Nanoparticle synthesis by marine resources

There have also been reports of using marine algae as a source to create silver nanoparticles. For example, Somasundaram *et al*. (2021) reported the application of silver nanoparticles (AgNPs) mediated by *Sargassum coreanum* (marine algae) in pollution detoxification, specifically in the degradation of dye (methylene blue dye). Algotiml (2022) evaluated the antimicrobial and anticancer properties of silver nanoparticles synthesized from the extracts of three distinct marine algae species: *Ulva rigida* (green alga), *Cystoseira myrica* (brown alga), and *Gracilaria foliifera* (red alga). Out of the these three, *U. rigida* silver nanoparticles were found to be effective substitute for antidermatophytes to treat skin infections and inhibitors of cancer by targeting the MCF-7 cell line. According to Singh *et al*. (2014), a marine invertebrate called a polychaete was used to synthesize silver nanoparticles. This suggests that marine invertebrates, in addition to plants and microbes, have the potential to synthesize nanoparticles in an environmentally responsible and cost-effective manner.

3.1.1.4 Nanoparticle synthesis by animal resources

There have also been reports of using animals and animal products to synthesize nanoparticles. The synthesis of AgNPs from cow milk has a number of benefits over other biological resources. These benefits include: (i) the synthesized AgNPs have potential applications in the medical and

pharmaceutical sciences because milk does not contain any toxic materials; (ii) the method is safe for the environment and does not release any harmful chemicals into the environment; (iii) the synthesis of AgNPs requires less incubation time; and (iv) despite the fact that milk is meant to be used for nutritional purposes, using a minimum amount of milk for non-nutrient applications may not restrict human availability (Kui *et al*., 2013). Despite being widely accessible, its range of applications may be limited.

3.1.1.5 Greener synthesis of silver nanoparticles using plants

Because of the wide variety of biomolecules found in plants, using them to synthesize nanoparticles is becoming more advantageous than using microbes or animal products. This is due to the fact that plants can act as capping and reducing agents, which speed up the process of reducing and stabilizing nanoparticles (Thakkar *et al*., 2010).

A number of plant biomasses or extracts have been effectively employed for the extracellular biosynthesis of silver nanoparticles among the different biological syntheses. The advent of new technologies and their recent development has ushered in a new era known as the "nano-revolution," which reveals the role of plants in the bio and environmentally friendly synthesis of nanoparticles. This has undoubtedly attracted attention with the aim of synthesizing stable nanoparticles (Kavitha *et al*., 2013; Premkumar *et al*., 2018).

The field of nanoparticle synthesis has completely changed as a result of the synthesis of silver nanoparticles through the use of green synthesis techniques. Plant extracts are used to synthesize nanoparticles by simply combining the extract with metal salt solution at room temperature. Within minutes, the reaction finishes, and silver nanoparticals are produced (Li *et al*., 2011; Mittal *et al*., 2013; Roy *et al*., 2013).

Benefits of using plants for nanoparticle synthesis:

i. Easily available, safe, and nontoxic
ii. Contain a large number of metabolites that can aid in the reduction of silver ions.
iii. The synthesis process is faster than that of microbes.
iv. The entire plant, pulp, fruit, seeds, roots, leaves, stems, bark, and secretory substances like latex—and

v. *In vitro* cultured calli have been reported for the green synthesis of nanoparticles.
vi. A variety of biomolecules found in the plant extract break down the monovalent silver ion into uncharged atoms, which then aggregate to form nanoscale particles. Other biomolecules in the extract then enclose or "cap" these particles to stop them from continuing to agglomerate (Ganaie *et al*., 2014; Rajoriya *et al.*, 2017).

The synthesis of AgNPs is largely dependent on phytochemicals found in plants, including amides, aldehydes, carboxylic acids, flavones, ketones, terpenoids, and different reducing sugars. According to some research, phytochemicals have a direct role in the ion reduction process and AgNP formation (Jha *et al*., 2009; Rajoriya *et al*., 2017). The extracellular synthesis of silver nanoparticles has been effectively applied to a number of plants and plant parts. Below are a few of the instances:

(i) Synthesis of silver nanoparticles using whole plant extracts

Mahitha *et al*. (2011) carried out the biosynthesis, characterization, and antimicrobial studies of silver nanoparticles made from the extract of the whole *Bacopa monniera* plant. The produced particles demonstrated strong inhibitory action against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumonia*. Shawkey *et al*. (2013) reported the anticancer activity of silver nanoparticles utilizing the *Citrullus colocynthis* aqueous extracts of fruits, seeds, leaves, and roots. When tested against human cancer cell lines from the colon (HCT-116), breast (MCF-7), liver (Hep-G2), and intestine (Caco-2), the synthesized silver nanoparticles demonstrated strong anticancer activity.

(ii) Stem extract mediated silver nanoparticles

Vanaja *et al*. (2013) described the environmental - friendly synthesis of silver nanoparticles using *Cissus quadrangularis* stem extract and evaluated the physical and chemical parameters that are vital to the synthesis of the nanoparticles, such as temperature, pH, time duration, and metal ion concentration. The highest amount of silver nanoparticle synthesis was achieved in one hour at 70°C, pH 8, and 1 mM $AgNO_3$ concentration. The antibacterial activity exhibited by the synthesized silver nanoparticles was maximum against *Bacillus subtilis* and *Klebsiella planticola*.

(iii) Root mediated synthesis of silver nanoparticles

Babu *et al*. (2013) investigated the biosynthesis of silver nanoparticles using the root of the Chinese white ginseng plant, *Panax ginseng* and reported that the silver nanoparticles wielded toxicity against Multidrug resistant bacteria. In another study, Kalidasan and Yogamoorthi (2014) described the reduction of silver nitrate to silver nanoparticles by aqueous root extract of the wild weed *Achyranthus aspera.*

(iv) Flower extract in nanoparticle synthesis

Kudle *et al*. (2013) used the flower extract of *Boswellia serrata* to assist in the green synthesis of silver nanoparticles. The produced silver nanoparticles had spherical and rod-shaped structure and ranged in size from 60 to 84 nm. Pavani *et al*. (2013) reported the phyto-synthesis of silver nanoparticles using *Ipomoea indica* flower extract. The average particle size of the pure crystalline silver structures was ranged between 13 and 16 nm, with cubic shape. According to Pavani *et al*. (2013), this attractive plant has the potential to serve as a phyto-factory for the production of silver nanoparticles, which are widely used in the fields of nanomedicine and nanodiagnostics. The synthesized particles are non-toxic and environmentally friendly.

v. Synthesis of silver nanoparticles from fruit extracts

Mittal *et al*. (2013) used fruit extract from *Syzygium cumini* to synthesize monodispersed, crystalline silver nanoparticles that were between 10 and 15 nm in size. The synthesized silver nanoparticles showed antioxidant and anticancer activity against Dalton Lymphoma cell lines up to 50%. The primary cause of the nanoparticle reduction and stabilization was attributed to the flavonoids found in *S. cumini*. Reddy *et al*. (2014) made 46 nm-sized spherical-shaped silver nanoparticles using fruit extract from *Piper longum*. The produced silver nanoparticles demonstrated strong cytotoxic activity against MCF-7 breast cancer cell lines as well as strong antioxidant properties.

Gnanajobitha *et al*. (2013) reported the fruit-mediated synthesis of silver nanoparticles utilizing *Vitis vinifera.* The fruit extract of this plant served as a reliable source for the synthesis of silver nanoparticles. When applied to *Bacillus subtilis* and *Klebsiella planticola*, the silver nanoparticles demonstrated outstanding antimicrobial efficacy.

vi. Synthesis of silver nanoparticles from peel extracts

Mango (*Mangifera indica*) peel extract was used in the greener synthesis of silver nanoparticles, by Yang and Li (2013). When the synthesized silver nanoparticles were applied to non-woven materials, they took on a crystalline form and showed antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*, with a size range of 7–27 nm. The synthesis of silver nanoparticles using aqueous extract of *Citrus sinensis* peel was reported by Konwarha *et al*. (2011). It is possible to envision orange peel's effective reductive potential to produce silver nanoparticles based on the compositional abundance of pectins, flavonoids, ascorbic acid, sugars, carotenoids, and numerous other flavones (Madhumitha and Roopan, 2013). The human leukemic monocytic cell line is cytocompatible with the silver nanoparticles. The nanoparticles exhibited bactericidal activity against *Bacillus subtilis*.

vii. Synthesis of silver nanoparticles from seed extracts

Ranjan *et al*. (2013) investigated the green synthesis and characterization of silver nanoparticles using *Nigella sativa* seeds and their use against bacteria that cause urinary tract infections (UTIs), specifically *E. coli* and *S. aureus*. The resulting spherical silver nanoparticles, ranged in size from 1.5 to 4.0 nm. Tho *et al*. (2013) reported the green synthesis of silver nanoparticles using *Nelumbo nucifera* seed extract and its antibacterial activity against Gram negative bacteria.

viii. Synthesis of silver nanoparticles from leaf extracts

Raja *et al*. (2012) used *Prosopis juliflora* leaf extract to help create triangular, pentagonal, and hexagonal-shaped silver nanoparticles with a size range of 35–60 nm. These nanoparticles were found to have antimicrobial activity against the sewage contained G-ve bacteria and decreased their population. They were also found to be useful in the effluent treatment process as they lowered the microbial load. *Polyalthia longifolia* (Annonaceae) leaf extract mediated silver nanoparticles, was carried out by Kaviya *et al*. (2011). The particles had spherical shape with an average size of 57.53nm. When compared to the standard antibiotics such as chloromphenical or ketoconazole, the synthesized silver nanoparticles demonstrated antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus cereus*, *Candida albicans*, *Candida tropicalis*, and *Candida krusei*.

3.1.1.6 Mechanism of nanoparticle synthesis

i. Plant extracts contain biomolecules that can be utilized in a one-step green synthesis process to reduce metal ions to nanoparticles (Sadeghi and Gholamhoseinpoor, 2015). At room temperature and pressure, this biogenic reduction of metal ion to base metal can be carried out quite quickly and easily (Khan*et al*., 2009). These days, a large variety of plants that are easily accessible, widely distributed, safe to handle, and have a wide range of metabolites make this technique popular. It also minimizes waste and energy costs (Park *et al*., 2011). When creating nanoparticles, plant extracts can function as stabilizing and reducing agents.

ii. Role of reducing agents

Metal ions are reduced into metal nanoparticles with the assistance of plant metabolites. According to Syed *et al*. (2013), the plant extract contains a variety of water-soluble metabolites, including alkaloids, phenolic compounds, terpenoids, flavones, quinines, organic acids, polysaccharides, proteins, and co-enzymes.

iii. Role of stabilizing agents/ligands/capping agents/passivating agents

The stabilizing agent prevent uncontrollable growth of particles, particle aggregation and control growth rate and particle size. They also allow particle solubility in various solvents. The rate of production of the nanoparticles, their quantity, and other characteristics are known to be influenced by the type of plant extract used, its concentration, concentration of the metal salt, pH, temperature, and contact time. Plant extracts are a cheap, easily scalable, and environmentally safe way to create metallic nanoparticles (Mittal *et al*., 2013).

