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REVIEW

From Citrus to Clinic: Limonene's Journey Through Preclinical Research, Clinical Trials, and Formulation Innovations

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Abstract: Limonene is a naturally occurring monoterpene found in oranges, lemons, grapefruit, fennel, and caraway, and is a part of essential oils of different plants. It has caught the eye of the research community owing to its innumerable health benefits. It exhibits significant antioxidant, anti-inflammatory, wound-healing, antidiabetic, anticancer, and immunomodulatory activities. These activities of limonene render it an indispensable compound in both traditional and modern medicine. This article presents a thorough compilation of the various therapeutic activities of limonene and the mechanisms underlying them. Furthermore, it delves into an in-depth discussion of the role of nanoformulations and novel drug delivery systems in ensuring the targeted delivery of limonene. To substantiate the safety and efficacy of limonene, a large number of preclinical and clinical studies have been conducted by researchers have also been discussed in detail in this review. Limonene is an unparalleled terpenoid with numerous therapeutic benefits. Incorporating it into sophisticated drug delivery systems and medical devices, together with personalised medicine strategies, signifies notable progress in its therapeutic use. Technologies, such as 3D printing, nanoformulations, and microneedles, can improve the ability of limonene to be absorbed by the body and targeted to specific areas.

Keywords: limonene, antioxidant, anti-inflammatory, antidiabetic, anticancer, preclinical studies, clinical studies, formulations, nanoparticles

Introduction

Herbal medicines anchored in ancient traditions and knowledge provide a holistic strategy towards health and wellness. The healing properties of plants and herbs have been harnessed for centuries to alleviate various ailments and to promote overall well-being. Essential oils (EOs), which are the concentrated essences extracted from aromatic plants, have potent healing properties. These volatile liquids carry the distinct aroma and therapeutic benefits of their botanical sources, and offer a diverse array of uses in promoting physical, emotional, and spiritual well-being. Essential oils are mainly terpenoids in nature and have the capability of depicting various pharmacological and therapeutic properties such as antioxidant, anticancer, antibacterial, and anti-spasmodic effects.[1,2] Limonene, a naturally available cyclic monoterpene, has attracted the attention of the research community owing to its numerous health benefits.

Chemically, limonene ($C_{10}H_{16}$) is 1-methyl-4-(1-methylethenyl) cyclohexane,[3] and is an olefinic hydrocarbon that exists as a colourless liquid at room temperature. It is an optically active compound that exists in the forms of D-limonene and L-limonene. Its racemic mixture is known as dipentene. Its (D) form is the main and most important form that has been found in natural sources such as oranges, lemons, grapefruit, fennel, and caraway, and is a part of the

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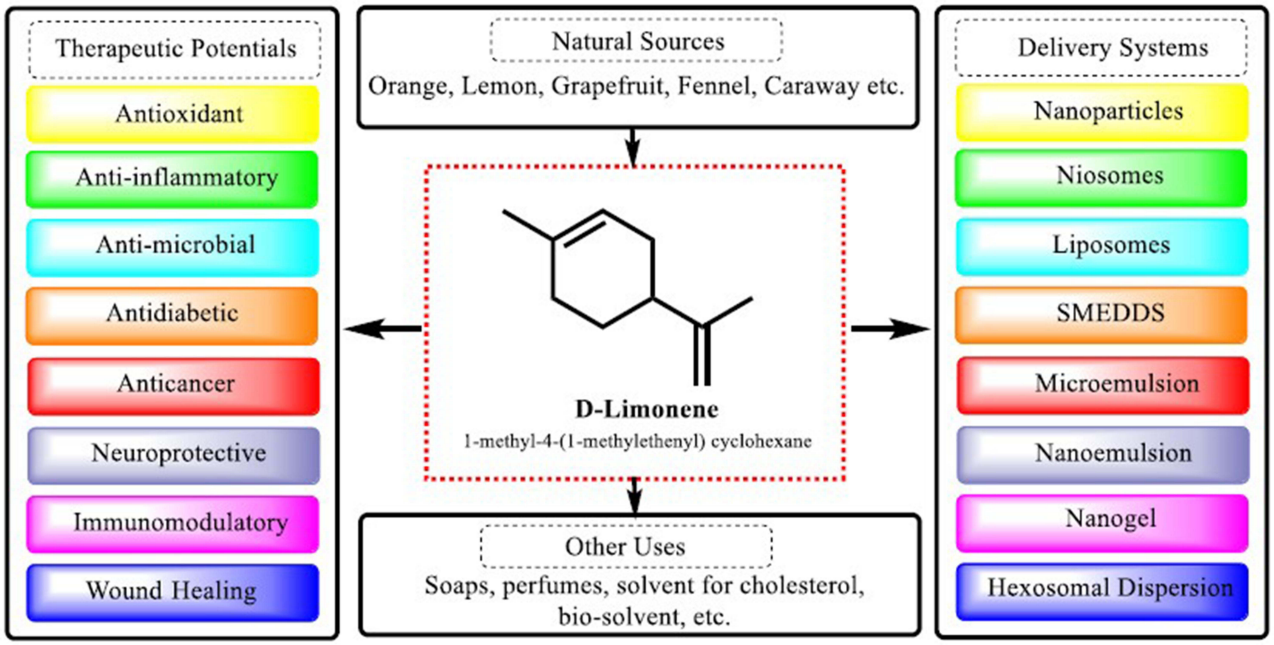
Graphical Abstract



essential oils of different plants. Limonene has applications in diverse fields.[4] It evaporates completely from the surface and does not leave any residues.[5] Dextrogyre form i.e. d-limonene is present in abundant amounts in nature and acts as a potent solvent. Levogyre form ie l-limonene is present in scarcer amounts and has a piney smell.[6] The specific gravity of D-limonene at 20°C is 0.84, and its refractive index varies between 1.450 and 1.590.[7] The biosynthesis of limonene occurs in the glandular trichomes of plants, where the condensation of isopentenyl pyrophosphate (IPP) and dimethyl allyl pyrophosphate (DMAPP) occurs to form geranyl pyrophosphate, which is then further converted to limonene.

Limonene offers a plethora of therapeutic and nontherapeutic applications. Conventionally, it has been utilised in the generation of soaps and perfumes, as it has the characteristic odour of lemons, and has also been used in the production of pesticides and insect repellents. Limonene can act as a solvent for cholesterol and has, therefore, been used curatively to dissolve gallstones. It can also be used to neutralise gastric acid produced in the body and provide relief from burning sensations and gastroesophageal reflux.[1] Limonene has also been found to be useful as an alternative bio-solvent to toluene and chlorinated organic solvents owing to its environmentally friendly properties. D-limonene, when blended with polylactic acid and polyhydroxy butane, can also be used as a biodegradable packaging material for food owing to its inflated oxygen barrier and hostility towards water.[4] It has also been used as an antimicrobial and an anticancer agent. During handling and storage, it is oxidised, but its products are allergenic.[1] Its noticeable and definite lipophilicity contributes to an increase in the integration of substances within cells, and exhibits satisfactory bioavailability in the blood.[3]

Limonene and its derivatives depict significant therapeutic and non-therapeutic properties. Limonene when subjected to a hydroxyl radical reaction produces 4-acetyl-1-methylcyclohexane, ketoaldehyde, formaldehyde, 3-oxobutanal, and C10 dicarbonyl. Another derivative of D-limonene, carvone, shows antimicrobial and anti-fungal properties and is also used as an active component in the pharmaceutical industry. Another derivative, thiosemicarbazone, exhibits anti-fungal, antimicrobial, and anti-tumour properties. p-cymene is another derivative of limonene, which is utilised as a flavouring agent and as an intermediate in the pharmaceutical industry because of its medicinal value. They are also used in the manufacture of pesticides and fungicides. It also acts as an analgesic and exerts anti-inflammatory effects in mice. Polylimonene (piccolyte C115), a derivative of limonene, is synthesised from citrus oil. It is used as a resin in adhesive

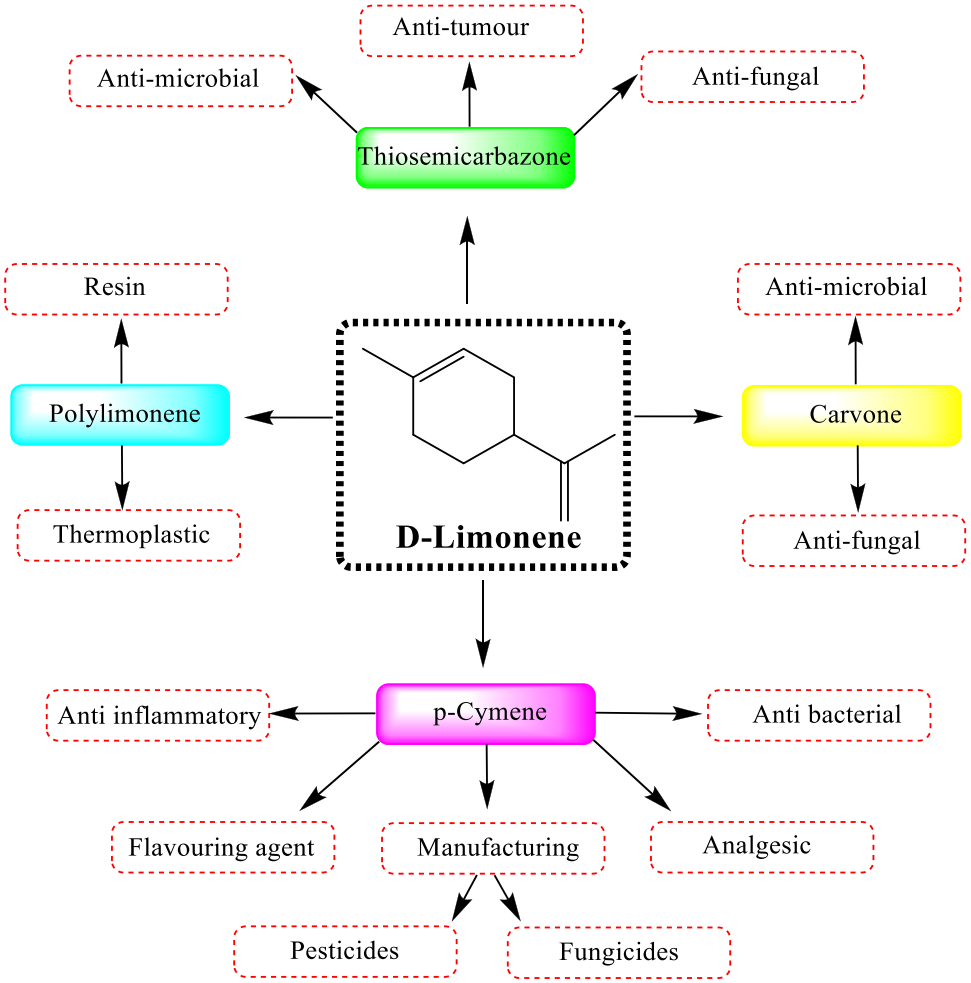


Figure 1 Structure of D-Limonene and Applications of its Derivatives in Health and Industry.

materials and as a thermoplastic in food packaging. It acts as a masticatory agent in chewing gum.[4] The use of different limonene derivatives is illustrated systematically in Figure 1.

This article delves into the remarkable therapeutic trajectory of limonene and navigates the realms of its therapeutic potential and pharmaceutical applications. This paper also provides insight into preclinical and clinical studies that have been carried out to explore the therapeutic potential of D-Limonene.

Therapeutic Potentials of D-Limonene

Limonene displays remarkable therapeutic potential in a diverse range of medical and pharmaceutical applications. It exhibits significant antioxidant, anti-inflammatory, wound-healing, antidiabetic, anticancer, and immunomodulatory activities. These activities of limonene render it an indispensable compound in both traditional and modern medicine. This section discusses the therapeutic potentials offered by limonene.

Antioxidant Activity

Antioxidants protect the body against the toxic effects of free radicals by the mechanism of scavenging them. As the substantial intake of vegetables and fruits in the diet have a protective effect against different pathologies, more phytoconstituents are being included in studies to determine their therapeutic effects in the diseased state.[8] D-limonene, a monocyclic monoterpene, is a naturally occurring phytoconstituent that protects against oxidative stress. As limonene is

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insoluble in water, it is administered as a dietary antioxidant with some oil moieties. Rehman et al reported that orally administered D-limonene shows complete absorption from the GI tract of both animals and humans.[9]

The defense mechanisms against free radical-induced damage are discussed hereunder. Free radicals are electrically charged molecules with unpaired electrons that drive them to seek and accept electrons from other substances to neutralise themselves. Although the first interaction neutralises the free radical, another free radical is produced throughout the process, resulting in a chain reaction. The antioxidant protective mechanism occurs by preventing the initiation of chain reactions that may be caused by free radicals or by interrupting chain sequences, scavenging free radicals generated in a chain reaction, or removing peroxidises, thereby preventing the further generation of reactive oxygen species (ROS), as depicted in Figure 2.[10]

Approximately 5% or more of the breathed O_2 transformed to ROS, such as superoxide, hydrogen peroxide, and hydroxyl radicals, by the univalent reduction of O_2.[11] Under oxidative stress conditions, superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) levels are reduced in the kidneys. A catalytic reaction for the removal of free radicals and ROS by factors such as SOD, Catalase (CAT), GPx, and thiol-specific antioxidants takes place. These catalytic reactions reduce the levels of enzymes in the cells and cause oxidation. Glutathione peroxidise (GSH-Px) reduces H_2O_2 while oxidising GSH. GSH-Px can also diminish other peroxides such as fatty acid hydroperoxides. These enzymes can be found in millimolar quantities in both the cytoplasm and mitochondrial matrix. Most animal tissues exhibit CAT and GSH-Px activity. This leads to protein denaturation and DNA damage.

