Research in Molecular Medicine

The Anti-inflammatory Effects of Eugenol and Its Derivatives: A Scoping Review of Preclinical Evidence

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ABSTRACT

Inflammation, whether aseptic or induced by pathogenic microorganisms, serves as a protective mechanism of the immune system. While the current pharmacological agents used to mitigate inflammation are effective, they are associated with numerous adverse effects. Thus, it is imperative to explore alternative therapies that offer greater efficacy and fewer side effects. Certain phytochemicals, such as eugenol, are effective in alleviating inflammation. This study aims to thoroughly review and synthesize existing literature on the effects of eugenol administration in managing various inflammatory disorders in laboratory and animal experimental models. Eugenol and its derivatives have been successful in reducing inflammation in laboratory studies but have been ineffective in reaching clinical trials. Issues include differences between in vitro and in vivo responses, poor bioavailability, a short biological half-life, and eugenol's instability.

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Introduction

Inflammation is known as a complex protective response of the immune system. This response may be either acute or chronic. Acute inflammation initially activates resident cells to release proinflammatory cytokines and chemokines. This message results in the recruitment of primarily neutrophils from blood to the site of injury, thereby promoting classic signs of inflammation such as pain, swelling, redness, and heat [1, 2]. However, chronic inflammation is a prolonged response characterized by a gradual shift in cell types at the site of inflammation, which can cause both permanent damage and tissue healing over time [3].

Several key transcription factors are involved in the inflammatory responses, including nuclear factor-kappa B (NF-κB) [4], nuclear factor of activated T cells (NFAT) [5], activator protein-1 (AP-1), and Signal transducer and activator of transcription-3 (STAT3) [6]. Biomarkers of inflammation include cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, IL-8, and monocyte chemotactic protein 1 (MCP-1) [7]. Additionally, enzymes and proteins, such as cyclooxygenase-2 (COX-2), 5-lipoxygenase (5-LOX), matrix metalloproteinases (MMP), C-reactive protein (CRP), and vascular endothelial growth factor (VEGF), can serve as biomarkers of inflammation [8].

Despite this, current anti-inflammatory medications are associated with significant adverse effects. Corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) are classified as anti-inflammatory medications, both of which have the potential to induce adverse effects [9, 10]. These adverse effects encompass those that are specifically associated with NSAIDs, such as indigestion, stomach ulcers, headaches, drowsiness, dizziness, and allergic reactions. Likewise, corticosteroids can elicit side effects, including increased appetite, acne, thinning of the skin, increased risk of bruising, muscle weakness, and delayed wound healing [11-13].

Today, the use of phytochemical compounds, such as eugenol, is significant in managing inflammation and inflammatory ailments owing to their low toxicity and high tolerability [14, 15]. This study reviews the effects of eugenol on the treatment of several inflammatory diseases in laboratory and animal models.

Eugenol

Eugenol, an organic compound also known as 4-allyl-2-methoxyphenol, is found in various plant families, including Lamiaceae (Holy basil or tulsi leaves), *Eugenia caryophyllata* (clove), *Zingiber officinale* (ginger), *Cinnamomum verum* (cinnamon) bark and leaves, *Curcuma longa* (turmeric), and Solanaceae (peppers) (Figure 1) [16]. Eugenol is a clear to pale yellow liquid with an oily texture and a spicy scent. It has limited solubility in water but dissolves well in organic solvents. Eugenol can be produced by combining guaiacol with allyl chloride or by using microorganisms, such as *Escherichia coli*, *Corynebacterium* sp., and *Bacillus cereus* in a biotransformation process. It is not chemically stable and prone to oxidation and other chemical reactions. When taken orally, it is rapidly absorbed, metabolized in the liver, and excreted almost completely in urine as sulphate or glucuronide conjugates. To prevent early absorption, enhance water solubility, and increase effectiveness, encapsulating eugenol appears to be the most effective method. Encapsulated eugenol complexes may exhibit improved thermal stability, leading to a gradual release of the compound [16-19].