4.0 CHARACTERIZATION OF SILVER NANOPARTICLES

Numerous techniques, including UV-visible spectroscopy, XRD, AFM, SEM & TEM, DLS, EDAX, DSC, LEIS, and AES, are commonly employed to investigate the physico-chemical properties of synthesized nanoparticles. The important characterization methods are presented in table 3 of this chapter (Kumar *et al*., 2017; Mourdikoudis *et al*., 2018; Almatroudi, 2020; https://www.phi.com/auger-electron-spectroscopy-analysis-of-fresh-and-ag.pdf).

Table 3. Characterization of silver nanoparticles using different methods

S.No.	Technique	Information gained
1	UV-Vis spectroscopy	Confirmation of synthesis of silver nanoparticles. Silver nanoparticles exhibit a UV-Vis absorption maximum in the range of 400–500 nm. Optical properties, size, concentration and agglomeration state, hint on nanoparticle shape.
2	X-ray diffraction analysis (XRD)	Crystal structure, composition, crystalline grain size
3.	Atomic force microscopy (AFM)	NP size and shape in 3D mode, evaluate degree of covering of a surface with NP morphology, dispersion of NPs in cells and other matrices/supports,precision in lateral dimensions of NPs, quick examination–elemental composition
4	Scanning Electron Microscopy (SEM)	Morphology, dispersion of NPs in cells and other matrices/supports, precision in lateral dimensions of NPs, quick examination–elemental composition
5.	Transmission Electron Microscopy (TEM)	NP size, size monodispersity, shape, aggregation state, detect and localize/ quantify NPs in matrices, study growth kinetics
7.	Dynamic light scattering (DLS)	Hydrodynamic size, detection of agglomerates. It is used to determine the average size of nanoparticles in liquids.
8.	Energy-Dispersive X-ray Spectroscopy (EDX)	Chemical characterization- elemental composition of a sample
9.	Fourier Transform infrared Spectroscopy (FTIR)	Detection of Surface composition, ligand binding
10.	Differential Scanning calorimetry (DSC)	Thermal stability of nanoparticles

11	Low-energy ion scattering (LEIS)	Thickness and chemical composition of self-assembled monolayers of NPs
12	Auger electron spectroscopy (AES)	Effective method for characterizing surface features with very high lateral resolution

5.0 APPLICATIONS OF SILVER NANOPARTICLES

Silver nanoparticles is used in various fields such as agriculture, medicine and industry. Few of the applications are listed below.

(i) Colorimetric sensor

SNPs are employed as colorimetric sensors to identify impurities and other pollutants. It is predicated on how particular analytes (pollutants) alter the optical absorption of silver nanoparticles. Effective optical sensors can be created by utilizing the gradual alteration of optical characteristics in response to the concentration of impurities. The type of functional unit causing changes in the vicinity of the silver nanoparticles, which change the observed SPR intensity, energy, and band shape and enable proper quantification of the target molecules and determines the sensitivity of such optical sensors (Prosposito *et al*., 2020).

(ii) Optical Probes

SNPs are used in various imaging techniques and in chemical and biological sensing applications (Pandey *et al*, 2012). For example, it is very useful in the detection of human serum antibody (Otamiri *et al*., 1999), real-time probing of membrane transport in living microbial cells (Xu *et al*., 2004), and glucose sensor for medical diagnostics(Mishra *et al*., 2007) etc.

(iii) Antibacterial agent

Since ancient times, silver has been utilized for its antimicrobial properties and has been used to treat and prevent a variety of illnesses. Many bacterial pathogens become multidrug resistant as a result of the widespread use of broad spectrum antibiotics. Currently, nanotechnology opens up new possibilities for nanomedicine. Numerous studies demonstrate the potential of Silver nanoparticles as an antimicrobial agent especially when it comes to multidrug-resistant organisms. Therefore, it is thought that SNPs are a good substitute for antibiotics (Franci *et al*., 2015; Bruna *et al*., 2021).

(iv) Insecticides

Nanoparticles have proven to be effective alternative to agrochemicals that can increase crop productivity and reduce pests. By increasing plant nutrient uptake and preventing plant-infecting pathogens, they aid in agricultural applications. As "magic bullets," nanoparticles have numerous applications, including as fertilizers, herbicides, and insecticides (Anand and Bhagat, 2019).

Conclusion

For the past two decades, nanotechnology field has been growing and reports are available on fascinating properties and applications of silver nanoparticles. In India, a plenty of reports are available regarding plant mediated synthesis of silver nanoparticles, characterization and application studies such as antimicrobial, antioxidant, insecticidal activities etc.The extensive application studies would give solution to many issues in effective manner.

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CRISPR-Cas9: Revolutionizing Medical Treatments in Genetic Diseases, Infectious Diseases, and Cancer

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Introduction

The remarkable and versatile therapeutic applications of the CRISPR-Cas9 gene-editing system across various fields, including genetic diseases, infectious diseases, and cancer. CRISPR-Cas9 is a revolutionary technology that allows precise and targeted modification of DNA sequences, making it a potent tool for tackling a wide range of medical conditions. In the realm of genetic diseases, the technology enables the correction of disease-causing mutations, offering hope for conditions such as Duchenne muscular dystrophy, α1-antitrypsin deficiency, and various hematopoietic disorders. It has the potential to revolutionize the treatment of these conditions by editing the genomes of affected individuals or by generating genetically corrected cells for transplantation. In the context of infectious diseases, CRISPR-Cas9 holds great promise for eradicating latent viral infections, such as HIV and oncogenic viruses like hepatitis B, Epstein-Barr virus, and human papillomavirus. Researchers have explored its use to disrupt viral genomes and reduce viral replication, providing a potential avenue for curing these infections. Furthermore, CRISPR-Cas9 is emerging as a powerful tool in the fight against cancer. It allows for the precise editing of oncogenes and tumor suppressor genes, enabling the development of targeted therapies. Additionally, the technology has been used to enhance the efficacy of immunotherapies like CAR-T cell therapy, offering new possibilities in the treatment of various malignancies. The chapter highlights the transformative potential of CRISPR-Cas9 in reshaping the landscape of medical treatments and underscores the continuous advancements in this field.

CRISPR-Cas

The structure of clustered regularly interspaced short palindromic repeats (CRISPRs) was initially identified in prokaryotic organisms. CRISPRs and CRISPR-associated (Cas) proteins constitute a crucial component of the adaptive immune system in archaea and bacteria, serving as a defense mechanism against invasive nucleic acids such as plasmids and phages. The discovery of the CRISPR structure was first documented by Ishino in 1987 and the acronym CRISPR was suggested by Jansen in 2002 following the identification of several analogous structures in various bacteria and archaea (Ishino *et al.,* 1987 & Jansen *et al.,* 2002). In 2005, a significant milestone was reached when hyper-variable spacers containing sequences similar to those found in foreign plasmids and viruses were uncovered. Mojica and his colleagues subsequently postulated that the CRISPR structure and its associated proteins might serve as a defense mechanism and have important roles in safeguarding against transmissible genetic elements (Mojica *et al.,* 2005). Subsequently, additional insights into the CRISPR system have been unraveled at an accelerated rate. Among the pivotal contributors in this field are Charpentier, Doudna, and Zhang. Charpentier was the first to clarify the workings of the CRISPR-associated protein 9 (Cas9) genome-editing system (Deltcheva *et al.,* 2011). Additionally, Charpentier and Doudna conducted a study that involved the biochemical characterization of Cas9-mediated gene editing and further optimized the system (Jinek *et al.,* 2012). Zhang was the pioneering scientist to apply the CRISPR-Cas9 system in eukaryotic cells for the purpose of genome editing (Cong *et al.,* 2013).

Cas proteins are endonucleases that utilize a single guide RNA (sgRNA) to establish complementary base pairs with target DNA and then cut the DNA at precise locations. Among the various types of Cas proteins, Cas9 stands out as the most widely utilized due to its simplicity, high efficiency, and user-friendliness. The Cas9/sgRNA two-component system proves highly efficient and specific in the realm of gene editing. The sgRNA is responsible for recognizing a particular sequence within the genome, while the Cas9 protein acts like a pair of molecular scissors, cleaving the DNA sequence. Theoretically, this system can be harnessed to modify nearly any DNA sequence within the genome, rendering the CRISPR-Cas9 system the most potent gene-editing tool to date. One notable application of this technology is the rapid generation of knockout cell lines or animal models.

When compared to zinc finger nucleases and transcription activator-like effector nucleases, the CRISPR-Cas9 gene editing system is a more effective tool for precisely manipulating specific DNA or RNA (Hsu *et al.,* 2014). Furthermore, it has a lower likelihood of causing insertional mutations or non-physiological expression of proteins. Over the past five years, CRISPR-Cas9 has found widespread use in researching the genetic underpinnings of human diseases (Kim *et al.,* 2014). Additionally, the CRISPR-Cas9 system holds immense potential for gene therapies aimed at addressing a wide range of diseases, including hematologic conditions (Wu *et al.,* 2018), acquired immunodeficiency syndrome (AIDS), malignancies, cardiovascular diseases and so forth.

Structure of CRISPR-Cas

The CRISPR-Cas system is comprised of Cas gene family proteins and a CRISPR array, which consists of repeats, spacers, and a leader sequence. The leader sequence is positioned upstream of the CRISPR array and plays a role in initiating CRISPR transcription. Repeats are short repetitive sequences, typically ranging from 21 to 48 nucleotides in length, and they have the ability to form hairpin loops. The number of repeats can vary depending on the species, generally spanning from just a few to several hundreds. Spacers are approximately 26 to 72 nucleotides in length and are located between two repeat sequences (Grissa *et al.,* 2007). Typically, the coding sequence of the Cas gene is found in the upstream region of the CRISPR array. This coding sequence encodes a highly conserved nucleic acid-related Cas protein, which possesses various enzymatic activities such as nuclease, helicase, and nickase functions. Importantly, this Cas protein can specifically cleave DNA sequences. (Richter *et al.,* 2018).