Rehman et al found that pretreatment of a group of male Wistar rats with D-limonene in induced oxidative stress conditions showed significant results compared to the group that was not treated with D-limonene. Figure 3 shows that the administration of D-limonene restored the activities of antioxidant enzymes, such as SOD, CAT, GP, and GSH level, further resulting in the reduction of oxidative stress by preventing DNA damage and protein denaturation. The increase in GSH levels in animals treated with D-limonene partially explains the protective mechanism. These increased levels can provide thiol groups for GSH-mediated detoxification reactions of GPx and glutathione transferase (GST). Administration of D-limonene is also capable of increasing the activities of renal GPx and GST. Moreover, the enhancement of SOD activity might also be involved

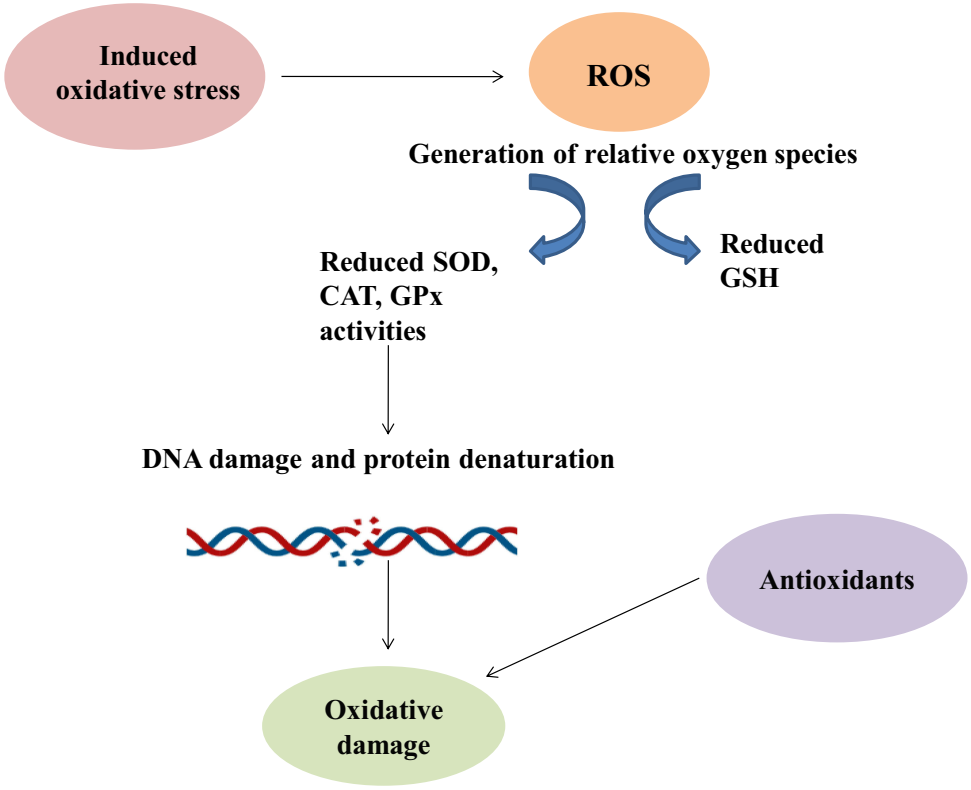

Figure 2 General Mechanism of Antioxidants to Inhibit Regeneration of ROS.

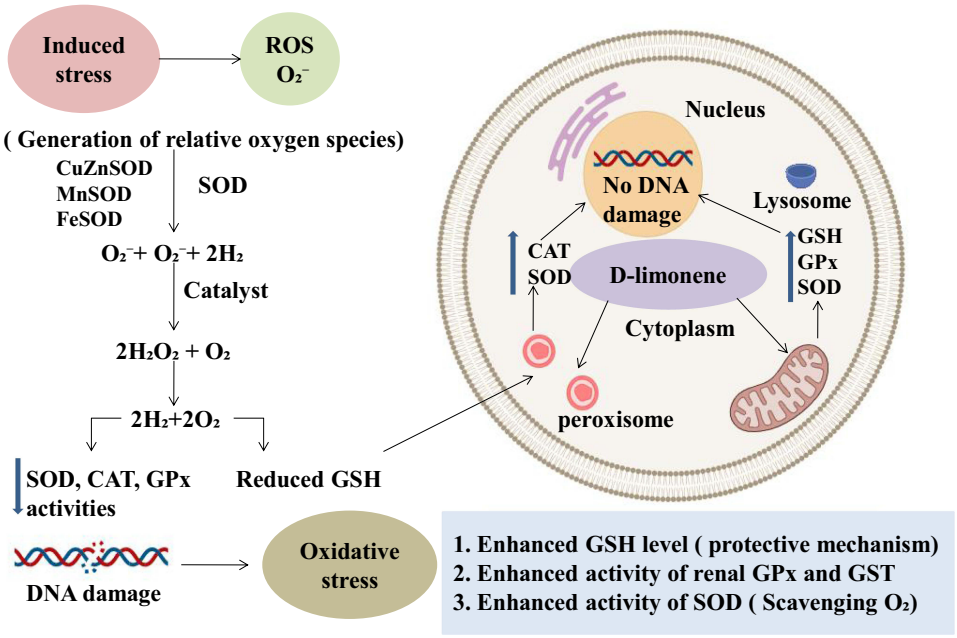

Figure 3 Role of Limonene in Preventing DNA Damage and Protein Denaturation.

in the scavenging of O_2 generated from doxorubicin (Dox) in the D-limonene-treated group. The protective mechanism was further supported by the heightened renal GST activity in the D-limonene plus Dox treatment group.[12]

Wound Healing and Anti-Inflammatory Action

Wound healing is a process of regeneration and tissue repair, comprising a series of molecular and cellular events that can be observed after tissue lesions to restore damaged tissue. Wound healing requires ongoing interactions between cells and the matrix, including inflammation, proliferation, and remodelling. The process of healing depends on three main factors involved in tissue to determine whether it is capable of regeneration, the extent of tissue injury, whether the extracellular matrix framework is intact, the nature of the injury, whether it is persistent and associated with chronic inflammation, or whether it is acute.

The healing mechanism involves four stages, of which homeostasis is the initial response to the injury where the clot is formed by the constriction of blood vessels, followed by inflammation which is the body's immune response to eliminate debris and bacteria from the wound, the proliferative phase where new tissues are formed to repair the wound, including granulation tissue and new blood vessels, and the maturation phase, where the wound undergoes remodelling and gains strength as collagen fibres are recognised. During proliferation phase some tissues will undergo regeneration only whereas some tissues exhibit regeneration along with fibrosis and some undergo just fibrosis. Vasospasm followed by the formation of a platelet plug prevents bleeding after homeostasis. Owing to the activation of the clotting factor, fibrin is deposited on the platelet plug to form a blood clot. Inflammation and regeneration may also occur. However, in some conditions such as diabetes mellitus, the formulation of granulation tissue is defective, resulting in a delay in the healing process.

Recent studies have claimed that D-limonene acts as an anti-inflammatory agent because it reduces inflammation via matrix metalloproteinase (MMP)-2 and −9 gene expression, which further helps in tissue remodelling.[13]

Plasminogen activator inhibitor-1 (PAI1) is a protein that plays a major role in the regulation of the fibrinolytic system. In wound healing, PAI1 has been shown to inhibit the activity of plasmin, an enzyme that degrades fibrin and promotes tissue remodelling.[11,14] As a drug target, PAI1 inhibition has the potential to promote wound healing by enhancing fibrinolysis and tissue regeneration.[15] Complex formation with the phytoconstituent D-limonene demonstrated the efficacy of PAI1 inhibitors in promoting wound healing in animal models (Figure 4). Clinical trials are currently underway to evaluate the safety and efficacy of these inhibitors in humans. PAI1 inhibitors have also been shown to have potential therapeutic applications beyond wound healing, including in the treatment of thrombotic disorders, cardiovascular diseases, and cancer. The main mechanism through which limonene exerts its effects during wound healing is by

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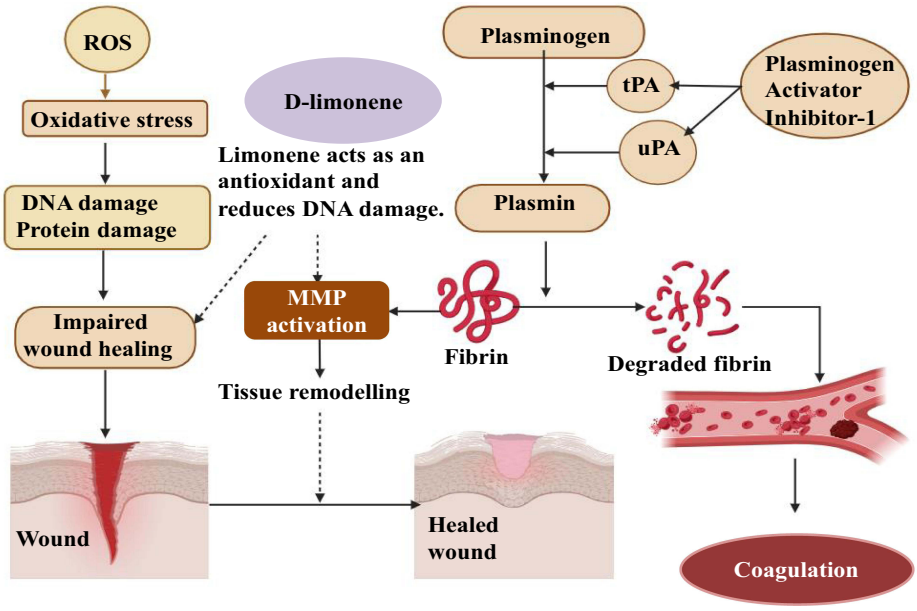

Figure 4 Complex Formation Between D-Limonene and PAI1 for Promoting Wound Healing by MMP Activation.

activation of (MPP)-2 and −9 for tissue remodelling. D-limonene is an effective antioxidant which prevents DNA damage and protein denaturation of the open wounds, and significantly promotes the healing process.[16]

Antimicrobial Activity

Contamination of food through microbes has been a major concern over the years across the globe.[17] Causing significant economical losses in food industry, it also is responsible for many foodborne diseases in humans, causing significant economic loss in the food industry.[18] Although most preservatives and antimicrobials are used to inhibit food contamination and spoilage, many antimicrobials have been reported to cause adverse reactions in humans and even show carcinogenic effects. Some antimicrobial substances can improve the preservative effects. D-limonene, a constituent in citrus-derived essential oils, such as orange, lemon, lime, and grapefruit, acts as a safe antimicrobial agent.[19] However, its lipophilic characteristics turn out to be a major drawback, as they are required at high concentrations to show equivalent antimicrobial effects in food.[20] Limonene has vast application prospects related to antibacterial activity and food preservation because of its broad-spectrum bactericidal activity and safety. D-limonene has the potential to inhibit gram-negative and gram-positive bacteria and fungal activity.[21]

A study showed the antibacterial sensitivity and mechanism of action of D-limonene on *L. monocytogenes*. D-limonene's anti-*L. monocytogenes* response was examined using minimum inhibitory concentration (MIC). The mechanism of action of D-limonene was explained by its effect on cell morphology, membrane permeability, and alterations in protein, acetic acid, nucleic acid, ATP, and various protein expressions in the respiratory chain complex of *L. monocytogene*.