Eugenol has various pharmacological benefits, including antimicrobial, anti-inflammatory, pain-relieving, neuroprotective, antidiabetic, and antitumor effects. Eugenol is recognized for its antibacterial properties, making it a popular choice for oral and dental hygiene (Figure 2). Its efficacy in reducing local pain and functioning as a disinfectant has led to its widespread application in the dental field. In dental medicine, eugenol-derived amorphous chelate compounds combined with zinc oxide are used for indirect pulp coverage. Furthermore, it is used in liquid form to fill root canals, often included in specific pastes. Sometimes, before inserting a denture, eugenol is rubbed on the gums to provide numbing [19-21].

Another therapeutic impact of eugenol, its anticancer properties, is mediated through various mechanisms, including apoptosis stimulation, cell cycle arrest, and inhibition of cell proliferation, migration, angiogenesis, and metastasis in different types of cancer cells [22-24]. These properties make it a versatile natural ingredient that can help prevent and treat various disorders. Also, the World Health Organization (WHO) has recognized eugenol as generally safe and non-mutagenic [17]. The safety of eugenol and its anti-inflammatory properties are key factors in using this phytochemical for treating inflammatory diseases (Figure 3, Table 1). Its ability to suppress NF-κB–mediated cytokine production, along with its low toxicity at therapeutic doses, highlights its curative promise.

Altogether, these features support eugenol as a safe and effective candidate for managing inflammation.

The function of eugenol in mitigating pulmonary inflammation

Eugenol and its derivatives can reduce lung inflammation, thereby enhancing lung viscosity and elasticity. Conditions such as asthma and acute lung injury contribute to the development and expansion of an inflammatory response. Chronic obstructive pulmonary disease is characterized by widespread, persistent lung inflammation that leads to destruction of alveoli. Eugenol and bis-eugenol have shown effectiveness in managing emphysema and lung inflammation. These beneficial effects can be attributed to the regulation of MMP-9 and metallopeptidase inhibitor 1 (TIMP1), a tissue inhibitor of metalloproteinases, as well as the reduction of inducible nitric oxide synthase (iNOS).

Additionally, both compounds have been observed to reduce alveolar damage induced by porcine pancreatic elastase solution for emphysema induction. However, no effects of these compounds have been observed on collagen deposition or on the expression of superoxide dismutase-1 (SOD-1) and SOD-2 in lung tissue [25]. Both eugenol and dehydrodieugenol, a dimeric derivative of eugenol, possess no cytotoxicity effect on cells. These compounds inhibit nitric oxide (NO) release and *IL-1β* and *IL-6* gene expression in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells [26]. Also, in the ovalbumin-sensitized asthma model, dehydrodieugenol has been shown to reduce the number of inflammatory cells in the lungs and the levels of IL-4, IL-13, IL-17, and IL-10. The JNK, p38, ERK1/2, vesicular acetylcholine transporter (a neurotransmitter transporter), and STAT3/SOCS3 signaling pathways are inhibited, resulting in the observed anti-inflammatory effects.

Furthermore, treatment with dehydrodieugenol effectively alleviates airway hyper-responsiveness. In mice, dehydrodieugenol has been shown to have a greater influence than eugenol in balancing allergic airway inflammation, particularly by inhibiting the JNK, p38, and ERK1/2 components of the mitogen-activated protein kinase (MAPK) pathway [27]. Furthermore, in a murine model of acute lung injury induced by intratracheal LPS instillation, both eugenol and dehydrodieugenol B reduce lung edema, inflammatory cell infiltration, and levels of proinflammatory cytokines IL-6 and IL-1β in bronchoalveolar lavage (BAL) fluid. Additionally, these compounds reduce inflammatory cell infiltration and the expression of markers, including iNOS, MMP-9, TIMP-1, collagen, and 8-isoprostane, in lung tissue. Additionally, eugenol and dehydrodieugenol B have been demonstrated to suppress Jc-Jun-NH2 terminal kinase (JNK) phosphorylation, a signaling protein implicated in the MAPK pathway. However, these compounds have no effects on the lung function [27].