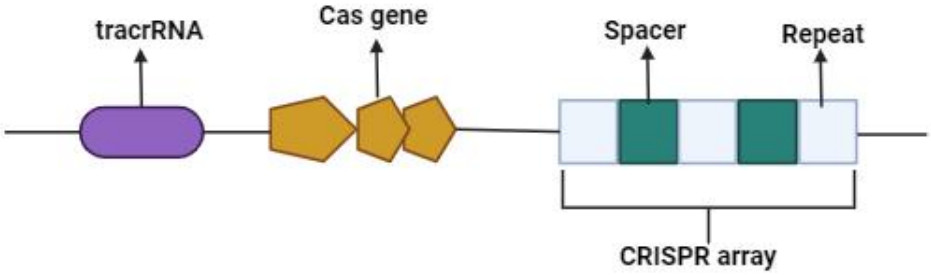

Fig. 1 Structure of CRISPR-Cas locus

Historical studies of CRISPR-Cas

The CRISPR-Cas system has its origins in the adaptive immune system that developed in bacteria and archaea over long periods of evolution. In 1987, a Japanese research group made a significant discovery when they identified a unique DNA sequence in the noncoding region of the alkaline phosphatase gene of *Escherichia coli* (Ishino *et al.,* 1987). This sequence consists of multiple repetitive DNA fragments arranged in a tandem fashion. In 2002, this DNA was initially termed "short regularly spaced repeats," and later, the name was modified to "clustered regularly interspaced palindromic repeats" (CRISPR) (Mojica *et al.,* 2013). In 2005, it was discovered that CRISPR spacer sequences exhibit significant homology with the DNA sequences of viruses or foreign plasmids. This finding suggested that CRISPR may have a specific function in defending against infection by foreign genetic material (Mojica *et al.,* 2005). In 2007, Barrangou and his colleagues observed that artificially modifying the repeats within CRISPR could regulate the immune response of *Streptococcus thermophilus* against specific phages. Through experiments, it was discovered that the CRISPR-Cas system has the ability to specifically recognize and capture exogenous gene fragments, forming a kind of "immune memory." When bacteria face re-infection by the same phage, the CRISPR-Cas system can then target and destroy the exogenous genes, providing the bacteria with resistance against that particular phage (Barrangou *et al.,* 2007). In 2012, Jinek and colleagues found that a single-guide RNA within the CRISPR-Cas system could effectively target specific DNA fragments. This discovery led to the proposal that the system could be harnessed for gene editing applications (Jinek *et al.,* 2012). In 2013, Cong and his team achieved a breakthrough by successfully utilizing the CRISPR-Cas system for targeted gene editing in animal genomes. This was a pivotal milestone in the development and application of CRISPR-Cas technology in genetic engineering (Cong *et al.,* 2013). Subsequently, the third-generation gene editing technology CRISPR-Cas was introduced and has found extensive use in various fields of molecular biology due to its notable technical advantages.

Mechanisms

Once bound to the single-guide RNA (sgRNA), the Cas9 endonuclease undergoes a conformational change that activates it to perform further functions. When a protospacer-adjacent motif (PAM) (5’ -NGG-30’) is present adjacent to the target site, Cas9 is directed to the desired location by sgRNA through base pairing between the CRISPR RNA (crRNA) and the target DNA. This leads to site-specific DNA double-strand breaks (DSBs). In the process of

DNA cleavage, the HNH domains cleave the DNA strand complementary to the 20-nucleotide sequence of the crRNA, while the RuvC-like domains cleave the DNA strand opposite to the complementary strand (Garneau *et al.,* 2013 & Jinek *et al.,* 2012). Subsequently, the DSBs induced by Cas9 trigger the endogenous cellular DNA repair mechanisms, resulting in targeted gene knockout, correction, or insertion. In many cases, the cell's genome will undergo self-repair through a process called non-homologous end-joining (NHEJ), which is error-prone and can lead to small mutations (Cong *et al.,* 2013). In contrast, homologous directed repair (HDR) is a more precise gene correction or replacement approach guided by an oligodeoxynucleotide template (Sander *et al.,* 2014).

For the CRISPR-Cas9 editing system to function, two conditions must be met: the presence of a DNA sequence complementary to the crRNA and the presence of a suitable PAM sequence. When these conditions are satisfied, CRISPR-Cas9 can technically edit nearly any DNA sequence. Importantly, since the target DNA site is determined by the 20-nucleotide sequence of the sgRNA through Watson–Crick base pairing (Jinek *et al.,* 2012), CRISPR-Cas9 genome engineering can edit a target sequence by reprogramming only the 20 nucleotides of a complementary sgRNA, rather than modifying the entire system. Additionally, CRISPR-Cas9 can edit different genomic loci with multiple sgRNAs, a capability known as multiplexing. These characteristics make the CRISPR-Cas9 system highly versatile and promising in the field of genome engineering, with a wider range of potential applications compared to earlier protein-guided genome editing tools.

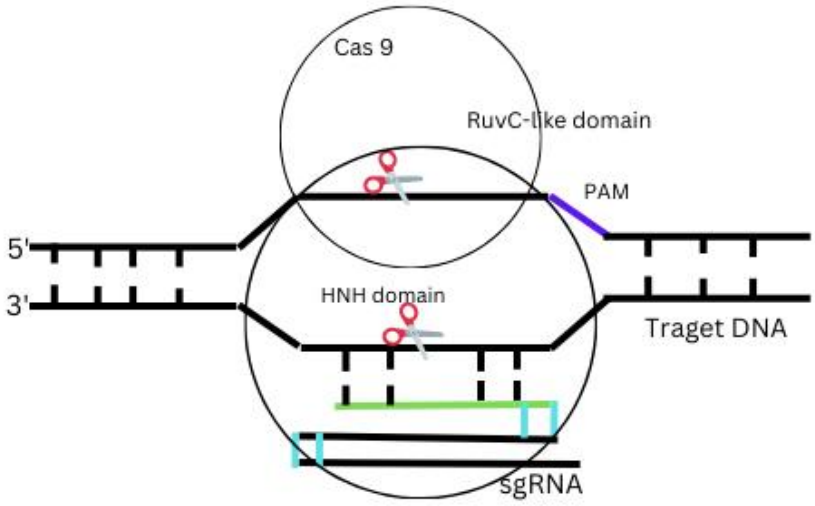

Fig. 2 Mechanism of CRISPR-Cas9

Therapeutic application of CRISPR-Cas9 for cancers

Cancer is generally regarded as a genetic disease that results from the accumulation of genetic or epigenetic mutations. CRISPR-Cas9, as a genomic editing tool, has the capacity to manipulate almost any target genomic sequence and correct cancer-causing abnormalities. This holds great promise for the treatment of cancer with minimal side effects (Xiao *et al.*, 2015). Initially, the CRISPR-Cas9 system primarily functioned as a detector to identify oncogenic factors, proto-oncogene mutations, and potential therapeutic targets. Previous research has unveiled a wealth of information about DNA mutations that lead to the promotion of uncontrolled cell proliferation, ultimately resulting in cancer. Based on these findings, the CRISPR-Cas9 system can be employed to knock out or edit target oncogenes as a treatment for malignancies. Researchers have utilized the CRISPR-Cas9 system to delve into the underlying mechanisms of tumorigenesis, identify potential therapeutic target genes, and address drug resistance in cancer treatment.

More importantly, numerous preliminary studies have been conducted to develop CRISPR-Cas9-based gene therapy against cancer. For instance, after the CD38 gene was knocked out via CRISPR-Cas9, A549 human adenocarcinoma cell lines exhibited reduced anchorage-independent cell proliferation and invasion, along with significantly slower xenograft growth in nude mouse models (Bu *et al.*, 2018). In a cellular model of bladder cancer generated through CRISPR-Cas9 genome editing, researchers identified the

function of MPT0L145 as a dual inhibitor of PIK3C3 and the FGFR pathway, unveiling a novel therapeutic strategy by promoting bladder cancer cell death (Chen *et al.*, 2018). Many similar studies, based on CRISPR-Cas9 gene editing technology, have been conducted to knock out proto-oncogenes both in vitro and in vivo, showcasing the therapeutic potential for treating various tumors.

In addition to correcting gain-of-function mutations in oncogenes, another important therapeutic strategy for cancer involves addressing loss-of-function mutations in tumor suppressor genes, a concept that was recognized back in 1986 (Friend *et al.*, 1986). Normally, tumor suppressor genes play a critical role in inhibiting tumor progression. However, mutations or loss of these genes can lead to the development of malignant tumor cells (Kodama *et al.*, 2000). In recent years, several studies have utilized CRISPR-Cas9 gene editing to repair mutations or induce overexpression of tumor suppressor genes, offering a novel approach to treating cancer. Cancer can, to some extent, be attributed to defects in immunosurveillance or the strengthening of self-tolerance to cancer antigens. Consequently, immunotherapy, including checkpoint inhibitors, vaccines, and cell therapy, has become an essential part of cancer treatment and has made significant advances in recent years. Chimeric antigen receptor-engineered T cell (CAR-T) therapy stands out as a potent and promising cell-based therapeutic approach (Garfall *et al.*, 2015). Importantly, CRISPR-Cas9 technology provides a simple and relatively efficient method for manipulating CAR-T cells. CRISPR-Cas9-edited CAR-T cells exhibit enhanced T cell functionality and improved efficacy in immunotherapy. For instance, Eyquem and colleagues directed a CD19-specific chimeric antigen receptor into the TRAC locus using CRISPR-Cas9, resulting in enhanced T cell potency that outperformed conventionally generated CAR-T cells. CRISPR-Cas9 technology has also been used to simultaneously knock out TRAC and B2M genes to generate universal CAR-T cells. Furthermore, multiplex genome editing with CRISPR-Cas9 in CAR-T cells can create gene-disrupted allogeneic CAR-T cells. CRISPR-Cas9 can also be applied to knock out inhibitory checkpoint molecules in T cells, such as programmed cell death protein 1, lymphocyte activation gene 3 protein (LAG-3), and cytotoxic T lymphocyte protein 4, thereby enhancing cytotoxicity against tumor target cells (Huang *et al.*, 2017).

Finally, CRISPR-Cas9 technology can be employed to treat or prevent cancer by targeting mutations caused by oncogenic viruses. As mentioned earlier, there is a close relationship between oncogenic viruses and carcinogenesis. An analysis of 12.7 million cancer cases in 2008 revealed that pathogen infection was a significant factor in cancer development, responsible

for approximately 16.1% (around 2 million) of cancer incidence (de Martel *et al.,* 2012). Therefore, clearing or inactivating these viruses represents a novel and essential therapeutic strategy that can potentially disrupt and even reverse tumorigenesis.

Therapeutic application of CRISPR-Cas9 in genetic diseases

With the continued improvement of CRISPR-Cas9 technology, its potential for protective and therapeutic applications has grown significantly. Remarkable advances in CRISPR-Cas9 technology have made it a more efficient, versatile, and user-friendly tool for genome editing. To date, CRISPR-Cas9 has been widely employed in addressing genetic diseases, including conditions such as Duchenne muscular dystrophy, α1-antitrypsin deficiency, hemophilia, hearing loss, and hematopoietic diseases. Take hematopoietic diseases as an example. β-thalassemia, one of the most common genetic diseases worldwide, is caused by inherited point mutations or small deletions in the human β-globin (HBB) gene (Fan *et al.,* 2012). β-thalassemia patients fail to produce sufficient functional β-hemoglobin, leading to a range of clinical features from asymptomatic cases to severe anemia. Similarly, sickle cell disease (SCD) is also caused by mutations in the HBB gene and is characterized by abnormal hemoglobin and malfunctioning erythrocytes (Orkin *et al.,* 2010). In the past, treating hematologic diseases through genome manipulation was extremely challenging due to the complexity of designing targeting vectors (Gonzalez *et al.,* 2019). Furthermore, previous research on therapeutic genetic correction strategies failed to achieve satisfactory long-term therapeutic effects (Chang *et al.,* 2017).