In an experiment performed by Yingjie Han Limonene and a positive control (Levofloxacin Hydrochloride) showed inhibitory action on bacterial reproduction, along with an increase in antimicrobial activity with an increase in the concentration of the drug.[20] It was detected that bacterial growth was significantly low when limonene concentration was 20 mL/L. However, only 0.625 mL/L Levofloxacin Hydrochloride was required to inhibit bacterial growth. Therefore, the minimum inhibitory concentration of limonene was higher than that of the drug. It has also been proven experimentally that antimicrobial activity can be improved using essential oils encapsulated in liposomes.[22]

The effects of limonene on cell morphology and cell membranes have also been studied in *L. monocytogene*. No cell damage was detected in the negative control group, as all cells were intact. In contrast, bacterial cells treated with D-limonene were damaged by the degraded cell membrane. The extent of cell damage was determined by the amount of time to which the cell was exposed to D-limonene. Severe ruptures and holes were observed as treatment time increased. The degree of cell damage can also

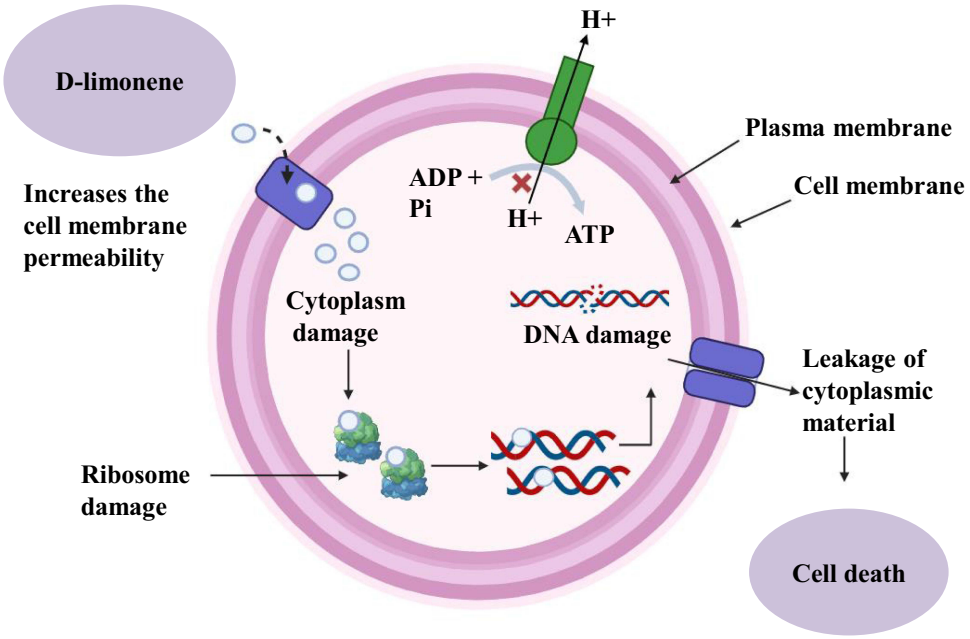

Figure 5 Effect of Limonene on The Cell Morphology and Cell Membrane of *L. monocytogenes* Leading to Cell Death.

be determined by the level of propidium iodide (PI) that enters the cell membrane of dead cells. Propidium iodide enters dead cells and binds to nucleic acids, resulting in a red fluorescence emission.[23] The change in optical density was measured in the D-limonene-treated bacterial suspension because of the leakage of nucleic acids (Figure 5). Hsouna et al discovered that limonene can be considered as a major component of *C. Limon* essential oil (CIEO) when analysed using GC-MS, and its antibacterial activity can be evaluated by measuring the inhibition zone and MIC values. Zhao et al concluded that the cell membrane is an essential barrier for bacteria and is naturally protective.

Effect on Gall Stones

Most cases of gallstones, also known as cholelithiasis, are treated surgically; however, removal of gallstones present in the bile duct is not always possible. Mechanical techniques, such as roentgenology, are used to remove gallstones, but the size and hardness of stones act as barriers to these techniques. Hirotsune et al, identified solubilising agents responsible for the dissolution of gallstones using a comparatively safe technique. The conducted study focused on an in-vitro study, along with an in-situ experimental study, to determine the effects of limonene. He preceded the experiment by vacuum drying five different types of gallstones obtained after surgery. The different types of gallstones used in the experiment included pure cholesterol, two cholesterol pigment-calcium gallstones, one bile pigment-calcium gallstone, and one fatty acid-calcium gallstone. The stones were submerged in 100 mL of D-limonene solution that was prepared by mixing 2.1 parts polysorbate 80 and 0.9 part sorbitan monooleate to 97.0 parts D-limonene in separate Erlenmeyer flasks at 37°C and monitored for dissolution and fragmentation. The insoluble residues were collected by filtration and dried at 85°C for 3 h for weight measurement.

The main mechanism involved in gallstone reduction was dissolution, as shown in Figure 6. Through this experimental study, Hirotsune observed that pure cholesterol stones completely dissolved in the preparation of D-limonene within a period of 2 h. The effect of D-limonene on cholesterol pigment-calcium stones was seen to be comparatively less, as the two dissolved by 85.4% and 90.1% of their initial weight, respectively. Both fatty acid-calcium and bile were dissolved by 16.8% and 25.2%, respectively.

Another scientist Sun et al demonstrated the dissolution of gallstones with the help of D-limonene, in which human gallstones were dissolved using in-vitro dissolution techniques. Infusion of D-limonene results in dissolution and disintegration of gallstones in animals, which are later excreted through the bile duct.[24] In patients who had gallstones removed through surgical procedures, 20 mL of D-limonene was infused every day, resulting in the dissolution of

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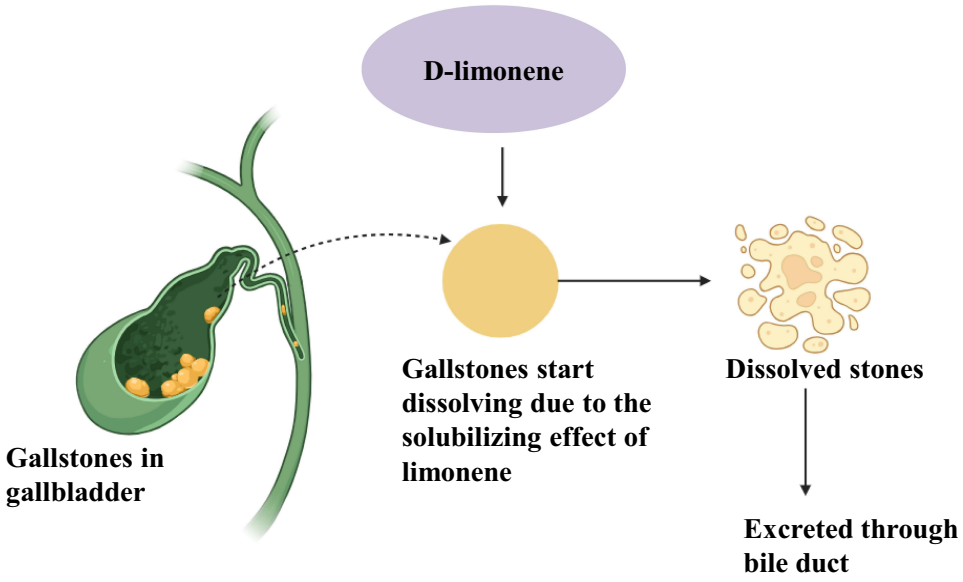

Figure 6 Dissolution of Gallstones by D-Limonene.

gallstones overlooked during the surgery. In most cases, D-limonene was found to completely or partially dissolve gallstones over a time of three weeks to four months.[24]

Antidiabetic Effect

Diabetes mellitus (DM) is a persistent, chronic metabolic disease. The major characteristic associated with it is impaired insulin secretion, which may lead to hyperglycaemia and is responsible for the production of reactive oxygen species (ROS), followed by the generation of high blood glucose levels. Diabetes has been classified into two categories: type 1 and type 2 diabetes, which occur earlier due to insufficient insulin secretion, and the latter which is very prevalent and is caused by insulin resistance and insufficient insulin secretion.[25] Glycation is a chemical reaction that produces advanced glycation end products (AGEs), which cause cell damage and are responsible for complications in diabetes mellitus such as nephropathy, neuropathy, and retinopathy. Inhibition of AGE can prevent these complications.[26] Reducing sugars react with amino groups of proteins (albumins, fibrinogens, and immunoglobulins) in the body, leading to the formation of a Schiff base. The Schiff base forms di-carbonyl compounds which further form AGEs that cause oxidative stress due to the destruction of pancreatic beta cells, resulting in diabetic complications.[27,28] In addition, AGEs form the RAGE (receptor for AGEs) complex which regulates diabetic complications. D-limonene is found to reduce oxidative stress and induce the potentiation of beta cells in the pancreas, thus showing beneficial effects in diabetes mellitus (Figure 7).[29]

Anticancer Effect

Cancer is characterised by uncontrolled cell growth, and if this abnormal growth is not controlled, it may result in death. Various factors are associated with cancer, and these factors can be external, including tobacco and chemicals, etc. or internal, such as hormones, mutations, and genetics.[30] Normally, apoptosis (programmed cell death) plays a critical role in maintaining homeostasis in organisms and is associated with other pathological processes such as cancer. Unbalanced apoptotic pathways can advance tumourigenesis and induce resistance in cancer cells towards pharmacological therapies. Thus, a controlled apoptotic pathway is required for effective cancer therapy.[31] Apoptosis may occur via two mechanisms: the intrinsic pathway, also known as the "mitochondrial" pathway, and the extrinsic pathway.[32]

In the Intrinsic pathway, the detection of internal stimuli, such as DNA lesions (acts as an initiation for apoptosis signal). DNA lesions are in the form of double-strand breaks, and the first molecule that is going to recruit is ATM serine/threonine kinase, which activates p53 protein (tumour suppressor protein) which in turn regulates another protein PUMA (p53

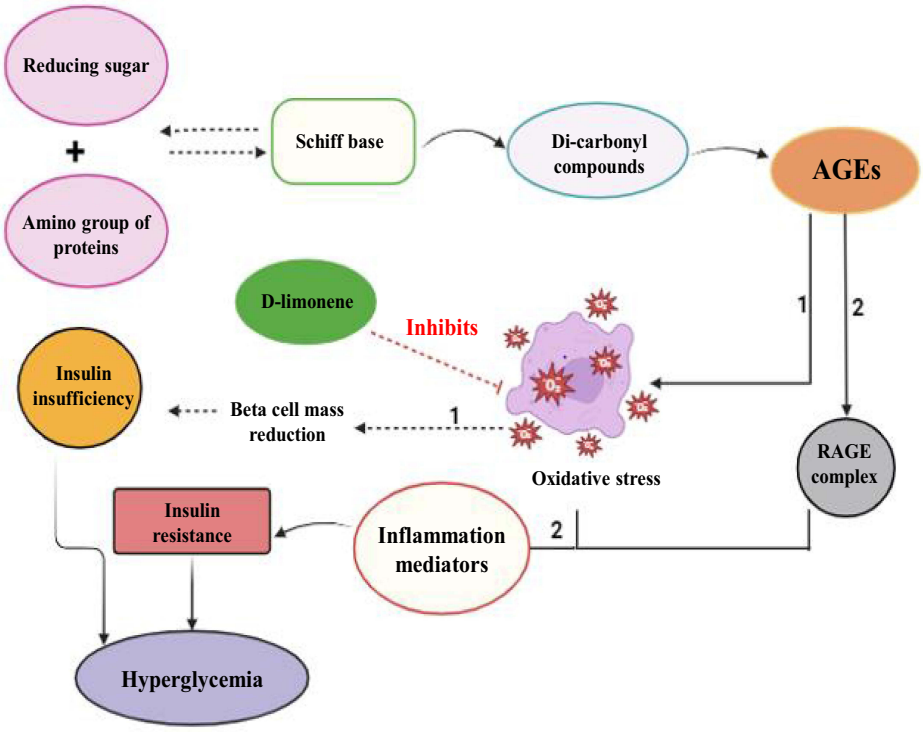

Figure 7 The Role of Limonene in Inhibiting Oxidative Stress in Case of Hyperglycaemia.

upregulated modulator of apoptosis) and is a pro-apoptotic protein. It activates two pro-apoptotic BCL2 (B-cell lymphoma) proteins BAX (BCL2 associated X protein) and BAK (BCL2 antagonist killer) these activated proteins open VDAC (voltage-dependent anionic channels) of mitochondria and form MAC (mitochondrial membrane apoptotic inducer channel) on mitochondrial membrane. After the series of reactions in the mitochondria in the presence of reactive oxygen species and calcium ions, cytochrome C is released into the cytosol which combines with APAF-1 (apoptotic protease activating factor 1) and in the presence of dATP, apoptosome are produced with dADP after utilising the ATP energy from dATP. The apoptosome acts on procaspase 9, an initiator caspase (cysteine aspartic protease), converts it to its active form, activated caspase 9 will activate caspase 3, an executive caspase, and causes cell death within minutes[32,33] (Figure 8).

Extrinsic pathway is divided into pathways based on the type of signalling molecules and receptors, one of which is the tumour necrosis factor (TNF) path and the other one is the FSN (Fatty acid synthetase) path. Previously, the extracellular signalling molecule was TNF-α which is a cell-signalling protein cytokine produced by activated macrophages, whereas in the latter, the extracellular signalling molecule is FAS-L (FAS ligand protein which initiates extrinsic apoptosis). Death receptors consist of TNF receptors which are identified by various protein motifs such as death domains (DD) and death effector domains (DED). On the surface of cell cognate ligands of the TNF family, CD95 (first apoptotic signal, Fas/Apo 1) and TNF-related apoptosis-inducing ligand (TRAIL) capture the death receptor to draw DD-containing molecules such as FADD (Fas-associated death domain protein) and TRADD (TNF receptor-associated death domain). FADD activates pro-apoptotic pathways and TRADD prompts antiapoptotic signals. FADD draws other DD/DED-containing proteins including procaspase 8 and 10, to encourage the formation of a death-inducing complex (DISC) in the cytoplasmic compartment. TRADD binds to receptor-interacting protein 1 (RIP1), TNF-associated factor 2 (TRAF2), and TRAF5 to form complex I. Complex II is formed by the binding of RIP1 and TRADD to FADD and caspase 8 and 10. As caspases 8 and 10 are activated, the death signal is amplified through the activation of caspases 3, 6, and 7, ultimately causing cell death.[34]

As mentioned earlier, dysregulation of the apoptotic pathway in major cancer studies has been shown to be an effective activator of apoptosis in animal tumour models. Hafidh et al (2018), Jia et al (2013), Yang et al (2008), and Ye et al (2020) reported that D-limonene activates caspases 3 and 9 (Figure 9), ultimately causing the death of tumour cells.