In another investigation, he adminstration of eugenol 6 hours after LPS-induced lung injury significantly improved the viscous, flexible, and viscoelastic components of lung mechanics. Animals injected with LPS and treated with eugenol have shown reduced alveolar failure and decreased collagen fiber deposition in the lung parenchyma. Eugenol treatment also effectively reduces TNF-α release in BAL fluid and inhibits NF-κB activation [28]. Moreover, bis-eugenol has demonstrated antiasthmatic effects in ovalbumin-sensitized mice, which serve as an experimental model of mixed-granulocytic asthma. This property has been observed through decreased inflammation, as evidenced by reductions in eosinophil, neutrophil, and total cell counts compared with an ovalbumin-sensitized model.

Nevertheless, there have been no significant differences in macrophage and lymphocyte levels [29]. It is worth noting that eugenol plays a role in preventing COVID-19 and improving lung inflammation caused by SARS-CoV-2. Eugenol has been found to strongly inhibit the entry of pseudotyped SARS-CoV-2 into human angiotensin-converting enzyme 2 (ACE2)-expressing HEK293 cells. Furthermore, eugenol reduces SARS-CoV-2 spike S1-induced NF-κB activation and the expression of IL-6, IL-1β, and TNFα in human A549 lung cells. Therefore, eugenol holds promise for therapeutic use in the treatment of COVID-19 [30, 31].

The role of eugenol in alleviating hepatitis and hepatic fibrosis

Eugenol has anti-inflammatory and anti-fibrosis effects on the liver. The ingestion of cadmium leads to a significant increase in hepatic marker levels, including lactate dehydrogenase, gamma-glutamyl transpeptidase, alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase, and bilirubin. The levels of albumin, glutathione (GSH), and activity of antioxidant enzymes significantly decrease in cases of oral intoxication with cadmium. Eugenol exhibits properties of a natural antioxidant and anti-inflammatory agent. The administration of eugenol is remarkably effective in reversing the cadmium-induced biochemical alterations [32].

Also, eugenol exhibits a hypolipidemic effect in rats that are subjected to a diet rich in cholesterol and fat. This compound significantly reduces total cholesterol, low-density lipoproteins (LDL), and the atherogenic index, without affecting high-density lipoproteins or triglycerides. Additionally, eugenol reduces fat accumulation and inflammation in liver cells, reduces liver enlargement, and lowers ALT and ALP activity, which are markers of liver function. Furthermore, eugenol enhances the activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in rats with high cholesterol levels [33]. The induction of liver fibrogenesis by iNOS protein can be mitigated by using eugenol and telmisartan.

The simultaneous administration of eugenol and telmisartan to mice with fibrosis can lead to a reduction in serum aminotransferases and oxidative parameters, as well as the down-regulation of NF-κB, TNF-α, IL-6, and iNOS protein expression [34]. Furthermore, eugenol protected against liver fibrosis in rats with non-alcoholic fatty liver disease induced by a high-fat diet (HFD). Notably, treatment with eugenol resulted in a significant decrease in HFD-induced hepatic transaminases and triglycerides, along with improvements in histopathological lesions [35]. Eugenol also reduced collagen and smooth muscle α-actin accumulation, as well as TGF-β expression, which are considered markers of fibrosis [35]. In this regard, eugenol has been shown to mitigate hepatic impairment in a rat model of fructose-induced metabolic syndrome. Moreover, these defensive effects may, to some extent, be facilitated by enhancing the antioxidant status, reducing oxidative stress, and limiting lipid peroxidation. Additionally, it might alleviate hepatic inflammation and lipid accumulation, as well as hepatic cell fibrosis [36].

Eugenol's role in reducing inflammation in the colon

Inflammatory bowel disease (IBD) is a persistent inflammatory condition affecting the gastrointestinal tract. This disease is divided into two main subtypes: Ulcerative colitis (UC) and Crohn's disease (CD). While UC specifically impacts the colon, CD can affect any part of the gastrointestinal tract, with a primary occurrence in the terminal ileum of the small intestine. IBD is a multifaceted disorder that involves genetic predisposition, dysbiosis of the intestinal microbiota, dysregulation of the host immune system, and environmental factors such as diet and stress. These factors collectively contribute to a continuous cycle of intestinal inflammation. In the immune system, disturbances in the epithelial layer increase permeability, allowing luminal antigens to pass into the underlying tissue. Antigen-presenting cells, such as macrophages and dendritic cells, become activated upon recognizing antigens via toll-like receptors. This activation triggers the NF-κB pathway in these cells, inducing the production of inflammatory cytokines, including IL-6, IL-12, IL-23, IL-1β, and TNF-α through enhanced transcription. Subsequently, these antigen-presenting cells process the antigen and present it to T cells, thereby promoting their differentiation and leading to the generation of IFN-γ and IL-17, which play a crucial role in regulating cell-mediated immunity [37].