The advent of CRISPR-Cas9 has brought about a revolutionary transformation in the field of genome engineering, enabling the effective modification of pathogenic genes and the cure of genetic diseases. Recent studies demonstrate that genetic errors in hematopoietic diseases can be corrected through CRISPR-Cas9-based hematopoietic stem and progenitor cell (HSPC) transplant therapy (Hultquist *et al.,* 2016). Correcting pathogenic mutations in induced pluripotent stem cells (iPSCs) can restore normal hemoglobin function and provide a rich source of cells for transplantation, now considered the definitive treatment for β-thalassemia and sickle cell disease (SCD). Correcting the HBB mutation with CRISPR-Cas9 is one of the most promising strategies for curing hematopoietic diseases and has been successfully carried out in patient-derived iPSCs without leaving any residual footprint in several studies. For instance, Park *et al*. employed the CRISPR-Cas9 system along with a single-stranded oligonucleotide to correct the HBB

gene mutation in HSPCs derived from peripheral blood or bone marrow of SCD patients. The results indicated a significant reduction in sickle cells, with the level of normal hemoglobin increasing to (25.3 ± 13.9) % (Park *et al.*, 2019). Additionally, Song *et al*. assessed the hematopoietic differentiation efficiency of iPSCs corrected with CRISPR-Cas9 technology, reporting a substantial increase in embryoid body formation and various hematopoietic progenitor cell percentages (Song *et al.*, 2015).

Furthermore, the BCL11A gene plays a pivotal role in the switch from fetal to adult hemoglobin and serves as a suppressor of fetal hemoglobin expression. Given its significance in hematopoietic stem cell functions and lymphoid development, targeted disruption of the BCL11A erythroid-specific enhancer is a natural therapeutic strategy for β-thalassemia and SCD. Several studies have demonstrated that increased fetal hemoglobin can alleviate the clinical symptoms of these diseases. Khosravi *et al*. applied CRISPR-Cas9 technology to delete a 200 bp segment of the BCL11A erythroid enhancer, including the GATAA motif, in K562 cell lines, resulting in a disruption of BCL11A expression. This research showed a significant increase in γ-hemoglobin, highlighting the therapeutic potential of CRISPR-Cas9 as a relatively precise genome editing tool for treating β-thalassemia. These studies represent a significant step toward the further application of stem cell-based gene therapy for monogenic diseases in clinical practice (Khosravi *et al.*, 2019).

Therapeutic application of CRISPR-Cas9 for infectious diseases

Oncogenic virus

Numerous viral infections, such as hepatitis B virus (HBV), Epstein–Barr virus (EBV), and human papillomavirus (HPV), are closely associated with carcinogenesis, leading to conditions such as liver cancer, nasopharyngeal carcinoma, and cervical cancer, respectively. Currently, CRISPR-Cas9-mediated genome editing has been trialed to inactivate the oncogenic viral genome or inhibit virus replication. Among these oncogenic viruses, HBV infection is the most common chronic viral infection, affecting over 250 million individuals. HBV is a known cause of hepatocellular carcinoma and is ranked as the 10th leading cause of death according to the 2010 Global Burden of Disease Study (Lozano *et al.*, 2010). Following infection, the double-stranded covalently closed circular DNA (cccDNA) serves as a template for the production of pregenomic RNA and poses a significant obstacle to the complete eradication of chronic hepatitis B. Current therapies can only suppress HBV replication but are unable to eliminate cccDNA. CRISPR-Cas9

technology introduces a novel approach to clear cccDNA and prevent hepatocellular carcinoma. Lin *et al*. conducted the first application of the CRISPR-Cas9 system to cleave HBV genomes, resulting in a significant reduction in the production of HBV core and surface proteins in Huh-7 cells transfected with an HBV expression vector. Furthermore, this system could cleave the intrahepatic HBV genome-containing plasmid and facilitate its disruption in vivo, leading to reduced serum surface antigen levels in mice (Lin *et al.,* 2014).

Subsequent research further demonstrated the therapeutic potential of CRISPR-Cas9 in disrupting cccDNA both in vitro and in vivo. Recently, CRISPR-Cas9 systems derived from *Streptococcus pyogenes* (Sp) and *Streptococcus thermophilus* (St) were used to block HBV replication, resulting in the degradation of over 90% of cccDNA six days after transfection. Importantly, off-target mutagenesis was compared between Sp and St CRISPR-Cas9 through deep sequencing, and the results indicated that the St CRISPR-Cas9 system could be an ideal candidate for the development of an HBV cure with minimal off-target nucleolytic activity (Kostyushev *et al.,* 2019).

In addition to HBV, CRISPR-Cas9 can also be applied to combat numerous other oncogenic viruses such as EBV and HPV. EBV can etiologically cause conditions like Burkitt's lymphoma, nasopharyngeal carcinoma, and several other human cancers. Wang *et al*. utilized the CRISPR-Cas9 tool on Burkitt's lymphoma patient-derived cells with latent EBV infection, resulting in the elimination of the EBV genome in a quarter of the cells and a concomitant decrease in viral load in half of the cells (Wang *et al.,* 2014). Similarly, Yuen *et al*. designed gRNAs targeting the EBV genome and transfected them into C666-1 cells, resulting in a 50% reduction in the levels of EBV DNA in the transfected cells (Yuen *et al.,* 2019). Although the suppression of EBV did not improve the survival of C666-1 cells, CRISPR-Cas9 sensitized them to chemotherapeutic killing by cisplatin and 5-fluorouracil, suggesting a potential new strategy to sensitize EBV-infected nasopharyngeal carcinoma cells to chemotherapy. High-risk HPV infection may lead to the development of cervical carcinomas and is also the causative agent of several other head and neck cancers. However, there is currently a lack of effective specific anti-HPV drugs. Disrupting the HPV genome using the CRISPR-Cas9 tool is one of the most promising antiviral therapeutic strategies. Kennedy *et al*. used the CRISPR-Cas9 tool to induce inactivating deletion and insertion mutations into the E6 or E7 gene, leading to cell cycle arrest and eventual cancer cell death in both HPV-16 and HPV-18 transformed cells (Kennedy *et al.,* 2014). In another study, Lao *et al*. non virally delivered

CRISPR-Cas9 with F127/PPO-NMe3/pCas9 micelle to disrupt the HPV E7 oncogene both in vitro and in vivo (Lao *et al.*, 2018). The results demonstrated that Cas9-mediated E7 knockout led to significant inhibition of HPV-induced cancerous activity with low off-target effects.

HIV

In the human immunodeficiency virus (HIV)-1 lifecycle, HIV DNA is reversely transcribed into the host cell genome, becoming the fundamental source of viral proteins as a latent reservoir. This reservoir poses a significant challenge in efforts to eradicate HIV-1 from infected individuals (Finzi *et al.*, 2014). Highly active antiretroviral therapy (HAART) is the current primary method for controlling HIV-1 replication and has transformed AIDS into a manageable chronic disease. However, HAART therapy cannot completely eliminate the HIV virus or target latent infections. AIDS patients still bear the burden of lifelong treatment and the ultimate incurability of the disease (Zaccarelli *et al.*, 2014). As a result, there is an urgent need to develop a permanent and effective antiviral therapy to eliminate latent HIV infections.

Ebina *et al*. were the first to report the application of CRISPR-Cas9 to manipulate HIV-1 integrated proviral DNA in HIV-1 long terminal repeat (LTR) expression-dormant and -inducible T cells. By transfecting gRNA and Cas9 targeting the HIV-1 LTR sequence, the system significantly suppressed latent HIV-1 provirus expression, demonstrating the therapeutic potential of the HIV-LTR targeting CRISPR-Cas9 system for eliminating HIV latency (Ebina *et al.*, 2013).

As of now, the CC chemokine receptor 5 (CCR5) gene, a major co-receptor for HIV and a potential target for AIDS treatment, is considered one of the most promising gene therapy targets for AIDS (Yukl *et al.*, 2014). Transplanting stem cells with homozygous CCR5 mutations into AIDS patients can result in a loss of detectable HIV-1 burden. This approach has been extensively studied in human primary T cells and CD34+ hematopoietic stem and progenitor cells (HSPCs). Cho *et al*. used the CRISPR-Cas tool to induce approximately 33% mutation at the CCR5 locus in human cells (Cho *et al.*, 2013). Later, Mandal *et al*. applied CRISPR-Cas9 in CD34+ HSPCs, achieving a biallelic inactivation frequency of up to 34% with low off-target mutagenesis (Mandal *et al.*, 2014). Importantly, the CCR5-edited CD34+ HSPCs retained multilineage potential both in vitro and in vivo. Furthermore, genome editing efficiency can be enhanced by co-delivering chemically modified sgRNAs with Cas9 mRNA or protein in both human primary T cells and CD34+ HSPCs, without significant toxicity associated with DNA delivery.

Recently, the HIV-1 prevention effect was evaluated through CRISPR-Cas9-mediated CCR5 disruption in long-term reconstituted mice and secondary transplanted repopulating HSCs. The results demonstrated robust CCR5 disruption in mice, along with a remarkable antiviral effect in vivo, opening the path for the clinical translation of CCR5 gene-edited HSCs as a potential cure for HIV infection. It's worth noting that while CCR5 mutation can confer resistance to CCR5-utilizing viruses, HIV-1 can still infect cells via the CXCR4 pathway and several other pathways. Consequently, CRISPR-Cas9 was also employed to disrupt the CXCR4 gene in human primary CD4+ T cells. The modified cells exhibited resistance to infection by X4 tropic HIV-1, with an efficacy of more than 40% mutagenesis (Hou *et al.*, 2015). To prevent HIV-1 infection through CD4 and CCR5 or CXCR4 independent methods, simultaneous manipulation of CCR5 and CXCR4 via CRISPR-Cas9 was further investigated in various cell lines. The results showed a significant decrease in HIV-1 infection in edited cells compared to unmodified cells, without any apparent non-specific editing or cytotoxicity (Yu *et al.*, 2018). Very recently, Dash *et al*. achieved viral clearance in HIV-1 infected humanized mice by employing sequential long-acting slow-effective release antiviral therapy together with CRISPR-Cas9 (Dash *et al*., 2019). Nested and digital droplet PCR, as well as RNAscope analysis, revealed no detectable HIV-1 virus in blood, lymphoid tissue, bone marrow, and brain. This marks the first successful HIV-1 clearance in humanized mice, representing a crucial step in the further development of gene therapy for AIDS.