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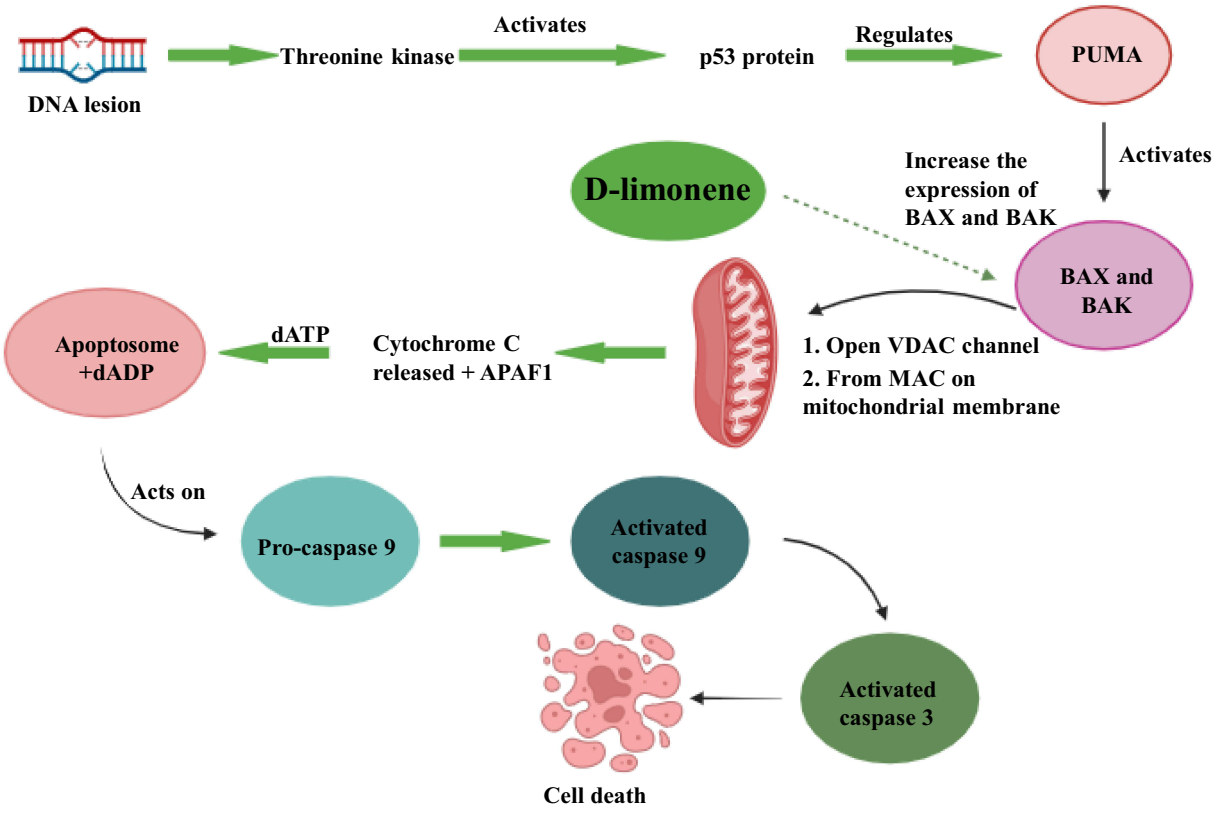

Figure 8 The Role of D-limonene to increase the expression of BAK and BAX proteins, hence leading to the of cancer cells.

Chaudhary et al, 2012; Jia et al, 2013; Lu et al, 2003; Yang et al, 2008; Ye et al, 2020; Yu et al, 2018; Zhang et al, 2014) reported that limonene participates in the mitochondrial pathway of apoptosis, increases the expression of BAX protein, and decreases BCL2 protein expression, thus confirming its pro-apoptotic activity.[31]

Immunomodulatory Effect

The immune system is widely associated with physiology and mechanisms underlying various medical disorders. The primary objective of immunology research is to determine whether it is immunomodulatory (modulation of immune response in the prevention of diseases) or immunosuppressive (suppression of unwanted immune responses). Immunomodulators have a major function in enhancing the body's defense mechanism against various diseases. Some plants and their phytoconstituents are mostly used to enhance the immune response of the body, and monoterpenes are the most commonly used. Monoterpenes are found in various plants, fruits, and essential oils (EOs). Among the monoterpenes D- limonene has been found to show the immunomodulatory effect.[35] D-limonene has also received attention because of its immunomodulatory effect against SARS-COV-2. Meeran et al described its potential effect on SARS-COV-2. It is beneficial in decreasing the epithelial expression of angiotensin-converting enzyme 2 (ACE2), a receptor that promotes the binding of SARS-COV-2 through its receptor-binding domain and releases its RNA, which is then converted into viral proteins.[36] Therefore, ACE2 inhibition in epithelial cells may block viral entry into the host, thereby preventing viral infection. In addition, limonene decreased the mRNA level of transmembrane protease serine 2 (TMPRSS2), which is expressed in human lung cells. TMPRSS2 is essential for S protein priming during the entry and dissemination of SARS-COV-2, but if it is inhibited, viral entry may also be blocked.[37] Macrophages have a pro-inflammatory phenotype (M1) which produces nitric oxide, IL-6, IL-8, IL-1β, ROS, MCP-1, CXCL-10, and TNF-α during the early stages of SARS-COV-2 infection. They produce a host defense against viruses and facilitate lung injury. The restoration of lung tissue is regulated by the activation of anti-inflammatory macrophages (M2) once the pathogenic

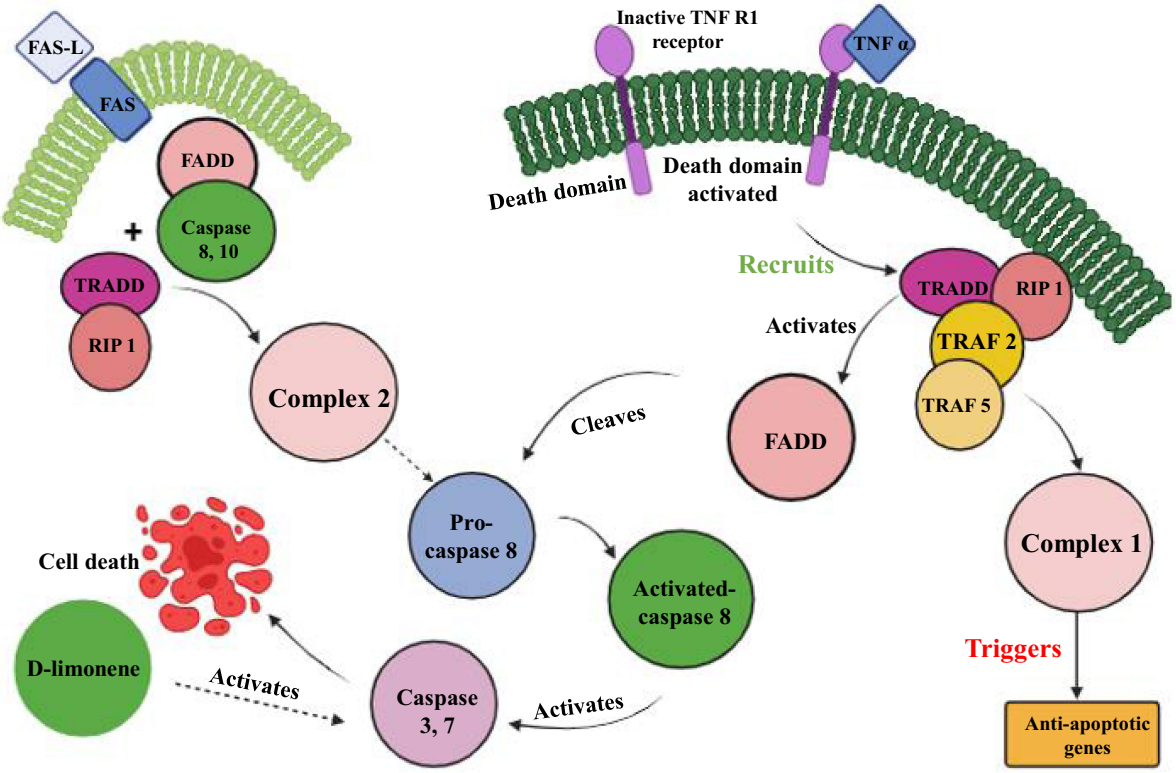

Figure 9 D-Limonene Has Been Found to Activating Caspases Leading to the Death of Cells.

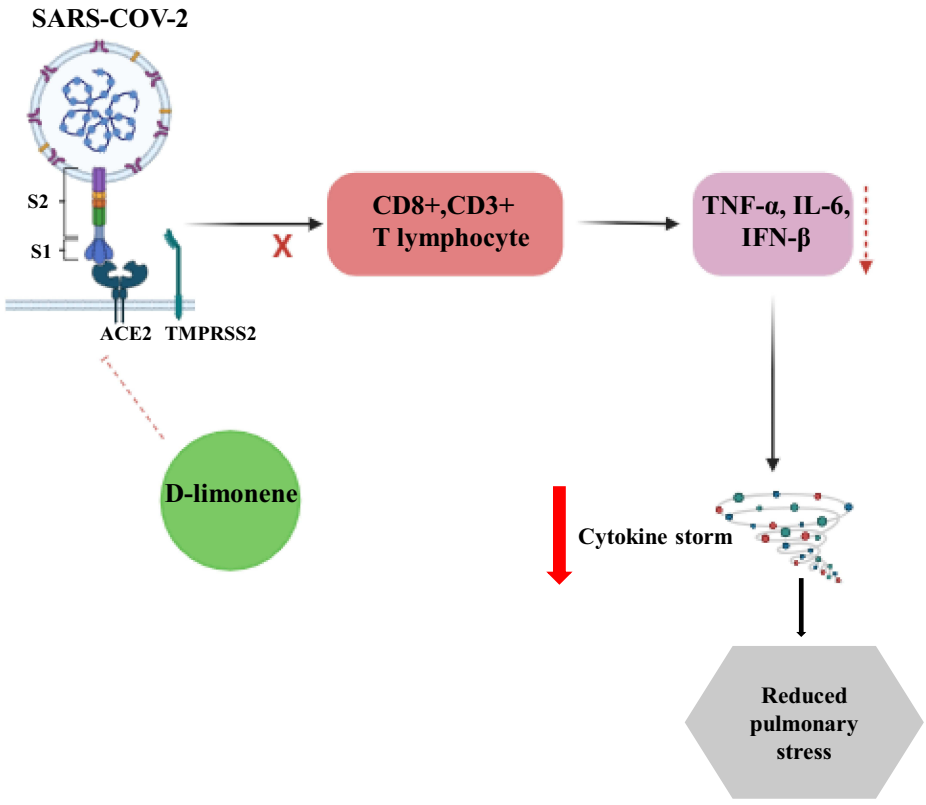

Figure 10 Immunomodulatory Effect of D-limonene in the Context of SARS-COV-2.

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agent is eliminated.[38] In some studies, D-limonene and its metabolites, D-limonene-1-2-diol and perillic acid, were also found to constrain the production of CD3+ CD4+ T cells for IFN-c, IL-2, TNF-α, IL-4, and IL-13, and the production of CD3+ CD8+ T cells for IFN-c, IL-2, and TNF-α (Figure 10).[39]

Neuroprotective Action of D-Limonene

Neurodegenerative diseases are a variety of disorders characterised by neuronal death or degeneration.[40] Alzheimer's disease (AD), multiple sclerosis (MS) and Parkinson's disease (PD) are some of the diseases that are associated with neurodegenerative diseases. Various mechanisms associated with neurodegenerative diseases include oxidative stress, damaged mitochondrial function, neuronal inflammation, abnormal protein deposition, and the induction of apoptosis.[41] Some of the phytochemicals are found to show Neuroprotective action against various neurodegenerative disorders and among the various phytochemicals, D-limonene draws attention towards itself due to its antioxidant and anti-inflammatory action.[42,43]

Alzheimer's disease (AD) is a neurodegenerative disease that causes cognitive impairment and memory loss due to reduced cholinergic neurotransmitter levels and the accumulation of Aβ and neurofibrillary tangles (NFTs).[44,45] To reduce AD symptoms, it is critical to increase Ach levels and prevent proteinaceous deposits from aggregating. Intrinsic techniques include inhibition of the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), which are responsible for Ach breakdown. Limonene inhibited AChE and BChE activities by 10% and 12%, respectively.[46] Alzheimer's disease (AD) is characterised by amyloid plaques that impair synaptic plasticity and cognitive functions.[47] A recent study showed that limonene can prevent neurotoxicity induced by Aβ42 in Drosophila, a fruit fly model of Alzheimer's disease. The study demonstrated that Limonene decreased Aβ42-induced neuronal cell death and reduced ROS levels, adversely affecting extracellular signal-regulated kinase (ERK) phosphorylation. Although limonene did not directly inhibit ERK, it did reduce its activation through its antioxidant effects. D-limonene was tested for its anti-inflammatory properties and was shown to drastically lower the number of activated glial cells and nitric oxide (NO) expression in the heads of flies (Figure 11).[48]

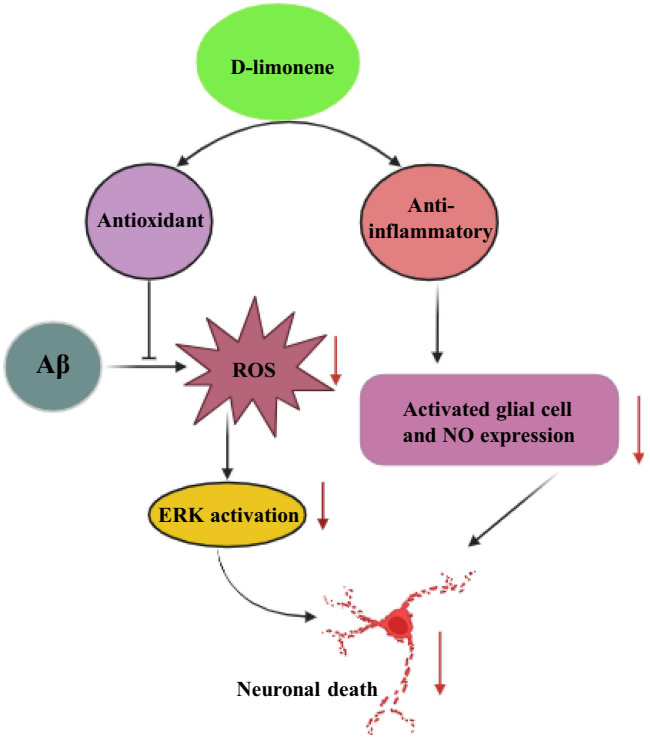

Figure 11 Neuroprotective Action of D-limonene in Alzheimer's Disease.