Given the substantial worldwide impact, with more than 4.9 million cases of IBD globally and a rising incidence of occurrence, there is an increasing demand for innovative treatments for this condition. The effectiveness of existing medications is constrained and subject to debate due to their restricted efficacy, unfavorable safety and tolerability profiles, and potential adverse effects [37]. Eugenol can alleviate colitis by reducing colonic inflammation and oxidative stress, regardless of the presence of intestinal microbiota. A thorough investigation was conducted to evaluate the effects of eugenol on dextran sulfate sodium (DSS)-induced colitis and to explore the underlying mechanisms. The results of the research indicated that eugenol effectively increased body weight while simultaneously decreasing the disease activity index score and colon pathological scores in DSS-treated mice.

Additionally, eugenol demonstrates a protective effect on proinflammatory cytokines, such as IL-6, IL-12, IL-21, and IL-23, and reduces colonic malondialdehyde (MDA) levels [38]. The Nrf2/heme oxygenase (HO-1) signaling pathway, considered essential in responding to oxidative stress, plays a role in anti-inflammatory, antioxidant, and apoptosis processes. Activation of HO by the diclofenac-eugenol hybrid inhibits NF-κB-mediated inflammation in both laboratory (in vitro) and clinical (in vivo) settings, including subjects with DSS-induced colitis. This hybrid compound shows promise as a potential new anti-inflammatory agent by disrupting the NF-κB signaling pathway and acting against oxidative stress by activating the HO-1 signaling pathway [39]. Additionally, eugenol has been found to reduce inflammation in human 3D epi-intestinal tissue [37]. In a separate investigation, nanovesicles containing eugenol and cinnamaldehyde (CA) were used to treat UC. The combined composite nanovesicles facilitated the delivery of eugenol and CA through the skin. The medicinal properties of eugenol/CA encapsulated in nanovesicles in rats with UC may be associated with the regulation

of neurotransmitters, such as NO, vasoactive intestinal peptide (VIP), and acetylcholine, as well as the replenishment of energy levels. Notably, eugenol/CA enhances the growth and specialization of interstitial cells of Cajal (ICCs) in colon tissues affected by UC by repairing the stem cell factor/c-kit signaling pathway, thereby increasing ICC numbers and restoring their function in controlling colonic motility. The SCF/c-kit signaling pathway, along with the levels of H_2O_2, VIP, and other cytokines, has been identified as being involved in the activation of STAT3, ERK, and AKT [40].

The impact of eugenol on the pathogenesis of rheumatoid arthritis (RA)