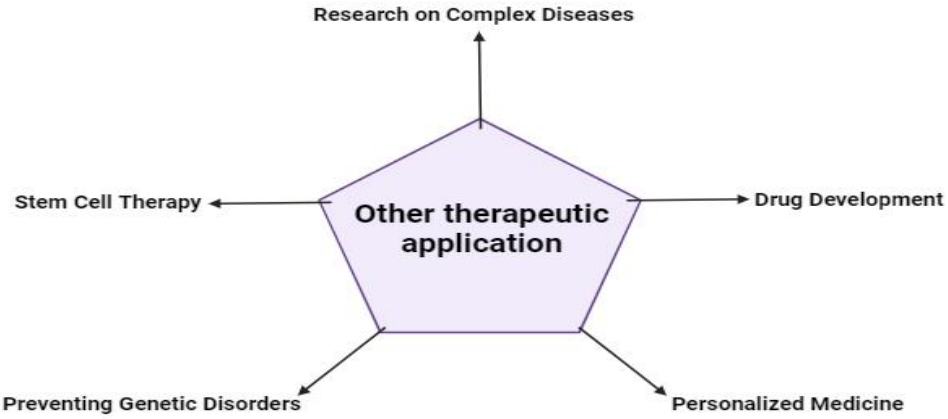

Fig.3 Other therapeutic application of CRISPR-Cas

Conclusion

The CRISPR-Cas9 gene-editing system stands as a ground breaking technology with transformative potential in the realm of medicine and molecular biology. Its evolution from a bacterial defense mechanism to a versatile tool for precise DNA editing has unlocked a myriad of therapeutic applications across various domains. In the field of genetic diseases, CRISPR-Cas9 offers unprecedented opportunities for targeted correction of mutations responsible for debilitating conditions. Its application in correcting genetic anomalies associated with diseases like Duchenne muscular dystrophy, α1-antitrypsin deficiency, and diverse hematopoietic disorders suggests a promising future for personalized and curative treatments. In the battle against infectious diseases, CRISPR-Cas9 has exhibited its potential to target and disable viral infections, including oncogenic viruses such as hepatitis B, Epstein-Barr virus, and human papillomavirus. By disrupting viral genomes and reducing replication, it introduces a new approach to potentially eliminate these infections and their associated risks. Furthermore, in the fight against cancer, CRISPR-Cas9 has emerged as a versatile tool for precisely targeting oncogenes, tumor suppressor genes, and other cancer-related mutations. It shows promise in enhancing existing therapies and developing novel, more targeted treatments for different types of cancers. The continuous evolution and advancements in the CRISPR-Cas9 system underscore its ongoing potential for revolutionizing medical treatments. However, challenges such as off-target effects, ethical considerations, and regulatory frameworks need to be addressed for its widespread clinical application. Overall, CRISPR-Cas9's remarkable versatility, precision, and potential for tailored, effective treatments indicate a future where this technology may significantly impact healthcare, offering hope for addressing previously challenging medical conditions and unlocking novel therapeutic avenues. Its journey from a bacterial immune system to a transformative therapeutic tool represents a testament to the power of innovation in advancing healthcare and molecular research.

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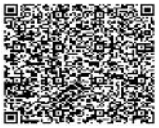	Subject: Gene therapy
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Siderophores – Booster for the biological systems

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Abstract

Siderophores are group of chemical substances produced by a variety of microorganisms in a stressed environmental conditions. These siderophores are having numerous applications, which is useful to living conditions of microorganism, plants and even human beings. They are acting as biocontrol agents, antimalarial substance, to treat Beta- thalassemia and cancer.

Key words: siderophores, hydroxamate, antimalarial, iron

Introduction

Iron is a necessary element for numerous biological activities, including the electron transport chain and as a cofactor for many enzymes (Litwin and Calderwood, 1993). Microorganisms thriving in aerobic circumstances require iron for a range of tasks, including oxygen reduction for ATP production, heme creation, and other critical processes. Plants use siderophores to help with respiration (Aznar and Dellagi, 2015), photosynthesis (Nagata *et al*., 2013), bioremediation (Saha *et al*., 2016), plant growth promotion (Yadav *et al*., 2011; Ghazy and ElNahrawy, 2021), and phytoremediation of heavy metals (Kong and Glick, 2017). Non-ribosomal peptide connections also generate siderophores (Hu and Xu, 2011), as do multidentate iron-chelating compounds that solubilize and chelate organic and inorganic forms of chemicals in soil (Singh *et a*l., 2017). Siderophore are low molecular weight (10 KD) iron chelating chemicals generated in significant quantities by numerous bacteria, including *Pseudomonas, Azotobacter, Bacillus, Enterobacter, Serratia, Azospirillum*, and *Rhizobium* (Glick *et al*., 1999, Loper *et al*., 1999). Siderophores form aggregates with free iron and transport it into the cell via membrane receptor molecules, which are produced by five genes in an operon that is shut off when enough iron has been absorbed into the cell (Lewin, 1984). Some bacteria make one or more siderophores, which can be used by

other microorganisms to acquire iron and other metals. This feature of siderophores boosted its use; also, siderophores have been connected with virulence processes in microorganisms that are detrimental to both animals and plants. They also have therapeutic, agricultural, and environmental uses. Currently, almost 500 siderophores have been described from various bacteria. The structure of the siderophore varies greatly between species. There are three types of siderophores: hydroxamate, catacholate, and carboxalate. These siderophores can be synthesized in liquid and/or solid environments in the laboratory. As a result, they may be discovered and recognized using various approaches. This work discusses the detection of several siderophores in microbes, as well as their interactions with metals such as Fe, Ni, Cd, Cu, and Zn. It is also explored how to use siderophore-producing microorganisms or siderophore-containing microbial preparations to improve plant development, enhance metalclean-up in contaminated areas and applications for medical purposes.

Siderophores: Types and Mechanism of action

The term comes from the Greek words sidero, which means "iron," and phore, which means "carriers" or iron-bearing compounds that absorb insoluble iron from numerous environmental sources (Nagoba andVedpathak, 2011).Based on the functional groups that bind to Fe^{3+} and the various topologies of siderophores, siderophores may be categorized into three major types (Wang *et al.*,2013): hydroxamate siderophores, catecholate siderophores, and carboxylate siderophores. Hydroxamate siderophores are the most common siderophores generated by bacteria and fungi. The fungal hydroxamic type is formed of hydroxylated and acylated ornithine groups, whereas the bacterial hydroxamic type is composed of hydroxylated and acylated alkyl amines (Chowdappa *et al*.,2020). Bacteria produce catechol. This siderophore is lipophilic, has a high affinity for iron, and can resist pH fluctuations in the environment. Furthermore, catecholate siderophores may be used as a powerful reducing agent to more effectively compete for iron with pathogenic fungus. Few bacteria are capable of producing carboxylate siderophores. Different siderophoreschelators chelate Fe^{3+} in different ways. Carboxylate, hydroxamate, and catecholate kinds are the most efficient Fe^{3+} chelators (Boukhalfa *et al*., 2002). Among the siderophores of the same functional group, the iron-chelating capacity of fungal siderophores is somewhat lower than that of bacterial siderophores (He *et al*., 2020). Metal ions in the soil have a large impact on the synthesis of siderophores. The concentration of soluble iron is the primary factor of siderophores production. While iron shortage is the

most important component in siderophore formation, other parameters such as carbon supply, nitrogen source, pH, and temperature are also important. Carbon supply influences not only cell growth but also siderophore production. According to Bendale *et al.,* maximal siderophore synthesis could only be accomplished with sucrose, but glycerol was the preferred carbon source for B. megaterium growth and siderophore production (Yu *et al.,* 2007)The influence of pH on siderophore synthesis is also critical (Yu *et al.,* 2007, Sebastian *et al*.,2017), since pH regulates iron solubility and availability for organism development. A neutral pH is generally preferable for most bacteria and fungi to produce the most siderophores (Ellermann *et al.,* 2017) Metal ions, in addition to environmental influences, have Iron-binding proteins like spermeases and ATPases are produced by siderophore-producing bacteria in gram-positive bacteria. These proteins chelate ferric iron (Fe^{3+}) and move Fe^{3+} ions throughout the cell membrane (Ahmed and Holmstrom, 2014). Bacterial species are the most widespread producers of catecholate forms of siderophores (Dave *et al.,* 2006). *Escherichia coli, Salmonella typhimurium*, and *Klebsiella pneumoniae* are the most prevalent bacterial species that create enterochelin subtypes of catecholate kinds of siderophore synthesis (Dertz *et al.,* 2006). Under iron-limiting circumstances, the bacterial species *Azotobacter vinelandii* produces a variety of catecholate siderophores, including monocatecholate aminochelin, dicatecholate azotochelin, and tricatecholate protochelin (Wittmann *et al.,* 2001).

Production of Microbial siderophores:

The initial stage in the manufacture of siderophores is the fabrication of a Fedeficient cell growth medium. To that aim, all glassware and utensils are cleaned in acid (e.g., 6M HCl) overnight, thoroughly rinsed in distilled water, and autoclaved before use. Glassware acid-rinsing aids in the removal of residual Fe contamination that may interfere with siderophore synthesis. A typical liquid growth media for siderophore formation is detailed step by step below as siderophore-inducing medium (SIM). This medium would be devoid of added Fe, yet Fe might be present as an impurity from the other reagents. As a result, the greater the purity grade of the chemicals utilized, the better the siderophore induction outcome. For control, 100 M Fe (e.g., $FeCl_3$) is generally enough to prevent siderophore production in most bacteria and fungi.

PIPES [piperazine-N,N′bis(2-ethanesulfonic acid)] is dissolved in 750 ml of a salt solution including KH_2PO_4 (0.3 g), NaCl (0.5 g), and NH_4Cl (1.0 g) in the SIM preparation process for liquid microbial cultures described by Alexander and Zuberer (1991). Due to the necessity to eliminate Cl in investigations using Ag, where the production of AgCl precipitates is undesired,this solution has been modified to replace NaCl and NH_4Cl with $NaNO_3$ and $(NH_4)_2SO_4$, respectively. To remove Fe in an earlier comparable medium used for detecting siderophore in ectomycorrhizal fungi, 8-hydroxyquinoline was added followed by chloroform extraction (Szaniszlo *et al.* 1981). Other modest changes in component chemical concentrations have also been observed (e.g. Oliveira *et al*. 2006). With an alkali (50% w:v in H_2O), the pH of SIM solution is adjusted to 6.8, and the volume is increased to 800 ml by adding distilled H_2O. This solution is known as solution-1. Following this is the preparation of a Fe-free trace elements solution (solution-2; final volume 70 ml) that would comprise of glucose (2.0 g), mannitol (2.0 g), $MgSO_4.7H_2O$ (493.0 mg), $CaCl_2$ (11.0 mg), $MnSO_4.H_2O$ (1.17 mg), H_3BO_3 (1.4 mg), $CuSO_4.5H_2O$ (0.04 mg), $ZnSO_4.7H_2O$ (1.2 mg) and $Na_2MoO_4.2H_2O$ (1.0 mg). To make it easier to measure out the chemicals in extremely small amounts, the amounts can be scaled up as needed, but with a corresponding increase in the ultimate volume of the solution. The first and second solutions are then autoclaved. The third step is to prepare casamino acids (10% w:v) in distilled H_2O (solution 3) by dissolving 3 g of the casamino acids in 27 ml of water thoroughly on an orbital shaker. To prevent damaging the proteins, the casamino acid solution is normally not autoclaved; nevertheless, it is filtered (0.2 m) to remove bacterial contamination. Finally, solutions 2-3 are combined with solution 1 to make the SIM (pH 6.8). At this stage, Fe can be introduced as a control to a subset of the flasks. Depending on the study, other changes may be made. Tryptophan, for example, can be added to the medium to stimulate the formation of both siderophores and auxins (Dimkpa*et al.* 2012).