Toxicity Studies of D-Limonene

D-limonene shows low toxicity effects and has not shown much affirmation in animal studies. Some studies have reported that they are non-carcinogenic in humans and do not show much toxicity, even after several years, when administered at low doses. However, exposure to, or administration of high doses may result in toxicity.[3]

Due to limonene's extremely lipophilic nature, it causes agglomeration of itself in the biological membrane. The mechanism of limonene toxicity involves disarrangement of the cell membrane and inhibition of essential membrane functions. When microorganisms, such as *Pseudomonas*, are exposed to organic solvents, some responses occur, such as differences in the homeostasis of the cell's energy, permutation of the structure of the cell membrane, enhanced generation of chaperones, induction of proteins involved with ROS, and actuation of efflux pump mechanisms. *Pseudomonas putida* exerts high endurance towards some organic molecules and acts as an efficient microbe for processes containing excess limonene. To prevent intracellular accumulation, the export rate of limonene must be high during production. *Alcanivorax borkumensis* when expressed in an *E. coli* strain responsible for producing an inhibiting quantity of limonene, showed a higher concentration of product due to diminished feedback inhibition of the limonene synthesis pathway.[6]

In addition to determining the lethal dose, some studies have focused on the renal pathology, dermal reactions, and mutagenesis.[49] Limonene also causes renal toxicity in male rats. A study was conducted to determine the effects of D-limonene on rat liver. D-limonene and L-limonene are metabolised by P450 enzymes. Studies have suggested that in the conversion pathway from d-carveol to d-carvone, some species-related differences occur during the metabolism of limonene by P450 enzymes. Initially, the enzyme that played a crucial role in the metabolism of d-carveol to d-carvone and vice versa was CYP2C because metabolism was inhibited by anti-human CYP2C12 in animal species. Another study was conducted to regulate the effects of an insecticidal dip containing 78.2% D-limonene on the skin. The recommended concentration are 1.5 Oz/gal of water. At this dose, no toxic effects or lesions were observed. Mild clinical symptoms such as excess secretion of saliva for a short time, ataxia, and muscle tremors were observed when the concentration was multiplied by five times the optimum concentration. By multiplying the concentration 15 times the optimum concentration, the symptoms included hypersalivation but for a comparatively longer time than the previous one, that is, 15 to 30 min, ataxia lasting for 1 to 5 hours, and muscle tremors for 1 to 4 hours. No death or long-lasting effects were observed at any stage.[7]

Renal Pathology

D-limonene actuates hyaline droplets that are the apical cytoplasmic vesicle in the renal pathway of male rats. The protein associated with the maturation of this physiology is alpha-2μ-globulin which can lead to carcinogenesis in the nephron. It is present in some specific strains, such as male rats and guinea pigs, which do not generate the protein alpha-2μ-globulin and produce hyaline droplets. It was discovered that humans generate different kinds of urinary proteins, but they also accord with the same family to which alpha-2μ-globulin accords with that of lipocalins. On the other hand, some evidence shows that the urinary proteins produced by humans are not connected with hyaline-inducing agent ie d-limonene. So it was considered that exposure to d-limonene at lethal doses may induce disturbance in renal pathology.[49]

Dermal Reactions

Occupational exposure to some limonene components resulted in eczema due to allergic contact, but some patch tests revealed that allergic sensitivity was caused by citral and geraniol which are constituents of terpene and are not specifically due to limonene. In association with d-limonene, some studies have revealed that when exposed to light or air, d-limonene undergoes oxidation and produces potent allergens. The transdermal delivery of drugs could have been a potential path if the skin was not a barrier. However, in rats, the percutaneous absorption of drugs such as indomethacin and diclofenac is intensified by the presence of D-limonene.[49]

Carcinogenesis

Some studies have reported that d-limonene has carcinogenic effects on the initiation and development of tumours. Some derivatives of limonene are more effective than limonene in extending tumour latency. Monoterpenes inhibit DNA

synthesis and stop the cell cycle in-vivo in human blood lymphocytes Perillyl alcohol, a derivative of limonene, completely inhibited the augmentation of human HT-29 colon cancer cells. The mechanism by which D-limonene interferes with carcinogenic processes to prevent and inhibit tumours is still under investigation.

D-limonene's versatility broadens from being used in aromatherapy, a fragrance in cleaning products, as a dietary supplement to a potential therapeutic agent in the pharmaceutical industry. To appreciate its diverse and extended roles it is important to understand the underlying mechanism of this versatile compound.

Preclinical Insights of D-Limonene

Preclinical studies serve as an essential foundation for understanding the potential therapeutic benefits and safety profiles of various compounds before they are tested in human subjects. These investigations typically involve in vitro experiments using cells or tissues as well as in vivo studies conducted in animal models. Preclinical research provides valuable insights into the pharmacokinetics, pharmacodynamics, and potential toxicity of compounds, helping researchers to identify promising candidates for further clinical development. Additionally, these studies play a crucial role in elucidating the underlying mechanisms of action and determining optimal dosing regimens. Overall, preclinical studies serve as a critical step in the translational process, bridging the gap between basic research and clinical trials and ultimately paving the way for the advancement of novel therapeutics.

Anticancer Activity

Guang Lu et al (2004) conducted a preclinical study in BALB nude mice to determine the effect of d-limonene on the inhibition of tumour growth in gastric cancer. Gastric cancer cells (BGC-823) were injected into mice to induce cancer. In comparison with other groups, the inhibitory effects of d-limonene and D-limonene on cancerous cells were significant.[50]

An animal study on Sprague-Dawley rats was conducted to ascertain the effect of D-limonene on regressing the growth of tumors in breast cancer by Elegbede et al,1984 0.7,12-dimethylbenz[a]anthracene (DMBA)was administered to induce tumour development. The mice were divided into three groups with variance in the quantity of D-limonene: group 1 (0 ppm), group 2 (1000 ppm), and group 3 (10000 ppm), which were fed one week before DMBA administration until the end of the experiment, and the dietary regimens continued for 27 weeks. D-limonene inhibited carcinogenesis, but there was some difference in inhibition with respect to diet, with a 72% decrease in the mammary tumour at the 18th week in the rats with 10,000 ppm when compared to the control post-DMBA treatment.[51]

Dwarakanath et al (2017) conducted a study on male Wistar rats to determine the additive effect of d-limonene administered in combination with testosterone enanthate, a marketed product used to treat hypergonadism in males. This study also compared the effects of TE+D-limonene and finasteride (therapeutic agents). Testosterone enanthate (TE) was used to induce tumours. After 21 days, the rats induced with d-limonene along with TE showed a significant decrease in prostatic weight, prostatic index, and inhibition of prostatic cell growth compared to finasteride.[52]

Antidiabetic Activity

Saravanan et al (2012) determined the antidiabetic effects of d-limonene in streptozotocin-induced diabetic rats. STZ (40 mg/kg body weight) was used to induce experimental diabetes in male albino rats. The animals were subdivided into seven groups of six animals each: group 1 (normal rats), group 2 (normal+D-limonene [200 mg/kg BW]), group 3 (diabetic rats), group 4 (diabetic+D-limonene [50 mg/kg BW]), group 5 (diabetic+ D-limonene [100 mg/kg BW]), group 6 (diabetic+ D-limonene [200 mg/kg BW]), and group 7 (diabetic+ glibenclamide [600 μg/kg]). The vehicle used was Saline, d-limonene, and glibenclamide were dissolved in a saline solution. D-limonene was administered at different doses (50, 100, and 200 mg/kg body weight) and glibenclamide (600 μg/kg body weight) once daily for 45 days via the oral route. It was observed that on administering D-limonene the blood glucose level decreased gradually and the maximal effect was observed at 100 mg/kg body weight. A decrease in the level of HbA1c and an increase in Hb level was observed upon the administration of D-limonene.[29]

Moradi et al (2021) investigated the antioxidant effect in diabetic rats exposed to d-limonene and showed that D-limonene has hypoglycaemic activity which is related to its antioxidant properties. It was observed that treatment with limonene reduced the levels of MDA (malondialdehyde) and NO (nitric oxide) which are the markers of oxidative stress

and antioxidant status in cancer patients. Limonene on the other side elevated the levels of GSH (glutathione), GPx (glutathione peroxidase), CAT (catalase), and SOD (superoxide dismutase). The results concluded that limonene has better effect on antioxidant markers in comparison to glibenclamide.[53]

Anti-Inflammatory Activity

A study was conducted by d'Alessioet al, 2013 to explore the anti-inflammatory properties of d-limonene. TNBS (2,5,6-trinitrobenzene sulfonic acid (TNBS) was used to induce inflammation in the animal models. D-limonene was administered as a dietary supplement to evaluate its anti-inflammatory activity. D-limonene-fed subjects had lower serum TNF-α concentrations. The anti-inflammatory properties of d-limonene showed both in vitro and in-vivo effects, suggesting its use as a dietary supplement for the reduction of inflammation.[54]

De Souza et al (2018) determined the involvement of d-limonene in reducing inflammation. D-limonene was administered at three different doses ie 25.50 and 100 mg/kg). In this study, it was observed that the most effective dose was 50 mg/kg, where D-limonene decreased the levels of the pro-inflammatory cytokines TNF-α, IL-6, and IL-1β and elevated the levels of the anti-inflammatory cytokine IL-10.[55]

Antioxidant Activity

A study on the effect of D-limonene on reducing acute kidney injury by inhibiting oxidative stress was conducted by Nasri et al, 2021. It was observed that D-limonene reduced oxidative stress by elevating the activity of renal catalase, renal glutathione peroxidase, and renal superoxide dismutase. It further decreased the amount of malondialdehyde and nitric oxide levels which are the markers of oxidative stress.[56]

Rehman et al,2014 conducted tests to determined the role of D-limonene in suppressing oxidative stress and inflammation by regressing COX-2, iNOS (inducible nitric oxide synthase), and NF κB. Doxorubicin (Dox) was used to induce oxidative stress and inflammation in rats for 20 days. Upon administration of Dox in rats, some effects were observed, such as elevation in renal lipid peroxidation; depletion in glutathione content; increase in levels of kidney injury molecule-1 (KIM), blood urea nitrogen (BUN), and creatinine which are kidney toxicity markers; and overexpression of COX-2, iNOS, and NF-κB. After 20 days, treatment with D-limonene restored the levels of antioxidant enzymes and reduced the levels of kidney toxicity markers KIM-1, BUN, and creatinine. The enhanced expression of COX-2, iNOS, and NF-κB also significantly decreased. This study concluded that d-limonene protects against doxorubicin-induced renal damage.[9]

Antimicrobial Activity

Yang et al, 2022 conducted a study to determine the anti-viral activity of D-limonene, a major ingredient of an essential oil, against the influenza virus. The in vitro and in vivo activities of the essential oil and D-limonene were assessed and they exhibited effectiveness against influenza A and B virus. It showed significant inhibitory activity against the virus strain, with an EC50 value of 1.87 μg/mL and a selective index of 85.13. In this study, D-limonene was administered via intragastric and intranasal pathways, and in vivo results showed that it protected 50% of mice from influenza virus as it inhibited the strain at the intracellular replication stage. It was observed that major inflammatory cytokines, such as IL-1β, IL-6, and TNF, decreased in subjects treated with D-limonene. Additionally, viral particles and viral genomic RNA were reduced by nearly 2-fold in subjects treated with D-limonene. Therefore, we concluded that D-limonene has the potential to be a potent anti-viral agent.[57]

To evaluate the anti-fungal activity and intravaginal fungal infection of D-limonene against different strains of Candida, Munoz et al,2020 conducted a study. This study was conducted in isogenic female BALB/c mice. Limonene at concentrations of ≥500 μM had the capability inhibited the growth of *C. albicans, C. krusei, C. glabrata* and *C. parapsilosis*. Transmission electron microscopy (TEM) analysis of Candida revealed damage to the cell wall, intracellular structure, nuclear alterations, specific changes in genetic material and mitochondria, and cell wall rupture. It was suggested that these structural changes occurred because of the limonene inoculation. D-limonene did not induce mutagenic, carcinogenic, or nephrotoxic effects. From all the study reports, it was concluded that d-limonene can be utilised as an anti-fungal agent for treating vulvovaginal candidiasis, along with other fungal disorders.[58]

Immunomodulatory Activity

Raphael et al (2003) determined the immunomodulatory effects of monoterpenes in BALB mice. Different monoterpene derivatives such as carvone, perillic acid, and limonene have been investigated. It was concluded that limonene exhibited immunomodulatory effects better than any other derivatives. They elevated the production of antibodies in the spleen and bone marrow more effectively than any other derivative.[35]