RA is a debilitating form of arthritis characterized by synovial tissue infiltration and hyperplasia, leading to progressive destruction of joint tissues. The production of proinflammatory cytokines by activated T cells triggers macrophages to release inflammatory mediators, which in turn results in secondary inflammatory damage in arthritis [1, 2]. TNF-α, a multifunctional cytokine generated by activated monocytes and macrophages, regulates immune cells and plays a crucial role in both chronic and acute inflammation. This cytokine triggers inflammatory responses that may lead to autoimmune diseases such as ankylosing spondylitis and RA. Conversely, IL-6 is implicated in damaging synovial cells by enhancing prostaglandin synthesis and fibroblast proliferation in synovial fluid. Moreover, IL-6 has been observed to contribute to joint and bone deterioration in RA patients. Dysregulation of TNF-α and IL-6 can significantly impact the immune system, thereby influencing the onset and progression of various inflammatory disorders. The excessive inflammation is also linked to the activation of oxidant-producing enzymes (NADPH oxidase, xanthine oxidase, myeloperoxidase), thereby promoting the generation of reactive oxygen (ROS) and reactive nitrogen (RNS) species. These reactive entities contribute to cellular damage in joint tissues through diverse mechanisms, including antioxidant depletion, lipid peroxidation, protein oxidation, and DNA impairment. These species inflict harm on joint tissues by diminishing antioxidants, inducing lipid peroxidation, oxidizing proteins, and causing DNA damage through distinct mechanisms. Consequently, inflammatory mediators and reactive species exert direct or indirect effects on the pathophysiology of RA [2]. NSAIDs and disease-modifying anti-rheumatic medications (DMARDs) utilized in the treatment of RA have been associated with various adverse drug reactions, such as gastrointestinal ulcers, cardiovascular complications, and the emergence of opportunistic infections due to immunosuppressive effects. In addition, biologics such as TNF-α inhibitors, IL-1β inhibitors, and IL-6 inhibitors are used in the treatment of RA [3, 4]. Nevertheless, their use is limited due to factors such as high cost, adverse effects, and immune system interference. Researchers have recently utilized eugenol or its derivatives in the treatment of RA in laboratory and animal models. In one study, fibroblast-like synovial cells exposed to TNF-α for 24 hours were treated with eugenol. The target genes of eugenol are significantly linked to the VEGF and NF-κB signaling pathway. Eugenol effectively counteracts TNF-α-induced stimulation of proteins involved in the NF-κB signaling pathway and prostaglandin-endoperoxide synthase 2 (PTGS2, also known as COX-2). It is suggested that eugenol can offer a new therapeutic approach to inhibit the progression of RA by targeting the NF-κB signaling pathway and COX-2 expression in fibroblast-like synovial cells [41]. Moreover, a separate study on a combination therapy involving two phytochemicals, CA and eugenol, reveals that eugenol is more effective than CA in reducing arthritis severity. Rats were induced with type II collagen for arthritis development and were orally administered CA (10 and 20 mg/kg/d) and eugenol (10 and 20 mg/kg/d) for 15 days. Both compounds exhibited significant decreases in ROS and NO, as well as in markers of biomolecular oxidation, and increases in enzymatic and non-enzymatic antioxidants. Additionally, treatment with CA and eugenol improves levels of TNF-α, IL-6, and IL-10 [42]. Furthermore, CA and eugenol are observed to possess strong anti-inflammatory and antioxidant properties in peripheral blood mononuclear cell (PBMC) cultures from patients with RA. Treatment of PBMCs from RA patients with varying concentrations of these compounds resulted in reduced levels of proinflammatory cytokines and oxidative stress markers. The compounds also mitigate ROS species formation, biomolecular oxidation, and antioxidant defense response. Fourier transform infrared spectroscopy, a non-destructive technique for analyzing cellular changes in various diseases, confirms the protective effects of CA and eugenol on biomolecules in PBMCs from RA patients. Molecular docking results indicate interactions between CA and eugenol, and between these compounds and key residues of TNF-α and IL-6 [43]. In a recent study, researchers explored a novel approach to treating RA by using essential oils delivered via a nanoplatform. The study successfully developed a customizable lipid system for controlled release, designed to efficiently deliver clove oil (CO), which contains eugenol, for RA treatment. Ultra-small nanostructured lipid carriers co-loaded with CO (CONCs) were created using an aqueous titration method followed by microfluidization. Results from in vivo studies indicated that the antiarthritic effects of CONC were similar to those of the standard treatment. The optimized formulation shows inhibitory effects on enzymes and reduces levels of IL-6 and TNF-α. These findings suggest that the CONCs formulation is effective in managing

Table 1. Eugenol modulates the anti-inflammatory and antioxidant response in the joint, lung, and liver