To facilitate siderophore generation, bacterial cells (108 cfu/ml; roughly OD 600nm = 0.1) or fungal mycelial plugs are seeded in the SIM over time for microbial cuture. Samples can be collected at defined intervals and analyzed for siderophore generation to gather information on the kinetics of siderophore production. To do this, the cells are centrifuged, and the supernatants are filtered (0.2 m) and either utilized immediately or frozen for later assessment of siderophore synthesis. Siderophores generated by bacteria or fungus during development in liquid or solid cultures can be detected using a variety of approaches. Chrome azurol S (CAS) is a common technique that is applicable to all siderophore kinds. Other techniques are more specific for

distinct siderophore types and are thus employed following CAS detection to specify the kind of siderophores generated. Colorimetric, spectroscopic, fluorescence, and chromatographic procedures are the most common.

Characterization of siderophores

The kind of siderophore was established based on the characteristics of siderophores in the fermentation broth. As seen, a shade of pink developed following the response in the Csaky test, confirming that these siderophores were of the hydroxamate type. The other two kinds were not found. Fungi and a few gram-positive bacteria from genera such as *Bacillus*, *Arthrobacter*, and *Nocardia* are known to manufacture such hydroxamate-type Siderophores.*Bacillus* sp. PZ-1 generated 32.24 g/ml hydroxamate-type siderophores in the fermentation broth, according to a hydroxylamine hydrochloride standard curve(Chakraborty *et al.*,2006).

Quantification of siderophores

Siderophore type and quantitative investigations were performed on the culture supernatant. (Csaky 1948) 0.5 ml of supernatant was combined with 0.5 ml of 6 mol/l H_2SO_4 and autoclaved at 121ºC for 30 minutes before being mixed with 1 ml of 1% (w/v) sulfanilic acid in 30% (v/v) acetic acid and 0.5 ml of 1.3% (w/v) iodine for 5 minutes. Then, a solution of -naphthylamine was added, and the emergence of a pink hue showed the presence of a hydroxamate-type siderophore. As negative and positive controls, distilled water and hydroxylamine hydrochloride were used. Arnow's test (Arnow,1937) was used to determine the catecholate type. In brief, 1 ml of HCl was added to 1 ml of culture supernatant, followed by 1 ml of nitrite-molybdate, and the shift in yellow hue to orange red indicated the production of a catecholate-type siderophore. Distilled water and catechol were used as negative and positive controls, respectively. A spectrophotometric test (Shenker *et al.*, 1992) was performed for carboxylate-type. In brief, 1 ml of culture supernatant was combined with 1 ml of 250 mol/l $CuSO_4$ and 2 ml of pH 4.0 acetate buffer, and the presence of a copper complex at 190-280 nm showed the existence of a carboxylate-type siderophore.

Application of siderophores

Siderophores in soil remediation

Soil metal contamination is a major continuing environmental hazard. While numerous solutions may be used to combat metal contamination, the employment of biological (as opposed to synthetic) approaches is being pushed (White 2001; Ullah*et al*., 2015). As a result of their affinity for a variety of metals, siderophores can be used to eradicate or reduce the presence of certain metals in polluted settings. In one case, Cd, Zn, and Pb bioavailability was enhanced by siderophore solubilisation of these metals and subsequent adsorption to biomass, which was subsequently separated from soil slurry by flocculation, resulting in lower soil concentrations (Diels *et al*. 1999).An additional instance of using siderophores to detoxify metal contamination was the use of a siderophores-producing strain of *Kluyveraascorbata* to reduce Ni toxicity in plants growing in Ni contaminated soil. Although bacteria-treated plants grew faster in the presence of Ni than untreated plants, both plants absorbed the same amount of Ni. Plant development was enhanced, however, by enabling the acquisition of sufficient Fe in the presence of inhibitory Ni levels (Burd*et al*. 1998, 2000; Dimkpa*et al*., 2008).

Cancer treatment

Dexrazoxane, O-trensox, desferriexochelins, desferrithiocin, and tachpyridine are examples of siderophore possibilities employed as iron chelators in cancer therapy (Miethke and Marahiel, 2007). To eliminate non-transferrin bound iron in serum, several chemotherapies uses siderophores (Chua *et al.,* 2003).

Siderophores as Biocontrol agent

Many bacteria restrict the growth of potentially harmful microorganisms through the release of siderophore, antibiotics, and cyanide (Edi Husane, 2005). *Phytophthoraparasitica* (Seuk*et al*., 1988*), Phythiumultimum* (Hamdan*et al*., 1991*), Fusariumoxysporumveridianthi* (Buysens*et al*., 1996), and *Sclerotiniasclerotiorum* (McLoughlin*et al.,* 1992) are all growth inhibitors of siderophores. Loepper*et al*. (1980) were the first to establish the relevance of siderophore synthesis as a biological control mechanism of *Erwiniacarotovora* by numerous plant-growthpromoting *Pseudomonas fluorescens* strains A1, BK1, TL3B1 and B10.

Siderophores acts as Anti-malarial substance

Some siderophores have been discovered to be effective in the treatment of *Plasmodium falciparum*causing malaria. *Klebsiella pneumoniae* produces a siderophore that acts as an antimalarial drug (Gysin *et al.,* 1991). Desferrioxamine B, which is generated by Streptomycespilosus (and is now being synthesized chemically), is active against *P. falciparum* both in vitro and in vivo. The siderophore penetrates the *P. falciparum* cell and depletes the intracellular iron. The same siderophore has been found to limit the growth of *Trypanosomabrucei*, another protozoic parasite that causes sleeping sickness in humans (Breidbach*et al.,* 2002).

Treatment of β –thalassemia using siderophores

Periodic whole blood transfusions are necessary in the treatment of β-thalassemia and some other anemias (Hershko *et al.,* 2002). Because there is no special physiological mechanism for iron elimination in humans, ongoing transfusion treatment results in a constant accumulation of iron. These iron excesses, as well as primary iron overload illnesses like hemochromatosis and hemosiderosis, and accidental iron poisoning, need iron elimination from the body, particularly the liver. Such diseases can be effectively treated using siderophore-based drugs, with the siderophore serving as the primary model (Pietrangelo, 2002).Desferrioxamine B has also been used to treat a variety of clinical diseases caused by aluminum overload. This hazardous metal commonly accumulates in persistently dialyzed patients who have lost the capacity to remove by renal elimination. Desferrioxamine B has also been suggested for the identification of this type of overload situation(Ackrill*etal*., 1980).

Conclusion

Siderophores are naturally occurring biopolymers which can be chemically synthesized whenever it is needed. They play a pivotal role in the development of plants as growth promotes and in human they used to treat cancer, Malaria, β – thalassemia.

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Modern Agriculture in Pest Control Management: A Comprehensive Overview

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Abstract

Amidst the complexities of contemporary agricultural challenges, the time-tested practices of crop rotation and diversity emerge as guiding beacons. With roots deeply embedded in ancestral wisdom, they intertwine seamlessly with the goals of modern agriculture: to sustainably nourish an ever-expanding global populace and preserve the planet's well-being. These practices evoke harmony between human cultivation and the intricate web of life on Earth, paving the way for a future where agricultural endeavors and ecological integrity are not opposing forces but harmonious partners. As humanity perpetually seeks innovation and adaptation, the enduring significance of crop rotation and diversity remains unwavering. They illuminate a path towards equilibrium between human ambitions and the sanctity of the natural world. In this journey towards harmonious coexistence, crop rotation and diversity serve as an inspiration, embodying the delicate balance that lies at the heart of sustainable agriculture. In a world where each harvest echoes with responsibility, these practices resonate as hopeful echoes of unity with the vibrant tapestry of life.

Introduction

In the ever-evolving landscape of agriculture, the role of pest control management has undergone a significant transformation. The conventional methods of pesticide application have given way to more sustainable and ecologically-friendly practices (1). This shift towards modern agriculture techniques in pest control management not only ensures higher yields but also safeguards the environment and human health. In this comprehensive blog post, we will delve into the various aspects of modern pest control management in agriculture (2).

Integrated Pest Management (IPM)

Integrated Pest Management (IPM) has emerged as a cornerstone of modern agriculture's approach to pest control. Unlike traditional methods that heavily rely on chemical pesticides, IPM integrates a variety of tactics to manage pests effectively. These tactics include biological control, cultural practices, and the targeted use of pesticides when necessary. By carefully monitoring pest populations and employing a holistic strategy, farmers can reduce the reliance on harmful chemicals while maintaining crop health (3)

Biological Control

Modern agriculture embraces the concept of using nature to control pests through biological control methods. This involves introducing natural predators, parasites, or pathogens that target specific pests. For instance, releasing ladybugs to combat aphids or using nematodes to control soil-dwelling insects. Biological control not only reduces the need for chemical pesticides but also maintains a more balanced ecosystem within agricultural landscapes (4)

In the quest for sustainable and environmentally friendly pest control solutions, modern agriculture has turned to nature itself. Biological control, a pillar of Integrated Pest Management (IPM), offers a natural alternative to chemical pesticides. By leveraging the power of predators, parasites, and pathogens, biological control has emerged as a pivotal strategy in reducing pest populations while preserving ecological balance. This article delves into the concept of biological control, its mechanisms, benefits, and applications in the realm of pest management.

Understanding Biological Control

Biological control involves the introduction, augmentation, or manipulation of natural enemies to suppress pest populations and mitigate crop damage. Unlike conventional pesticides, which can have unintended consequences for non-target organisms and the environment, biological control focuses on targeted and sustainable solutions (5)

Mechanisms of Biological Control

Predation: Predators are organisms that consume pest species. Ladybugs, lacewings, and predatory mites are examples of beneficial insects that feed on a range of pests, such as aphids and spider mites (6). These predators help keep pest populations in check, preventing outbreaks.