Lappas et al (2012) conducted an animal study to investigate its immunomodulatory effects. This study reported inhibition of cytokine production by CD4 and CD8 T cells by d-limonene and its derivatives. They modulate the expression of activation markers in T-cells. It has been observed in some reports that D-limonene elevates the total antibody production and bone marrow cellularity. Depending on the administration time and dose, it may either suppress or enhance the proliferation of lymphocytes and antibody response in animals. Elevated NO production and phagocytic activity. Additionally, it reduced the lipopolysaccharide-induced production of prostaglandin E2 and pro-inflammatory cytokines in RAW264.7 macrophage. Furthermore, high concentrations of D-limonene and its derivatives induced T-lymphocyte death. Thus, this limited dataset suggests that D-limonene can be considered as a candidate for immunomodulatory effects for therapeutic purposes.[39]

Arreola et al (2005) performed animal studies to evaluate the effects of D-limonene on the immune system of tumour-bearing BALB/c mice. For evaluating the effect of D-limonene it was incorporated into the diet of the mice and were sensitized with 2.4-dinitroflurobenzene (DNFB). The results showed that D-limonene elevated the survival of tumour bearing mice, delayed the hypersensitivity reaction to DNFB, decreased phagocytosis and microbicidal activity. It also elevated the production of nitric oxide (NO) in the macrophages of the tumour bearing mice. It was concluded that D-limonene efficiently modulates the immune response and can be utilised for its immunomodulatory effect.[59]

Table 1 Tabular Representation of Preclinical Studies of D-Limonene

S. No	Application	Animal Model	Outcome	Ref.
1	Gastric cancer	BALB nude mice	Inhibition of BGC-823 gastric cancer cell line significantly	[50]
2	Breast cancer	Sprague-Dawley rats	72% regression in mammary tumors induced by DMBA in the 18th week w.r.t diet	[51]
3	Prostate cancer	Male Wistar rats	Additive effect of D-limonene with testosterone enanthate led to downfall in prostatic weight and prostatic index further inhibiting prostatic cell growth.	[52]
4	Anti diabetic	Male albino rats	Significant decrease in blood glucose level and HbA1c level along with an increase in Hb level with maximum effect w.r.t diet	[29]
5	Anti diabetic	Wistar rats	Reduced the levels of MDA and NO and elevated GSH, GPx, CAT, and SOD levels in diabetic rats	[53]
6	Anti inflammatory	Wistar HsdBrlHan female rats	Decreased the level of serum concentration of TNF-αsuggesting D-limonene as a dietary supplement for reduction in inflammation.	[54]
7	Anti inflammatory	Male Wistar rats	Significant decrease in the levels of pro-inflammatory cytokines, TNF- α, IL-6, and IL-1β and elevation in levels of IL10 anti-inflammatory cytokine.	[55]
8	Antioxidant	Male Wistar rats	Decreased the levels of oxidative markers, malondialdehyde, and nitric oxide and uplifted the activity of renal catalase, renal glutathione peroxidase, and renal superoxide dismutase leading to the reduction of oxidative stress.	[56]
9	Antioxidant	Wistar rats	Decline in overexpression of COX-2, iNOS, and NFκB	[9]
10	Antiviral	Mice	Significant inhibitory activity against virus strain with EC50 value of 1.87 μg/mlat intracellular replication stage and selective index of 85.13.	[57]

(*Continued*)

Table 1 (Continued).

S. No	Application	Animal Model	Outcome	Ref.
11	Antifungal	Isogenic female BALB/c mice	Inhibit the growth of *C. albicans, C. krusei, C. glabrata*, and *C. parapsilosis* at concentrations ≥ 500 μM and damage the cell wall and genetic material.	[58]
12	Immunomodulatory	BALB mice	Elevated the production of antibody in spleen and bone marrow	[35]
13	Immunomodulatory	Female C57BL/6J mice	Elevation of antibody production and bone marrow cellularity further inhibiting CD4 and CD8 T cells.	[39]
14	Immunomodulatory	Tumour bearing BALB/c mice	Increased the production of nitric oxide in the macrophages of the tumour bearing mice and modulated the immune response	[59]

The preclinical studies on D-limonene are summarized in Table 1, which highlights its biological activities, experimental models, and potential therapeutic applications.

Limonene in the Clinic: Clinical Trials and Studies

Clinical studies represent a critical phase in the journey of biomedical research, where the efficacy and safety of a compound are evaluated in human subjects. These investigations, conducted following promising results from preclinical studies, aimed to translate laboratory findings into real-world applications. Clinical studies have provided essential insights into how a compound interacts with the human body, its therapeutic potential across different conditions, and any potential side effects or adverse reactions. Typically organised into phases, these trials progress from initial safety assessments in small groups to larger-scale studies examining efficacy and long-term effects. By rigorously assessing the performance of a compound in diverse patient populations, clinical studies can play a pivotal role in determining its suitability for widespread therapeutic use, ultimately shaping medical practice and improving patient outcomes.

As limonene has a very low toxicity level, it has been tested for carcinogenicity in mica and rats. It has been reported that initially D-limonene elevated the occurrence of renal tubular tumors causing mutagenic effect in male rats, female rats. When this study was established in humans, it was seen that D-limonene does not exhibit mutagenic, carcinogenic or nephrotoxic risk to humans. D-limonene is a solvent of cholesterol; therefore, it has been used in the dissolution of cholesterol containing gallstones. Another extensive clinical application of D-limonene is its ability to relieve heartburn, as it is a potential for gastric acid neutralization and is responsible for healthy peristalsis. Apart from that, chemotherapeutic activity of D-limonene against many types of cancers has been well-established.

Igimi et al performed clinical trials to dissolve gallstones by infusing different quantities of limonene. Infusion of D-limonene into the animal gall bladder dissolved and disintegrated the gallstones, which were later excreted through the bile duct. In humans, following gallstone surgery, an infusion of 20 mL D-limonene dissolved the residual stones.[21] This concentration was infused every other day until dissolution was complete. However, in some patients, gallstone dissolution was seen after three infusions. This study was performed on a larger group of 200 people where a straight infusion of 20–30 mL D-limonene (97% solution) effectively eliminated gallstones of the size range between 0.5 and 1.5 cm with an average diameter of 1.0 cm in 141 individuals. Complete dissolution of gallstones was observed in 96 cases (48%), partial dissolution in 29 (14.5%), and complete dissolution with hexametaphosphate (a chelating agent known to be a good solvent for the dissolution of bilirubin calcium stones) in the remaining 16%.[60]

In 1973, a 36-year-old housewife weighing 56 kg was hospitalised for upper right quadrant colic jaundice due to cholelithiasis. The stones removed during the surgery were mostly cholesterol stones. However, four stones 10–15 mm in diameter were found below the tube. The D-limonene preparation (5 mL of D-limonene preparation was gradually introduced into the CHR-tube for the infusion of D-limonene. Following the infusion, which continued for a few weeks, two stones dissolved after 6th infusion and the rest, after the 13th infusion.[24]

D-limonene is also useful for relieving occasional heartburn and gastroesophageal reflux disease (GERD). In this clinical study, 19 patients with persistent heartburn or GERD were asked to use D-limonene to alleviate their symptoms.

All patients experienced chronic heartburn or GERD for at least five years, with symptoms ranging from mild to severe. Depending on the severity and frequency of symptoms, a capsule of 1000 mg of D-limonene was administered every day or every other day. 32% of participants experienced relief from symptoms after D-limonene administration. As the day passed, the relief rate gradually improved and the patients achieved complete relief of symptoms.[61]

Similarly, in another placebo-controlled study, 13 volunteers with mild, moderate, or severe heartburn/GERD were randomly assigned to either the D-limonene or placebo groups. Seven subjects received 1000 mg of D-limonene every day or every other day, whereas six received a placebo pill containing soybean oil. Depending on the severity and frequency of symptoms, D-limonene was administered with a significant reduction in the symptoms of gastric reflux. 29% of participants in the D-limonene group received considerable improvement of symptoms (severity rating = 1–2), compared to no reduction of symptoms in the placebo group. With the ongoing administration of D-limonene by day 14, 86% of the subjects confirmed a reduction in symptoms compared with 29% of the participants in the placebo group. These two studies indicate that the positive benefits of D-limonene gradually emerge over time, with the highest outcomes observed with the 10-capsule regimen. However, the mechanism of action of D-limonene has not been entirely understood. An in-vitro research reveals that it may protect the stomach wall and mucosal lining against gastric acid exposure.[61]

D-limonene is a natural monoterpene with significant chemotherapeutic activity and a low toxicity in preclinical studies. In a Phase 1 clinical study based on D-limonene anticancer activity in a female patient with advanced breast cancer, D-limonene demonstrated partial beneficial response at a dose of 8 g/m^2/day. During the first five treatment cycles axillary and supraclavicular lymph nodes that contain metastatic infiltrating ductal carcinoma remained stable. More than 50% reduction in supraclavicular lymphadenopathy was determined at the beginning of the sixth cycle and by 14th cycle bone pain decreased and axillary lymph nodes were no longer palpable.[1]

In a study three individuals having colorectal carcinoma, were able to halt illness over a period of six months because of the action of D-limonene. Similarly when D-limonene was consumed at a dosage of 0.5 g/m^2/day, cancer was controlled in a patient affected by locally advanced mucinous cystadenocarcinoma of the appendix. A minor reduction in tumour size was observed in a patient with presacral recurrence of adenocarcinoma of the sigmoid colon.[62]

An epidemiological study reported that D-limonene levels were higher in individuals without epithelial cell carcinoma after consuming significantly more citrus peel than in those with epithelial cell carcinomas. This study concluded that citrus peel, which is a source of d-limonene, has the potential to prevent cell carcinoma.[63]

Formulation Innovations: Harnessing the Power of Limonene

The challenges related with the delivery of D-limonene generally rotates around its physicochemical properties which impacts its stability and bioavailability and makes its delivery a major issue of concern. D-limonene's solubility issues make its formulation into aqueous based system a challenging task as it is hydrophobic in nature. It has a vigorous citrus odour and taste which can be unacceptable in some formulations and the masking of these properties and on the other hand maintaining the integrity of the delivery system and therapeutic efficacy can be challenging. It is also found to have caused dermal allergy in the formulations that enhance its penetration into the skin. This can pose as a challenge in synthesising safe and effective topical products. Addressing these challenges for enhancing D-limonene's stability, solubility, and overall delivery efficiency requires innovational formulation approaches.[5]

Novel and nanoformulation strategies for limonene delivery represent cutting-edge advancements in pharmaceutical and biomedical research, aiming to enhance the compound's therapeutic efficacy and bioavailability while mitigating potential side effects.[64] These innovative approaches leverage nanotechnology and advanced formulation techniques to encapsulate limonene within nanoscale carriers, such as liposomes, nanoparticles, or micelles. By encapsulating limonene, these formulations offer several advantages including improved solubility, controlled release kinetics, and targeted delivery to specific tissues or cells. Moreover, nanoformulations can protect limonene from degradation and facilitate its passage across biological barriers, such as the blood-brain barrier, thereby enhancing its therapeutic potential for various applications, including drug delivery, cancer therapy, and the treatment of inflammatory diseases. In this section, we delve into the exciting developments and promising applications of novel and nanoformulation strategies for limonene delivery.

Anticancer Activity

Self-assembled nanoparticles containing doxorubicin and d-limonene, which passively target cancer cells, have been formulated by Assali et al. The clinical use of Dox is hindered by its dose-dependent cumulative cardiotoxicity caused by free radicals. D-limonene is a potent antioxidant agent and possesses the ability to reduce the generation of free radicals. It is hydrophobic, because it interferes with its use and efficacy. Nanoparticles have been formulated to increase their solubility and bioavailability and enhance their anti-cancer effects. In this study, the EPR effect was utilised to formulate nanoparticles, and three different self-assembled nano delivery systems were formulated: nanoemulsion, niosomes, and polylactide nanoparticles, to determine their capacity to load Dox and D-limonene, release Dox data, and their anti-cancer and antioxidant properties. Nanoemulsions were prepared by two surfactants Tween 80 and Span 80, with an HLB value of 12 and the addition of d-limonene as an antioxidant formulated by ultrasonic emulsification. Various nanoemulsions were formulated with varying amounts of surfactant and a fixed amount of D-limonene and analysed to determine their polydispersity index (PDI). The formulation had a mean size of 52 nm with a PDI of less than 0.3, and a zeta potential of-33.8 mV which is an indicator of high surface charge repulsion which leads to stability. It also demonstrated the highest loading efficiency owing to the large surface area and solubility of Dox in the core of nanoemulsion. Niosomes were prepared using Tween 80 and cholesterol via the film hydration method. The size of the formulation was 180 nm, with a PDI value of less than 0.3 and a zeta potential of-37.29 mV indicating high stability. Polylactide nanoparticles were formulated by a nanoprecipitation technique with a size of 257 nm, zeta potential of −23.86 mV and PDI value of 0.153, indicating improved polydispersity. In addition, it exhibited the highest release rate. DPPH assay was used to determine D-limonene's and nanoparticles loaded with d-limonene's antioxidant capability. It was observed that while Dox was loaded inside nanoparticles, they retained the antioxidant activity of d-limonene. Therefore, they might decrease the cardiotoxic effects of Dox owing to the generation of free radicals. The anti-cancer properties of the nanoparticles were evaluated in liver cancer cells (HepG2) and normal liver cells (LX2) after incubation at different concentrations, and the Dox-loaded PDLLA nanoparticles exhibited the highest activity. It was concluded that encapsulating Dox with D-limonene resulted in antioxidant activity which reduced the production of free radicals and cytotoxicity. The limonene-loaded nanoemulsion and limonene-loaded polylactide nanoparticles exhibited distinct anticancer activity. Thus, these two nanocarriers could be a potential candidate for anticancer therapy.[65]