Disease	Animals and or Cell Lines	Eugenol	Biological Effect	Ref.
Chronic obstructive pulmonary disease	C57BL/6 mice	Eugenol and bis-eugenol (20 mg/kg, IP)	Eugenol and bis-eugenol improve emphysema and pulmonary inflammation by regulating matrix MMP-9 and metallopeptidase inhibitor (TIMP)-1 and reducing iNOS activity.	[25]
Lipopolysaccharide-induced lung inflammation	RAW 264.7 macrophages and BALB/c mice	For in vitro, eugenol and dehydrodieugenol (5, 20, and 50 µg/mL) For in vivo (5,10 and 20 mg/mL)	Dehydrodieugenol shows superior efficacy to eugenol in reducing allergic airway inflammation by suppressing components of the MAPK pathway (JNK, p38, ERK1/2).	[26]
LPS-induced experimental acute lung inflammation	RAW 264.7 macrophages and BALB/c male mice	For in vitro, eugenol and dehydrodieugenol (10, 20, 30, and 60 µg/mL) For in vivo (10, 20, 30, and 60 mg/mL)	Dehydrodieugenol B preserved cell viability, inhibited LPS-induced NO, IL-1β, and IL-6, and both compounds reduced lung edema, inflammatory cells, and markers, including iNOS, MMP-9, and TIMP-1.	[27]
LPS-induced lung injury	BALB/c mice	.	Eugenol reduces TNF-α release in BAL fluid and inhibits (NF-κB) activation.	[28]
Asthma mouse model of mixed-granulocytic asthma	BALB/c mice	Bis-eugenol (20 mg/kg)	Bis-eugenol reduced airway and tissue damping similar to dexamethasone but was less effective against methacholine-induced airway hyperresponsiveness.	[29]
COVID-19	ACE2-expressing HEK293 cells	-	Eugenol blocked pseudotyped SARS-CoV-2 entry via ACE2 and reduced infection-induced systemic inflammation by targeting viral proteins and proinflammatory mediators.	[30]
COVID-19	C57/BL6 mice	Eugenol (25 mg/kg)	In A549 cells, eugenol suppressed NF-κB and proinflammatory cytokines induced by SARS-CoV-2 spike S1, and in mice, it alleviated lung inflammation, fever, and cardiac dysfunction while improving activity.	[31]
Hepatic oxidative stress and inflammation induced by cadmium	Wistar albino rats	Eugenol (3 mg/kg)	Eugenol co-administered with cadmium protects against cadmium-induced toxicity.	[32]
Rats fed a HCFD	Wistar rats	Eugenol (10 and 100 mg/kg)	Eugenol lowered total cholesterol and LDL, reduced liver steatosis and inflammation, decreased ALT and ALP, and boosted antioxidant enzymes SOD and CAT in hypercholesterolemic rats.	[33]
CCl_4-induced hepatic injury	Albino rats	Eugenol (10 and 100 mg/kg)	Eugenol or telmisartan reduced CCl4-induced oxidative and inflammatory markers and improved liver histopathology in fibrotic rats.	[34]
Liver fibrosis in HFD-induced experimental non-alcoholic fatty liver disease	Albino rats	Eugenol (10 mg/kg)	Eugenol protected against HFD-induced oxidative stress and inflammation, normalized GSH and Nrf-2, reduced lipid peroxidation, blocked NF-κB, and decreased fibrosis markers like collagen, α-SMA, and TGF-β.	[35]
Liver damage caused by fructose-induced metabolic syndrome	Male Wistar rats	Eugenol (50 and 100 mg/kg)	Eugenol alleviates liver damage in rats with metabolic syndrome by enhancing antioxidant defenses and reducing oxidative stress and lipid peroxidation.	[36]
DSS-induced colitis	C57BL/6 mice	Eugenol (20 mg/kg body weight)	Eugenol improved body weight, reduced disease and colon pathology scores, lowered proinflammatory cytokines and MDA, and inhibited UCP2 expression and p65 phosphorylation in DSS-treated mice.	[38]
DSS-induced colitis	Rats	Diclofenac-eugenol hybrid (50 mg/kg)	The diclofenac-eugenol hybrid activated HO-1, reducing NF-κB-mediated inflammation in vitro and in vivo.	[39]
The colon of rats with trinitrobenzene sulfonate-induced UC	Rats	Eugenol/CA -loaded ethosomes	Eugenol and CA promote colon ICC growth and function in UC by restoring SCF/c-kit signaling.	[40]
Rheumatoid arthritis	Fibroblast-like synovial cells	Eugenol	Eugenol inhibits TNF-α-driven proliferation and COX-2 via NF-κB in synovial cells.	[41]