Parasitism: Parasitic organisms lay their eggs on or inside pest hosts. Once hatched, the parasites consume the host, effectively reducing its numbers. Parasitic wasps are a classic example, laying eggs inside caterpillars or aphids, leading to their eventual demise (7)

Pathogens: Microbial pathogens, such as bacteria, fungi, and viruses, can be used as biological control agents. These pathogens infect pests and cause diseases that lead to their death. *Beauveria bassiana* and *Bacillus thuringiensis* (Bt) are well-known examples of microbial agents used in biological control (8)

Benefits of Biological Control

Reduced Chemical Dependency: One of the primary advantages of biological control is its potential to reduce the reliance on chemical pesticides. This results in safer food, improved soil health, and a healthier environment (9)

Targeted Action: Biological control agents tend to be specific to certain pest species, minimizing harm to beneficial insects and other non-target organisms. This precision enhances ecological balance (10)

Long-Term Effectiveness: Once established, natural enemies can provide long-term pest control. They adapt to changing conditions and can suppress pest populations over multiple seasons (11)

Preventing Resistance: Pests can develop resistance to chemical pesticides over time. Biological control offers an alternative approach that doesn't contribute to the development of resistant pest populations (12)

Applications of Biological Control (13)

Agricultural Crops: Biological control is widely used in various crops, including fruits, vegetables, and grains. For instance, releasing parasitic wasps to control whitefly infestations in greenhouse tomatoes is a common practice.

Forestry: In forestry, biological control agents can combat invasive insect species that harm trees, such as the use of parasitoid wasps to control the pine sawfly.

Urban Landscapes: Biological control has applications beyond agriculture, including urban gardens and parks, where natural enemies can be introduced to manage pests without the need for chemical interventions.

Crop Rotation and Diversity (14)

Crop rotation and diversification are fundamental practices in modern pest control management. By alternating crops in a specific field, farmers disrupt the lifecycle of pests that are dependent on a single crop. Additionally, planting a variety of crops creates an environment that is less conducive to the rapid spread of pests. This approach enhances soil health, minimizes pest pressure, and promotes sustainable agricultural practices.

In the intricate dance of modern agriculture, the age-old practice of crop rotation and diversity has regained the spotlight. As we grapple with the challenges of pest pressure, soil health, and sustainable food production, crop rotation and diversification emerge as powerful strategies. This article explores the significance of these practices in the realm of pest control management, shedding light on their benefits, implementation, and impact on agricultural landscapes.

The Power of Crop Rotation (15)

Crop rotation involves the systematic planting of different crops in a sequence over time on the same piece of land. This practice breaks the cycle of pests and diseases that target specific crops, as different plants have different nutritional needs and vulnerabilities. Here's how crop rotation contributes to modern pest control management:

Disruption of Pest Cycles: Many pests have specific host plants. By changing the type of crop grown, farmers interrupt the lifecycle of pests that depend on a continuous supply of their preferred hosts. This curtails the buildup of pest populations.

Nutritional Imbalance for Pests: Different crops require varying nutrients from the soil. Rotating crops helps prevent the depletion of specific nutrients essential for pest proliferation, making it harder for pests to establish themselves.

Weed Suppression: Certain crops can naturally suppress weeds, reducing competition for resources between crops and invasive plants. This reduces the habitat available to pests and their potential hosts.

Improved Soil Health: Different crops have different root structures, which can improve soil structure, prevent erosion, and enhance soil fertility. Healthy soil supports healthy crops and can discourage certain pests.

Diversification for Pest Control

Diversification involves planting a variety of crops in close proximity. This approach capitalizes on the interactions between different plants to manage pests:

Companion Planting: Certain plants emit chemicals or odors that repel pests or attract beneficial insects. Marigolds, for example, can deter nematodes and aphids, while attracting pollinators.

Trap Cropping: This strategy involves planting attractive crops that lure pests away from main crops, acting as sacrificial plants. This prevents pests from heavily infesting the primary crops.

Benefits and Real-World Applications

Reduced Reliance on Pesticides: Crop rotation and diversity minimize the need for chemical pesticides by creating less hospitable environments for pests. This reduces the environmental impact and potential harm to non-target organisms.

Enhanced Ecosystem Resilience: Diverse agricultural landscapes are more resilient to disturbances. When one crop is affected by pests or adverse conditions, others may thrive, maintaining overall yield stability.

Long-Term Sustainability: By preserving soil health, conserving biodiversity, and reducing pest pressure, crop rotation and diversity contribute to the sustainability of agriculture for future generations.

Local Adaptation: Tailoring crop choices to local conditions and pest profiles enhances the success of rotation and diversification strategies (16)

Trap Crops and Companion Planting

Trap crops and companion planting are innovative techniques in modern agriculture that capitalize on plant interactions to deter pests. Trap crops are sacrificial plants strategically placed to attract pests away from the main crops. Companion planting involves growing different crops in close proximity to enhance each other's growth or repel pests. For example, planting marigolds alongside vegetables can deter certain insect pests (17)

Use of Technology

Technology plays a pivotal role in modern pest control management. Remote sensing, drones, and satellite imagery assist farmers in identifying pest hotspots across large fields. This enables targeted interventions, reducing the

overall use of pesticides. Furthermore, precision agriculture techniques allow for precise pesticide application, minimizing wastage and environmental impact (18)

Organic Farming Practices

The rise of organic farming aligns well with the principles of modern pest control management. Organic farming avoids synthetic pesticides and prioritizes natural alternatives. Techniques such as composting, cover cropping, and the use of biopesticides are common in organic agriculture (19). This approach not only produces healthier crops but also promotes soil fertility and overall ecosystem health.

Conclusion

The paradigm of pest control management in agriculture has shifted dramatically towards more sustainable, eco-conscious, and effective methods. Modern agriculture embraces Integrated Pest Management, biological control, crop rotation, and innovative technologies to strike a balance between pest control and environmental conservation. By adopting these approaches, farmers can ensure bountiful harvests while safeguarding the planet for future generations. As the realm of agriculture continues to evolve, the principles of modern pest control management stand as a testament to human ingenuity and responsibility. In a world facing complex agricultural challenges, crop rotation and diversity emerge as time-tested tools that promote pest control while fostering sustainable and resilient farming systems. These practices honor the wisdom of our ancestors while aligning with the goals of modern agriculture—to feed a growing global population while safeguarding the health of our planet. As we continue to innovate and adapt, crop rotation and diversity stand as beacons of hope, illuminating a path toward harmony between human cultivation and the intricate web of life on Earth.

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Immunosenescence and Aging

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Abstract

Immunosenescence is the term for the gradual deterioration of immunity brought on by ageing naturally. Compared to the innate immune system, the adaptive immune system is more affected. Immunosenescence has two components: the formation of long-term immune memory and the host's capacity to fight infections. Immunosenescence has been associated with increased rates of morbidity and mortality in the elderly. Adults suffering from chronic inflammatory conditions such as rheumatoid arthritis may display signs of rapid immunosenescence. The development and course of age-related illnesses, such as cancer, cardiovascular, metabolic, and neurological conditions, may also be influenced by the aging immune system. Notably, several age-related diseases are associated with T cell ageing and low-grade inflammation. Increased blood levels of autoantibodies, pro-inflammatory cytokines, late-differentiated T cells (CD28−), and regulatory T cells have all been connected to morbidities associated with aging. Interactions between immunosenescence and age-related diseases have been demonstrated.

Introduction

Roy Walford first proposed the idea of immunosenescence in 1969, speculating that immune system malfunctions were linked to natural ageing in both humans and animals (Walford 1969). It is now evident that aging causes changes to nearly every facet of immune function, in both the innate and adaptive immune compartments. A portion of these modifications have been directly associated with aging, which can occasionally show up as immunological dysregulation, increased morbidity and mortality from noncommunicable diseases, and age-related illnesses. Ageing causes many changes that affect the immune system and physiological systems of the body. Immunosenescence is a factor in the immune system's diminished ability to fight off infections or immunisations in the aged. While the exact nature of the

molecular alterations leading to immunosenescence is uncertain, aging is known to cause phenotypic and functional changes in several cell types involved in both innate and adaptive immunity. Dysregulation at the genetic level mediates these cellular symptoms of immunosenescence, which in turn cause alterations in the immune system as a whole.As we age, the immune system is also greatly impacted by environmental variables including nutrition. Ageing causes many changes to the body that affect the immune system and physiological systems. The immune system's diminished ability to react to vaccinations or illnesses in the aged is a result of immunosenescence. Numerous cell types involved in innate and adaptive immunity show altered phenotypes and function as a result of aging; nevertheless, the full scope of the molecular alterations leading to immunosenescence is unknown. These cellular manifestations of immunosenescence are caused by genetic dysregulation, which in turn causes observable alterations in the immune system.As we age, environmental factors like nutrition also have a big impact on our immune systems.

Immunosenescence involves several changes in immune system, Such as:

Thymic Involution: The thymus, an organ responsible for the development of T cells, shrinks with age, leading to a reduced production of naive T cells.

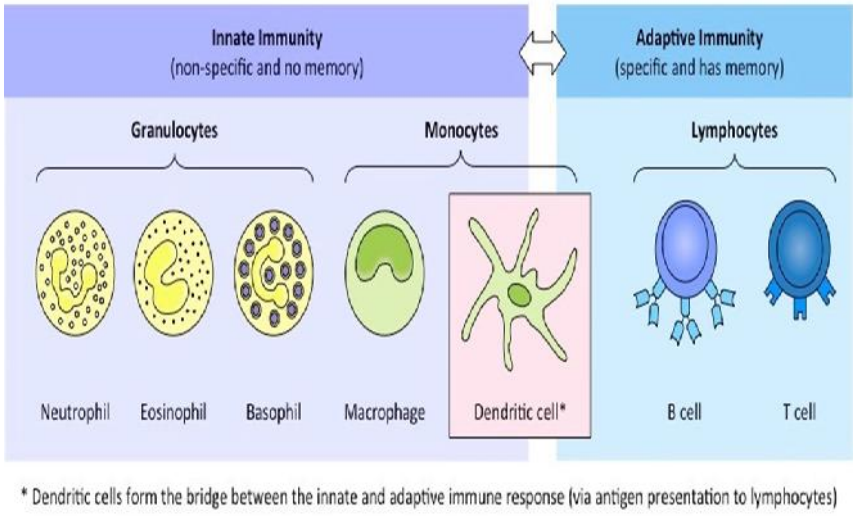
Reduced Immune Cell Function: Aging can lead to a decline in the function of immune cells, including macrophages, dendritic cells, and natural killer cells.

Decreased Vaccine Efficacy: Older individuals may have reduced responses to vaccinations, making them more vulnerable to vaccine-preventable diseases.

Chronic Inflammation: Senescent cells and chronic low-grade inflammation, known as inflammaging, can disrupt the balance of the immune system.

Impaired T-Cell and B-Cell Function: T cells and B cells may become less effective in recognizing and fighting infections over time.

Aging and Immune System



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Understanding immunosenescence is crucial in developing strategies to support the health of aging populations and improve vaccination and healthcare practices for the elderly.