Hajizadeh et al (2019) formulated D-limonene niosomes to enhance solubility. D-limonene is a potent anti-cancer agent; however, its low solubility poses a problem in cancer therapy. The formulation was prepared by mixing Span 40, Tween 40, and cholesterol using the film-hydration technique. The formulations were spherical unilamellar with sizes < 200 nm. The vesicle size elevated from 90–102 nm to 110–1100 nm after loading with D-limonene. The PDI of the formulated niosomes was 0.37, indicating appropriate size homogeneity. The zeta potential of the loaded niosomes was −45 mV. When D-limonene into niosome it exhibited greater negative values. The entrapment efficiency of the formulation was satisfactory (87%) because of the strong interaction between the surfactant, cholesterol, and D-limonene which made the walls of the vesicles rigid and prevented leakage of D-limonene from the formulation. The release profile of D-limonene from niosomes was analysed and it was observed that it got released in a sustained manner as a function of time. The zeta potential of niosomes lied in a range that exhibits high stability owing to electrostatic repulsion. The anticancer effect was studied in three different cell lines, HepG2, A549, and MCF-7, incubated at different concentrations with empty niosomes, D-limonene, and loaded niosomes, and assessed using the MTT assay. These results indicate that the loaded niosomes were more efficient than free d-limonene. The cytotoxic effect of D-limonene-loaded niosomes was attributed to the synergistic properties of solubility, cellular internalisation, and controlled release of d-limonene. It was concluded that D-limonene-loaded niosomes exhibited better anti-cancer activity than free phytochemicals, and that their cancer-treating ability could be enhanced by loading them into niosomes.[66]

Rani et al, 2023 synthesised a pH-responsive drug delivery system for liposomal carriers loaded with D-limonene for targeting glioma cells in humans. Cancer cells have acidic pH so this study formulated pH-sensitive liposomal carriers which would target the cancer cells efficiently at acidic pH. It was observed that the drug-loaded liposome got dispersed and easily penetrated into the cells and showed action. This study concluded that the formulation induced early apoptosis and displayed anti-cancer activity effectively.[67]

Chou et al,2020 designed and formulated self-microemulsifying drug delivery system (SMEDDS) containing D-limonene for the treatment of colorectal cancer. The anti-cancer drug 5-Demethyltangeretin (5-DTAN) was dissolved in anhydrous ethanol and hydrochloric acid and the obtained solution was heated to reflux for 16 hours. The solubility of 5-DTAN was studied in various oils, surfactants, and cosurfactants such as orange oil, D-limonene (5%), ethyl oleate (10%), and PEG400 (25.5%). 5-DTAN exhibited more solubility in D-limonene i.e 1.28 ± 0.004 mg/mL with mean droplet size of 97.1±6.50 nm determined by TEM and showed spherical shape and uniform size with PDI value 0.390 ±0.008. The zeta potential of D-limonene loaded SMEDDS was −38.37±0.11 mV and highest loading 3.01 ± 0.38 mg/mL. Based on these results, the ability of the D-limonene-containing SMEDDS to form stable microemulsion droplets in the GI digestive system was evaluated. Studies have concluded that SMEDDS is an effective carrier for augmenting the in vivo efficacy of 5-DTAN containing D-limonene as an oil. Improvement in oral absorption and pharmacokinetic properties led to the enhancement of therapeutic efficacy for SMEDDS of 5-DTAN containing D-limonene against colon cancer and serves as a promising nutraceutical candidate as anti cancer.[68]

Antioxidant Activity

A nanoemulsion of D-limonene was synthesised by Kaur et al (2020 to determine its antioxidant activity. The effectiveness of D-limonene is limited by its high volatility, poor stability, sensitivity to sunlight, and easy degradability. To overcome these challenges nanoemulsion of d-limonene has been formulated. A nanoemulsion was prepared by mixing D-limonene as the oil phase, Tween 80 as the surfactant, and ethanol as the co-surfactant using the spontaneous emulsification method. TEM analysis showed that the nanoemulsion was spherical in structure and uniformly distributed, with a size of 59.06 nm and PDI value of 0.125, indicating a homogenous colloidal solution. The zeta potential was −14.9 mV suggesting a higher degree of stability. DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed to determine the radical scavenging rate of DPPH in the presence of antioxidant compounds in the loaded nanoemulsion. The IC50 value of loaded nanoemulsion was found to be 4.74 µL/mL and free d-limonene was found to be 5.98 µL/mL. Similarly, the ABTS assay showed IC50 value of 4.53 µL/mL for the loaded nanoemulsion and 6.51 µL/mL for free D-limonene. This indicated that the nanoformulations had a greater ability to scavenge free radicals at lower concentrations than free D-limonene. The formulation scavenged OH free radicals in a dose-dependent manner. The release profile of the formulation suggested that 80% of the drug was released in the first 6 h and 20% was released at other times. Stability studies of the loaded formulations suggested that for a specific period of time, the loaded nanoemulsion had a higher stability percentage index than free limonene. From all analyses and studies, it was concluded that the antioxidant activity and stability of the formulated nanoemulsion were greater than those of limonene itself, and the D-limonene-loaded nanoemulsion can be an effective alternative nanoformulation for exhibiting antioxidant properties.[69]

Sarjono et al (2019) formulated D-limonene encapsulated in chitosan to enhance antioxidant activity. D-limonene exhibited the property of inhibits free radicals; however, its use is limited by its hydrophobicity. Therefore, it was encapsulated with chitosan to protect it from degradation and increase its solubility which would further result in an increase in the inhibition of free radicals. Limonene was prepared by mixing limonene, ethanol, and Tween 80 as nonionic surfactants using the spontaneous emulsification nano-method. The prepared emulsion was encapsulated in chitosan by adding modified chitosan as a coating agent and was freeze-dried. The nanoparticles were prepared using the ionic gelation method by mixing chitosan polymer with the polyanion sodium tripolyphosphate (Na-TPP). This procedure was performed to increase solubility in water. The particle size was 339.5 nm in limonene emulsion and 2249.7 nm on chitosan modification using Na-TPP with PDI of 0.772. Based on this information, it was characterised as micro-sized but not homogenous. % encapsulation efficiency at 100 ppm concentration with absorbance of 0.372 was 46% antioxidant tests were performed using DPPH assay. The encapsulated limonene exhibited an IC50 value of 116 ppm. Based on the above data, it was concluded that limonene encapsulated in chitosan showed better antioxidant activity.[70]

Antimicrobial Activity

Polymeric nanoparticles loaded with d-limonene for enhancement of the antimicrobial nature of d-limonene were designed and formulated by Andriotis et al,2021. The nanoparticles were synthesized for potentiating the application of D-limonene in food packaging owing to its antimicrobial activity. They were prepared mini emulsion polymerization

technique by mixing of methyl methacrylate (monomer), trimethylene glycol dimethacrylate (cross-linking agent), hexadecane (co-stabilizer), d-limonene and benzoyl peroxide (radical initiator). The synthesised nanoparticles contained 10.9% w/w d-limonene with entrapment efficiency close to 100%. The TGA analysis were performed to determine the release pattern of volatile oil and it revealed the presence of both d-limonene and hexadecane due to the penetration enhancement property of d-limonene. The antimicrobial activity was performed against four species ie *Escherichia coli, Salmonella enterica, Campylobacter jejuni*, and *Staphylococcus aureus*. The efficiency of the loaded formulation as antimicrobial agent was greater even though the total amount of d-limonene was low. Additionally it was observed that antimicrobial efficiency of d-limonene nanoformulation was elevated on addition of ε-polylysine. After all the above analytical tests it was concluded that d-limonene when encapsulated within any nanoformulation exhibited greater antimicrobial property even at low concentrations.[71]

Zahi et al,2015 synthesized d-limonene containing organogel-based nanoemulsion to determine their antimicrobial activity. These were synthesized as under normal storage conditions d-limonene undergoes oxidative degradation which results in loss of flavour and formation of flavours. To prevent this, its antimicrobial properties needed to be enhanced. D-limonene organogel nanoemulsion was synthesised by mixing of D-limonene organogel (oil phase), Milli-Q water containing Tween 80 (water phase) by high-pressure homogenisation. Tween 80 played a crucial role in the particle size as more surfactant dissolved the dispersed phase (D-limonene organogel) in the dispersing phase (water). The formulated nanoemulsion showed a small droplet size with a diameter of 36.60 nm and good stability for approximately 5 months. An antimicrobial study was performed against *E. coli, S. aureus, B. subtilis and S. cerevisiae*. The MIC value was measured by a broth dilution assay and revealed that 1 mg/mL was sufficient to stop the growth of all four microorganisms. The MIC values were 0.5 mg/mL against *E. coli* and *B. subtilis*, 0.125 against *S. aureus* and 0.25 mg/mL against *S. cerevisiae* suggesting that organogel based nanoemulsion can enhance the inhibitory ability of D-limonene as its small size can easily fuse with the microorganism and cause death. From all the above results it was concluded that on comparing free D-limonene and nanoformulated d-limonene it was clearly found that there was improvement in the antimicrobial activity of D-limonene after incorporating it into nanoemulsion.[72]

Umagiliyage et al (2017) formulated liposomes containing D-limonene for enhancing its antimicrobial property. They were synthesised by mixing of dimyristoylphosphatidylcholine (DMPC), poly-diacetylene, and N-hydroxysuccinimide dissolved in dichloromethane. The mean radius of the liposome was found to be 100.2 nm and after five weeks of storage, it was reduced to 100 nm because limonene protected the particles from oxidative degradation, resulting in fewer chances of losing activity. The antimicrobial activity was evaluated against *E. coli* and *L. monocytogenes*. The results suggest that the antibacterial effect was greater on the gram-positive bacterium E. coli than on the gram-negative bacterium *L. monocytogenes*. Differences in the susceptibility of gram-positive and gram-negative bacteria to limonene may be due to differences in the permeability of bacterial envelopes and the degradation of bioactive ingredients. This study concluded that D-limonene exhibited satisfactory physical stability and stable in vitro antimicrobial activity compared with free D-limonene. It was concluded that D-limonene-loaded liposomes could be utilised as effective antimicrobial agents rather than D-limonene itself.[73]

Wang et al, 2023 formulated nanostructured lipid carriers containing D-limonene for enhancing its anti-fungal activity. These were synthesized as D-limonene is an unstable substance and gets easily volatilized and oxidized which leads to its loss in activity. It was observed that the formulated lipid carrier droplets were well dispersed and exhibited excellent storage stability. It was concluded that D-limonene when encapsulated within nanostructured lipid carriers enhanced the anti-fungal property.[74]

Sedeek et al, 2021 formulated a hexosomal dispersion to enhance anti-fungal properties of d-limonene. This formulation was synthesised by a hot emulsification method using glyceryl monooleate (1 g), oleic acid (0.5 g), and volatile oil (1 g). The mixture was allowed to melt at 70°C, added to the aqueous phase, and homogenised for 5 min, leading to the formation of a milky white dispersion. The mixture was allowed to cool to room temperature. The anti-fungal properties of different citrus peel essential oils, such as *C. lemon, C. aurantifolia, C. maxima and C. sinensis* against some phytopathogenic fungi, such as *Rhizoctonia solani, Sclerotium rolfsii, Fusarium solani, Fusarium oxysporum and Alternaria alternata*, and it was determined that the percentage of D-limonene was highest in *C. lemon* (64.13%) and *C. aurantifolia* (80.34%). The amount of oxygenated hydrocarbons responsible for anti-fungal activity was

higher in *C. lemon* (33.53%) and *C. aurantifolia* (14.59%), which additively enhanced the antifungal activity of D-limonene. The formulated D-limonene-loaded hexosomal dispersion had a particle size of 210.35± 3.18 nm and acceptable PDI range of ie 0.31 ± 0.0.6. The formulated dispersion exhibited a negative zeta potential (−16 ± 0.84 nV which is attributed to the presence of carboxylic acid groups in oleic acid. TEM evaluation of the formulation revealed hexagonal non-aggregated particles. The lowest IC50 values were observed for *F. oxysporum* (36.92 and 41.72 µL/mL) for *C. lemon* and *C. aurantifolia* respectively. And the highest IC50 value was exhibited with *B. cinerea* (78.60 µL/mL) for *C. lemon. C. lemon* and *C. aurantifolia* both with high concentrations of D-limonene, exhibited potential anti-fungal effects. This study concluded that a hexosomal dispersion containing D-limonene as an active ingredient exhibited anti-fungal properties and could also be utilised as a nano-fungicide against plant fungal pathogens.[75]