Disease	Animals and or Cell Lines	Eugenol	Biological Effect	Ref.
Rats were induced with Type II collagen for arthritis development	Rats	CA and eugenol	CA and eugenol reduced ROS species and NO levels, boosted antioxidant levels, and balanced TNF-α, IL-6, and IL-10 levels.	[42]
RA	PBMCs from RA patients	CA and eugenol	CA and eugenol were non-toxic to PBMCs and dose-dependently reduced TNF-α, IL-6, NO, and ROS.	[43]
Rheumatoid arthritis	Rats	Ultra-small nanostructured lipid carriers CO-loaded with CO (CONCs)	CONC showed antiarthritic activity slightly higher than CO but slightly below the standard.	[44]
Rheumatoid arthritis	Wistar rats	Encapsulated eugenol by chitosan nanoparticles	Eugenol-loaded chitosan nanoparticles reduced *TGF-β* and *MCP-1* (*CCL2*) gene expression.	[45]

arthritis, resulting in decreased paw volume and biochemical parameters. Furthermore, both CRP and RF levels in serum significantly decrease with CONCs and Voltaren gel treatments [44]. Additionally, a separate study investigated the effects of chitosan nanoparticles eugenol, a potent nano-herbal agent, on healing neonatal RA, compared with methotrexate. Both the nanoparticle herbal agent and methotrexate reduced the expression of *TGF-β* and *MCP-1* genes. Monocyte chemoattractant protein (MCP-1)/CCL2 is a chemokine that promotes monocyte migration and activation, and its expression is elevated in synovial cells and RA-infiltrated monocytes, with a notable positive correlation observed between MCP-1 and TGF-β [45].

Toxicity

High concentrations of eugenol can be pro-oxidative and detrimental, and exposure or ingestion of large amounts, as in overdose, can result in tissue injury and a syndrome of acute onset of seizures, coma, and damage to the liver and kidneys. At the same time, measurements below 2.5 mg/kg body weight are considered safe by the Food and Agriculture Organization of the United Nations (FAO). The toxic effects of eugenol depend on its concentration and are dose-dependent [21, 46]. Furthermore, it may exhibit varying toxicity depending on the histological structure exposed to this compound [47]. Eugenol may cause hypersensitivity, particularly contact dermatitis, in some cases, especially among dental professionals [48]. Medeiros et al. reported that the toxicity of eugenol is related to protein inactivation caused by the binding of eugenol to the lysine residues. The cytotoxicity of eugenol may be due to its metabolic reactions. The reactive metabolites then react further with DNA, creating adducts that can damage nuclear DNA [48].