Here we discuss some basics and changes in immune system

(A) Innate immune system

When an infection occurs, the innate immune system is the first to respond. Neutrophils, monocytes, macrophages, natural killer cells (NK cells), mast cells, and dendritic cells (DCs) are innate immune system components that aid in the adaptive immune response, trigger an inflammatory response, and phagocytose and destroy pathogens.

Neutrophils

The first innate immune cells to be drawn to the scene of tissue injury or infection are neutrophils.Neutrophils have several roles at the infection site, including phagocytosis, the production of antimicrobial chemicals (such as lactoferrin, myeloperoxidase, and protease), and the creation of neutrophil extracellular traps (NETs) to stop the invasion of bacteria.(Cancro M. P. (2020)) The microorganisms go through apoptosis, or programmed cell death, after being removed from the infection site [Feng E., Balint E].There is no

discernible variation in the frequency of neutrophils between young and elderly people in terms of absolute numbers [Eggert T., Wolter K.].

Monocyte/macrophage

In addition to processing and presenting antigen, monocytes and macrophages are key players in the battle against invasive microorganisms and also trigger the adaptive immune system [Borgoni et al]. Macrophages are found all across the body, concentrating in various organs and tissues, and they are essential for both illness and homeostasis. When monocytes from older people (over 65) were stimulated in vitro, their production of IL-6 and TNF-α was shown to be lower than that of young adults (21–30 years old) [Gil et al]. Comparing monocytes from young individuals to those from older adults, the latter revealed unchanged expression of TLR2 and TLR6, increased expression of TLR5 and decreased expression of TLR1 and TLR4. [Nauent et al]. Additional research has revealed that older people had less TLR expression [Almanan et al], less macrophage infiltration at the site of infection, and a reduced capacity to mount an adaptive immune response in comparison to younger people [Feng E., Balint E]. Taken together, these data imply that greater focus is required to comprehend the function of macrophages and monocytes in organ-specific immunity.

Dendritic cells

DCs, or dendritic cells, are crucial for the innate and adaptive immune systems' coordination. They fall into two primary categories: myeloid DCs (mDCs) and plasmacytoid DCs (pDCs). Elderly people typically exhibit phenotypic and numerical alterations as well as dysregulation of intracellular molecules in mDCs and pDCs.

Natural killer (NK) cells

These cells are natural killers. The first description of natural killer (NK) cells appeared in 1975.These lymphocytes are cytotoxic, meaning they may destroy target cells without any prior exposure [Basterd et al]. Based on CD56 density, there are two primary subgroups of human NK cells: 1) mature and highly cytotoxic CD56dim NK cells, and 2) immature CD56bright NK cells with an immunoregulatoryrole. The frequency of NK cells is influenced by ageing; as people age, there is an increase in NK cells, a drop in the proportion of CD56 bright people, and an expansion of CD56dim subgroups. In a similar vein, NK cell proliferation rates decline with ageing [Molony et al]. Age-associated immunosenescence is shown in Fig. 2.

(B)Adaptive immune system

Variations in the adaptive immune system are one of the primary changes linked to ageing. The morphological changes seen in maturation organs, such as the thymus for T cells and the bone marrow for B cells, suggest that immune system dysregulation with ageing may also be viewed as an adaptation to the evolving organism in addition to the effects of memory cell accumulation in the periphery brought on by ongoing exposure to pathogens. [Iwasaki A, Medzhitov]

Hematopoietic stem cells (HSC)

Hematopoietic stem cells (HSC) are essential for maintaining an organism's ability to produce blood cells throughout its whole life. The ability of HSC to differentiate and self-renew is essential for a healthy hematopoietic system. HSCs undergo phenotypic and molecular changes as they age. Proteostasis and metabolic alterations, together with intrinsic aging-related changes as chromatin architecture and epigenetics, diminish the potential of HSCs as people age [Franceschi C, Salvioli S]. As people age, their HSCs' ability to proliferate diminishes and they start to produce more myeloid progenitors.

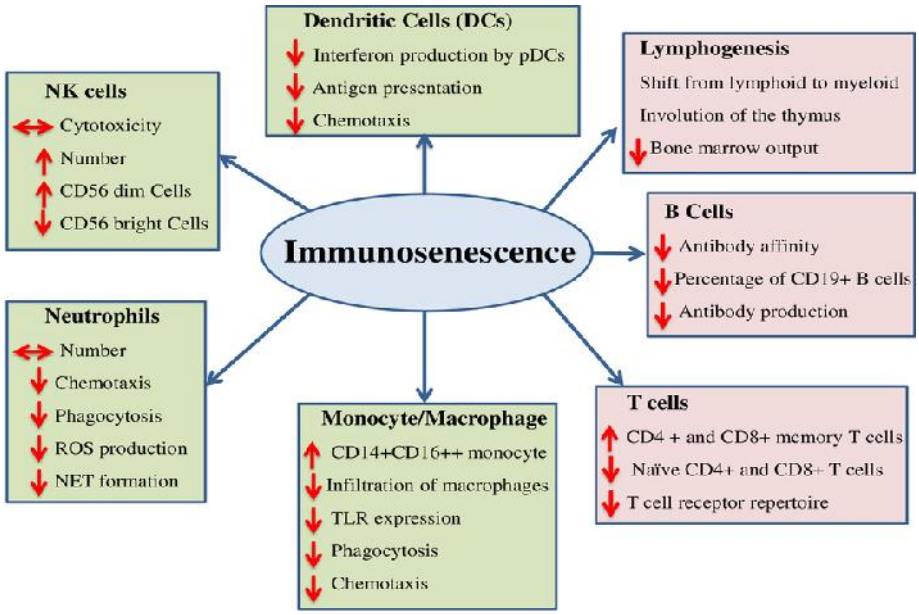

Figure 2- available from: Immunity & Ageing Online ISSN: 1742-4933.

B cells

B cells, an essential part of the adaptive immune system that produce antibodies and take part in the immunological response, are significantly impacted by ageing. [Flores R. R.,] The following are some alterations that take place in B cells as they age: Reduced Antibody Diversity: As people get older, the variety of antibodies that B cells make decreases. This decrease is partially brought about by a decrease in the production of fresh B cell clones and a buildup of memory B cells that are unique to pathogens that have already been encountered. The capacity to successfully combat emerging illnesses may be hampered by the decreased diversity.

Altered Antibody Production: Antibodies produced by B cells may have a lower affinity and effectiveness. Older people may have less efficient antibody affinity maturation, which makes their antibodies less capable of binding and neutralising infections.

Reduced B Cell Output: B cells are also derived from the bone marrow, much like T cells. The generation of new B cells may decline with age, which could add to the immune system's general decline in performance.

Impaired B Cell Activation: Activation signals are necessary for B cells to start producing antibodies. A weakened immune response can arise from B cells' diminished capacity to react to these activation cues as a person ages.

Increased Autoantibodies: With age, there is an increased likelihood of B cells producing auto antibodies—antibodies that mistakenly target the body's own tissues. This is associated with autoimmune diseases and can lead to chronic inflammation and tissue damage.

Memory B Cell Accumulation: As people get older, memory B cells—which have been exposed to and generated antibodies against a variety of pathogens—accumulate. This can limit the capacity to properly respond to novel, unfamiliar pathogens, even while it can be advantageous for treating illnesses that have already been encountered.

Decline in B Cell Signaling: Ageing may cause B cell signalling pathways to lose some of their effectiveness, which would lower B cell activation and proliferation in response to infections.

Impact of Chronic Diseases: B cell activity and antibody production can also be impacted by age-related chronic disorders like diabetes or chronic inflammatory conditions.It's crucial to remember that immune system ageing, which includes B cell ageing, is a complicated and diverse process with a wide

range of effects on an individual's health. Age-related deterioration of the immune system is possible, although lifelong exposure to viruses can also instill some degree of protection due to experience and memory. Immunisations, a healthy lifestyle, and regular exercise are strategies to lessen the impact of ageing on the immune system and can help sustain immune system function to some degree. Furthermore, scientists are still looking into ways to strengthen immune responses in the elderly, such as by creating new vaccinations and immunotherapies.

T cells

Immunosenescence involves several changes in T cells, Such as:

Thymic Involution: The thymus, an organ responsible for the development of T cells, shrinks with age, leading to a reduced production of naive T cells. The thymus is an organ where T cells mature and develop. As individuals age, the thymus gradually shrinks in size, leading to a reduced output of new T cells. This results in a decrease in the diversity of the T cell receptor (TCR) repertoire and can limit the ability to respond to new pathogens.

Reduction in Naïve T Cells: Naïve T cells are those that have not encountered their specific target antigen. With age, the number of naïve T cells declines, partly due to reduced thymic output and partly due to their conversion into memory T cells following exposure to pathogens. This can impact the ability to respond to new infections effectively.

Accumulation of Memory T Cells: As individuals age, there is an increase in the number of memory T cells. These cells are specific to previously encountered pathogens and can provide a faster and more effective response upon re-exposure. However, an overabundance of memory T cells may lead to a reduced ability to respond to novel pathogens.

T Cell Exhaustion: Chronic infections, such as cytomegalovirus (CMV), can lead to T cell exhaustion in older individuals. Exhausted T cells lose their ability to function properly and may contribute to increased susceptibility to infections.

Reduced T Cell Proliferation: Aging can lead to reduced proliferative capacity in T cells. This means that T cells may not divide and expand as rapidly in response to an infection, potentially leading to slower and less effective immune responses.

Changes in T Cell Function: The functional properties of T cells may also change with age. For example, there can be alterations in cytokine production, reduced cytotoxic activity, and impaired cell signaling pathways, all of which can affect the immune response.

Increased Inflammatory T Cells: Older individuals may have an increase in pro-inflammatory T cells, which can contribute to chronic low-grade inflammation, a condition known as inflammaging. This chronic inflammation is associated with age-related diseases.

Inflammaging

Chronic inflammation is another immune system alteration brought on by ageing. Inflammaging is the term used to describe the overexpression of the inflammatory immune response in the aging body. This also contributes to aging and illness, since inflammation is linked to many chronic diseases. [Zhao.J. et. al] A particular kind of chronic inflammation known as "inflammaging" is defined as a sterile inflammation, which means it lasts even when the body isn't fighting an infection. Inflammaging is low-grade inflammation as opposed to infection-driven inflammation. Chronic inflammation is brought on by the following kinds of stimuli: Self (molecules and remnants of cells) Non-self (pathogens) and quasi-self (gut microbiota). This kind of inflammation is brought on by a limited number of malfunctioning receptors, which detect stimuli and launch the innate immune response.

Conclusion

It's crucial to remember that as people age, their immune systems become less efficient in some areas and are more likely to overreact to certain stimuli, which might result in autoimmune diseases. The human immune system is surprisingly flexible and continues to offer important protection against infections throughout life, even in the face of these alterations. Certain age-related decreases in immune function can be reduced by adopting healthy lifestyle practices and receiving immunisations.

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