Anti-Inflammatory Activity

Elghani et al (2023) formulated a nano-hexosomal formulation of D-limonene to determine its anti-inflammatory properties by inducing peptic ulcers associated with Helicobacter pylori. Limonenes are a predominant class of compounds. The anti-inflammatory properties of D-limonene were compared with those of the standard marketed drug, clarithromycin. The MIC 90 of clarithromycin was 1.95 ± 0.002 µg/mL and that of the formulated nano hexosomal was 1.95 ± 0.0029 µg/mL. Thus, d-limonene exhibited more efficient anti-inflammatory activity than the standard drug, clarithromycin. A anti-inflammatory assay via COX-2 inhibition in comparison with another standard Celecoxib (IC50 = 0.28 µg/mL) suggested that the formulated nanoformulation is more potent than D-limonene alone with IC50 value 0.85 µg/mL and 2.04 µg/mL, respectively. An in silico study of D-limonene using *H. pylori* urease enzyme revealed that D-limonene, when formulated with nanoformulation, exhibited promising anti-inflammatory activity to combat Helicobacter pylori and is safe, natural, and cheap.[76]

Topical microemulsions of herbal substances, such as limonene, were formulated by Leanpolchareanchai et al (2023) to determine their anti-inflammatory properties in the skin. D-limonene is a plant extract with limitations, such as low skin absorption and stability issues. To overcome these challenges, microemulsions have been synthesised. It was concluded that the D-limonene-loaded topical microemulsions were more stable, showed enhanced absorption on the skin, and exhibited potent anti-inflammatory activity.[77]

Effect on Gall Stones

Zou et al, 2022 formulated docosahexaenoic acid-coupled limonene bovine serum albumin nanoparticles (LIM-DHA-BSA-NPs). The formulation was spherical in shape with a uniform distribution. Compared with free D-limonene, the formulated nanoparticles showed greater uptake by RAW264.7 cells. The fluorescence intensity of the formulated nanoparticles was greater than free D-limonene which suggested that the uptake of DHA conjugated BSA nanoparticles by RAW264.7 cells was stronger than free D-limonene. In contrast, the semi-quantitative fluorescence intensity showed that the uptake of the formulated nanoparticle was 4.5 times greater than that of free D-limonene. These nanoparticles can target the gallbladder. Additionally, the nanoparticles decreased the concentrations of nitric oxide (NO), aspartate transaminase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and glutamyl endopeptidase (GGT and total bilirubin, TBIL) enzymes. The formulated nanoparticles exhibited higher uptake rates, a uniform distribution, and lower cytotoxicity. Thus, LIM-DHA-BSA-NP can be utilised as an effective anti-inflammatory agent and can be absorbed by megalin receptor-mediated endocytosis; thus, it can be an effective treatment for cholecystolithiasis.[78]

Neuroprotective Effects

A propranolol-loaded intranasal limonene-based microemulsion mucoadhesive nanogel was designed and formulated by Abla et al,2022 to elevate the efficiency of propranolol, a first-line drug for the treatment of migraine. The formulation was prepared by incorporating limonene oil into Gelucire (oily phase), Labrasol (surfactant), and Labrafil (co-surfactant) via spontaneous emulsification. The loaded nanogels showed a nanometric size of 133.7 nm with a PDI score of 0.112 because of the administration of Labrasol and Labrafil which elevated the entropy of the system and reduced the interfacial tension and free energy of the system. The zeta value of the nanogels was −7.2 mV which was quite low because of the addition of Labrasol, a non-ionic surfactant. They exhibited high entrapment efficiencies and drug-loading

capacities of 81.9% and 117 mg/g, respectively. The optimum gelation temperature range was 30–34°C with a time range of 35–48 seconds. A short gelling time is crucial to avoid rapid clearance of the formulation and to increase its residence time in the nasal tissue. The optimum gel strength is 42s, as gels with less than 25s may erode rapidly, and gels with more than 50s may irritate the nasal mucosa. The pH of the formulated nanogel was 6.08, suggesting that it did not cause irritation during application. The drug content of the formulation was approximately 89% which is satisfactory. The mucin suspension had zeta value of −10.3 mV which decreased to −3.9 mV on addition of the prepared nanogel indicating its mucoadhesive property. The brain uptake study revealed that AUC $_{(0–7h)}$ of the intranasal groups was 4884.15 ng.h/g and $t_{1/2}$ was about 1.5 times more than that of the oral treated groups. Relative brain availability was 382.4%, suggesting an efficient brain-targeting feature of the formulation.

The improved brain targeting of the microemulsion of propranolol could be because limonene has penetration-enhancing capabilities that elevate the fluidity of the nasal and brain lipid bilayers. P-gp exists abundantly in the olfactory region, and Labrasol can inhibit P-gp activity by interrupting the hydrophobic environment and improving drug permeation into the brain. From these studies, it was concluded that the formulated nanogel improved the availability of propranolol in the brain and could be used as a potential formulation for the treatment of migraine.[79]

Zakharova et al, 2023 designed and formulated nanocarriers of limonene-loaded rivastigmine to enhance AD treatment. A major reason for the formulation of nanocarriers is the insufficient efficacy of the recently available therapeutic strategy. It was concluded that these formulations enhanced the bioavailability of the drugs, increased circulation, and crossed the BBB.[80]

El Shagea et al, 2024 formulated novasomal gels of rasagiline mesylate containing limonene for the treatment of Parkinson's disease via a transdermal route. Nanovesicles with small sizes and large surface areas are advantageous for enhanced drug permeability and efficacy across biological membranes. Additionally, the transdermal route is advantageous for delivery, as hydrophilic drugs have very limited penetration efficacy via the skin, which can be overruled by elevating their lipophilicity by incorporating them into lipid vesicles. They were prepared by mixing limonene as a penetration enhancer, cholesterol, surface-active agents, and free fatty acids by ethanol injection. The formulation was then incorporated into a gel system comprising of 2% HPMC and 2% CMC. TEM results showed well-defined spherical nanovesicles with a particle size of 265.90 nm and a zeta potential value of −33.45 mV. The entrapment efficiency of the formulation was found to be 83.09% and the release was controlled. The novasomal gel decreased the levels of interleukin-17, pro inflammatory cytokine, and inhibited NLRP-3 inflammasome activation, leading to a reduction in dyskinesia. Thus, it was concluded that the novasomal

Table 2 Delivery Systems of D-Limonene

S. No.	Application	Delivery System	Inference	Ref.
1	Anticancer	Self-assembled nanoparticles	Significant tumor growth delay and anti-cancer activity in HepG2 and LX2	[65]
2	Anticancer	Niosomes	Synergistic effect of solubility, internalization of cells and controlled release of D-limonene led to enhanced anti-cancer effect against HepG2, A549, and MCF-7 cell lines	[66]
3	Anticancer	pH-responsive liposomal carriers	Instigate early apoptosis in the glioma cells by targeting the cancer cells at an efficient pH	[67]
4	Anticancer	SMEDDS	Improvement in oral absorption and pharmacokinetic parameters with solubility of 1.28 ±0.004 mg/mL, PDI value 0.390±0.008 and zeta potential of 38.37±0.11 mV leading to an effective therapeutic efficacy as a nutraceutical	[68]
5	Antioxidant	Nanoemulsion	Decreased the IC50 value and scavenged free radicals at lower concentrations in comparison to free D-limonene	[69]

(*Continued*)

Table 2 (Continued).

S. No.	Application	Delivery System	Inference	Ref.
6	Antioxidant	Chitosan-encapsulated D-limonene nanoparticles	Enhanced solubility and antioxidant effect with IC50 value of 116 ppm and %EE of 46%	[70]
7	Antimicrobial	Polymeric nanoparticles	Tested against *E. coli, Salmonella enterica, C. jejuni*, and *S. aureus* with %EE close to 100 exhibiting antimicrobial properties even at low concentration	[71]
8	Antimicrobial	Organogel-based nanoemulsion	Performed against *E. coli, S. aureus, B. subtilis* and *S. cerevisiae* with MIC value 0.125–0.5 mg/mL indicating enhanced inhibitory and antimicrobial ability	[72]
9	Antimicrobial	Liposomes	Performed on *E. coli* and *L. monocytogenes* exhibiting satisfactory physical stability and stable in vitro anti-bacterial activity	[73]
10	Antimicrobial	Nanostructured lipid carriers	Well-dispersed and improved storage stability with increased anti-fungal activity	[74]
11	Antimicrobial	Hexosomal dispersion	*C. lemon* and *C. aurantifolia* (the most active essential oil) was loaded into the hexosomal dispersion and exhibited powerful fungicidal activity against *F. oxysporum* (36.92 and 41.72) for *C. lemon* and *C. aurantifolia* respectively, so was utilized as natural nano fungicide and a potent anti-fungal agent	[75]
12	Anti-inflammatory	Nano hexosomal formulation	Showed decreased MIC90 (1.95± 0.0029 μg/mL) and IC50 (0.85 μg/mL) values in comparison with marketed drugs Clarithromycin and Celecoxib respectively against *H. pylori*	[76]
13	Anti inflammatory	Topical microemulsions	Enhanced stability, absorption on skin and potent anti inflammatory activity	[77]
14	Neuroprotective	Propranolol loaded intranasal D-limonene-based microemulsion mucoadhesive nano gel	Improved brain availability of propranolol i.e 382.4% with $AUC_{(0-7h)}$ of 4884.15 ng.h/L, t1/2 1.5 times more than orally treated groups and enhanced zeta potential from −10.3 to −3.9 mV for treatment of migraine	[79]
15	Neuroprotective	Nanocarriers of limonene loaded rivastigmine	Increase the bioavailability of the drug, circulation time, and effectiveness in crossing BBB	[80]
16	Neuroprotective	Novasomal gels of rasagiline mesylate	Slowed down the level of IL-17 and pro-inflammatory cytokine when administered via a transdermal route with %EE of 83.09% and zeta potential −33.45 mV	[81]
17	Gall stones	Docosahexaenoic acid coupled D-limonene bovine serum albumin nanoparticles	Increased the uptake by RAW 264.7 cells by about 4.5 folds and produced less cytotoxicity and uniform distribution	[78]

gels of rasagiline mygalate when formulated along with d-limonene and administered via the transdermal route led to the efficacious treatment of Parkinson's disease.[81]

Table 2 provides an overview of various delivery systems of D-limonene, including their applications, mechanisms, and potential therapeutic outcomes.

Conclusion

Limonene, a naturally available cyclic monoterpene, has demonstrated phenomenal potential for use in diverse fields. Its antioxidant, anti-inflammatory, wound healing, antidiabetic, anticancer, and immunomodulatory activities highlight its

versatility as a therapeutic agent. With its complex therapeutic benefits and improved delivery via cutting-edge formulations, limonene is a strong contender for next-generation therapeutics. Its incorporation into contemporary medicine opens the door to safer, more efficient, and patient-focused therapies. Preclinical and clinical trials have been conducted comprehensively to ensure its safety and efficacy. The use of novel and innovative drug delivery systems, particularly nanoformulations, has also demonstrated significant potential in improving the bioavailability, stability, and precise delivery of limonene, thereby resolving impediments related to its clinical use. The advent of these innovations has helped enhance the effectiveness of treatments and opened new avenues for personalised medicine. Ongoing advancements indicate that the amalgamation of limonene with contemporary healthcare has a substantial potential to improve patient care across diverse health conditions. Further research and development will continue to unleash its full potential in treating a broad range of health issues.

Future Perspectives

Limonene is an unparalleled terpenoid compound that endeavours a myriad of therapeutic benefits such as antioxidant, anti-inflammatory, wound healing, antidiabetic, anticancer, and immunomodulatory activities. This review article offers an intricate discussion on the various therapeutic applications of limonene. The utilisation of novel nanoformulations has fascinated scientists to deliver medicinal drugs both locally and systemically. The ingrained lipophilicity and volatility of limonene pose impediments in its formulation. Traditional methods of drug delivery may not address these issues competently and may lead to suboptimal therapeutic outcomes. Thus, advanced and novel drug delivery systems have been developed. Novel drug delivery systems have significantly transformed the administration of various medicinal drugs; limonene is no exception to this trend. Nanoformulations shield limonene from early deterioration, guarantee regulated release, and enable precise transportation to certain tissues, thereby optimising its therapeutic effectiveness while minimising adverse reactions.[82] The use of metallic nanoparticles such as gold nanoparticles also provides a new and promising avenue.[83] Another obstacle in translating limonene therapies from the research phase to clinical practice involves navigating through the regulatory guidelines.

A captivating strategy entails the convergence of customised medicine with the administration of limonene. Comprehending the genetic variations is crucial for enhancing patient compliance and therapeutic success. The advent of advanced cutting-edge technologies such as 3D printing has the potential to completely transform drug delivery systems.[84] This innovative idea provides meticulous control over the production of dosage forms and customised compositions. These emerging technologies can completely transform the way limonene is administered, by improving its ability to be absorbed by the body, precisely targeting specific areas, and enhancing its therapeutic effectiveness.

Integration of limonene into medical devices can also be another innovative technique to help offer personalised treatment regimens. The use of specific botanical extracts can synergistically enhance the medicinal effects of limonene. Furthermore, 3D printing can simplify the manufacturing process of intricate drug delivery devices that can provide limonene along with other therapeutic substances. For instance, drug-eluting stents and implants that gradually release limonene can be employed to treat chronic inflammatory disorders to enhance therapeutic results. Novel and innovative drug delivery systems also include the use of microneedles.[85] Microneedles provide a less intrusive approach for delivering limonene through the skin. These miniscule needles can effortlessly pierce through the skin barrier, allowing limonene to be directly delivered into the dermal layers. This is advantageous improving overall absorption throughout the body.

In conclusion, incorporating limonene into sophisticated drug delivery systems and medical devices, together with personalised medicine strategies, signifies a notable progress in its therapeutic use. Technologies such as 3D printing, nanoformulations and microneedles improve the ability of limonene to be absorbed by the body and targeted to specific areas.

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Disclosure

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