Figure 1. Plant sources of eugenol

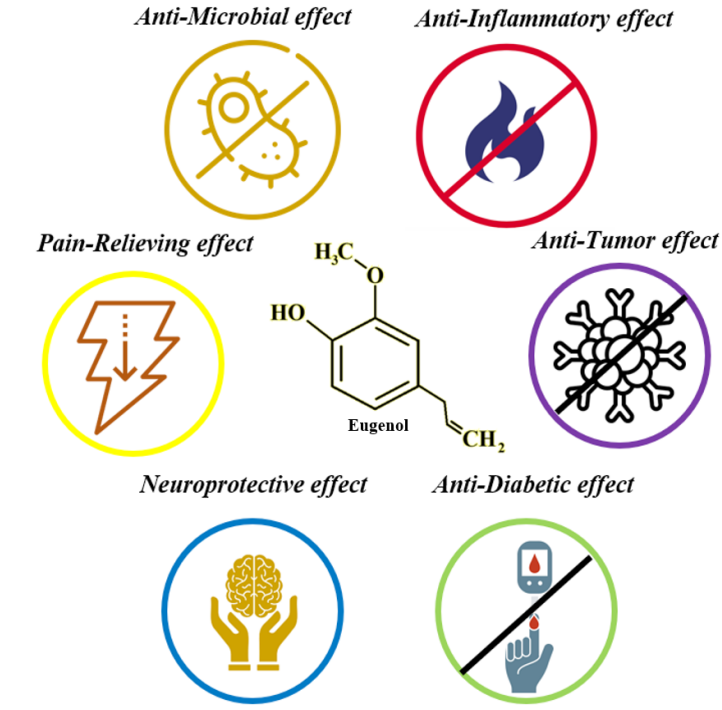

Figure 2. Therapeutic properties of eugenol

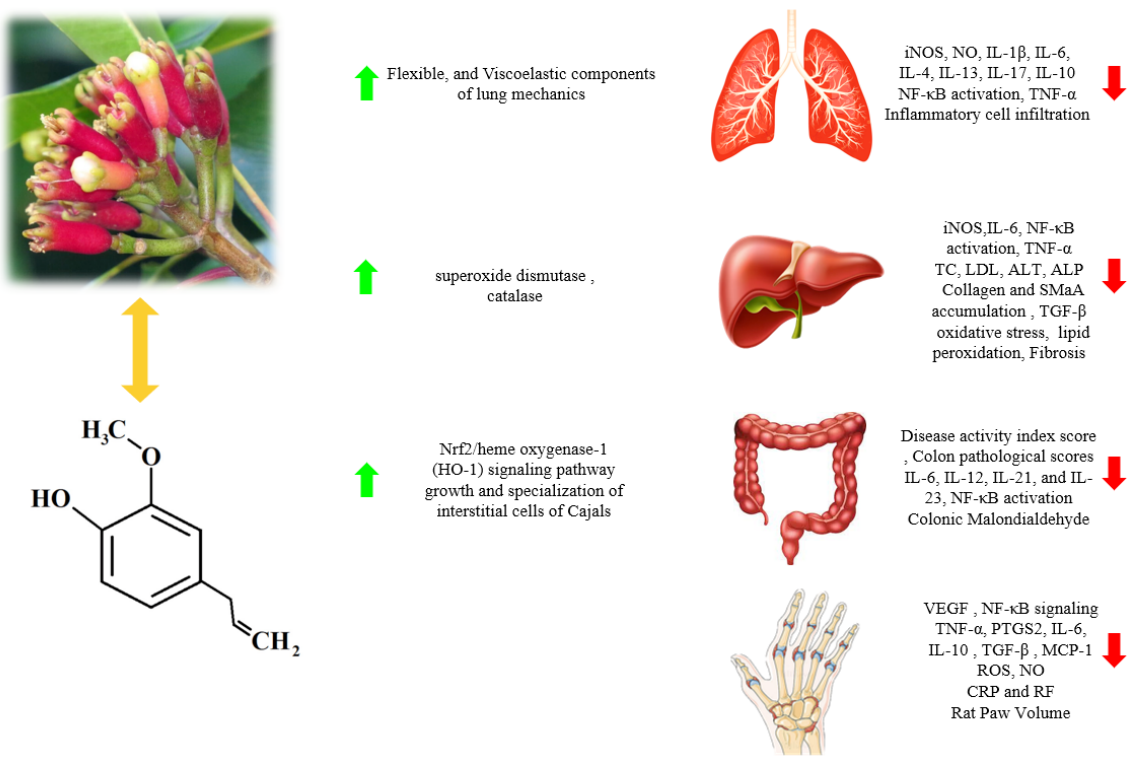

Figure 3. Anti-inflammatory effects of eugenol on inflammatory diseases

Conclusion

Despite numerous successes from in vitro and in vivo studies, there has been minimal progress in advancing natural phytochemicals into clinical trials. These challenges are related to both in vitro assays and bioavailability. In vitro, cells are directly exposed to eugenol, so the response is somewhat different than in vivo. The human body readily processes phytochemicals; eugenol has a short biological half-life in vivo; it is poorly water-soluble and volatile, and it is prone to oxidation, factors that prevent it from entering clinical trials. Despite these challenges, the growing body of preclinical evidence highlights eugenol's substantial therapeutic potential, particularly through its anti-inflammatory and antioxidant activities. Therefore, future research should prioritize improving its stability and delivery. Advances in nanoformulation, encapsulation technologies, and targeted delivery systems may help overcome these barriers and enhance its pharmacological performance.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors contribution's

Conceptualization: Nazanin Joudaki and Alireza Rafiei; Methodology: Nazanin Joudaki, Abolghasem Ajami, and Reza Jafari-Shakib; Investigation: Nazanin Joudaki; Writing the original draft: Nazanin Joudaki, and Alireza Rafiei; Review & editing: All authors; Supervision: Alireza Rafiei, Abolghasem Ajami, Reza Jafari-Shakib.

Conflict of interest

The authors declared no conflict of interest.

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