EUGENOL: A POTENTIAL PHYTOCHEMICAL WITH MULTIFACETED THERAPEUTIC ACTIVITIES

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Summary

In recent years, research on plant extract or fraction based on traditional knowledge are showing efficacy almost comparable to that of synthetic counterpart. Eugenol is an interesting molecule due to its wide spectrum of activities like analgesic, anti-inflammatory, antioxidants antifungal, antibacterial, antiviral, central and neuroprotective action. In last decade many pharmacological actions and therapeutic actions of eugenol have been investigated especially where conventional drugs are ineffective in the treatment of disease or requirement is unmet such as in treatment of Alzheimer's disease. In nervous system, eugenol is neuroprotective against excitotoxicity, ischemia and amyloid-β peptide. Eugenol also exhibit its antidepressive activity in a different manner from that of imipramine, which may provide an alternative treatment to patients who are resistant to typical antidepressant. Eugenol also showed potential therapeutic implication against the micro-organisms which become resistant to conventional antimicrobials. In addition to activity against the resistant microbes such as *H. pylori* and biofilm of *C. albicans*, it possesses the analgesic, anti-inflammatory and antioxidant action which can be beneficial in treatment of the microbial diseases associated with the inflammation and pain. In the present review attempt is made to review pharmacological activities of eugenol like central and neuroprotective, anthelminthic & antileishmanial activities with possible mechanism of action. The efficacy of eugenol against resistant microbes and its toxicities are also included in the present review.

Keywords: Eugenol, antioxidant, antimicrobial, anthelminthic, antidepressant

Introduction

Plants are one of the most important and traditional sources of medicines. Several prototype drugs used in modern system are derived from plants, like morphine from *Papaver somniferum,* aswagandha from *Withania somnifera,* ephedrine from *Ephedra vulgaris,* atropine from *Atropa belladonna,* reserpine from *Rowalfia serpentina* etc. For the diseases where current drugs are either little efficacious or ineffective plant extract may be alternative to NCEs. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability [1-2]

In recent years, research on plant extract or fraction are showing efficacy almost comparable to that of synthetic counterpart. The plants are rich in secondary metabolites (which are potential sources of drugs) and source of innumerable phytochemicals of therapeutic importance.

Essential oils and extracts of various species of edible and medicinal plants, herbs, and spices constitute very potent natural biologically active agents. Eugenol is the principal chemical component of essential oil from *Eugenia aromatica*, *Cinnamomum zeylanicum* and *Ocimum basilicum & O. gratissium* [3-4]. Eugenol belongs to the class of essential oils that is Generally Recognized As Safe (GRAS) by the Food and Drug Administration. Eugenol is an interesting molecule due to its wide spectrum of activities like analgesic, anti-inflammatory, antifungal, antibacterial and antihypertensive activity [5]. However, surprisingly, literature survey revealed that eugenol apart from the peripheral action also acts at central level and can be useful in stress, epilepsy and depressive disorders, as well as it has antipyretic activity [6-8]

Recently, many pharmacological and therapeutic actions of eugenol have been investigated especially where conventional drugs are ineffective in the treatment of disease. For example, conventional antimicrobial agents exhibit myriad side effects and further, microbes become resistant on irrational use of them. Eugenol showed potential therapeutic implication for biofilm-associated candidal infections when these infections are refractory to the conventional antifungal agents[9]. Eugenol also exhibit its antidepressant activity in a different manner from that of imipramine, which may provide an alternative treatment to patients who are resistant to typical antidepressants [8].

The present review article mainly updates the existing researches reported regarding eugenol, be it in clove oil or any other medicinal plant. The previously published review articles focused only on in-vitro studies regarding antimicrobial, insecticidal and antiviral actions of clove oil [10]. However, equally important other pharmacological actions had not been much discussed in the previous review. The present review also deals with other activities of eugenol which have been recently published like central and neuroprotective, anthelminthic & antileishmanial activities. The efficacy of eugenol against resistant microbes and its toxicities are also included in the present review. Mechanism of action, comparison of pharmacological activity of eugenol to that of standard compound and in-vivo studies regarding these pharmacological activities are also the part of present review.

Source and Chemistry of Eugenol

Eugenol occurs in essential oils and is a major constituent of Ocimum, Cinnamon, and Clove oils. Primarily, eugenol is obtained from the clove buds of *Eugenia aromatica*, E. *caryophyllata* belonging to family Mytraceae indigenous to the Molluca Islands, and which are also cultivated in other parts of Indonesia, Zanzibar, Madagascar, and Ceylon. Eugenol can also obtained from the leaves and barks of *Cinnamomum zeylanicum* belonging to family Lauraceae. The essential oil extracted from the leaves of *Ocimum sanctum* and *Ocimum basilicum* belonging to family Lamiaceae by steam distillation largely contains eugenol. Eugenol is also isolated as the active principle of volatile oil fraction of rhizome *acori graminei* (RAG) [11]. Its extraction was performed using hydrodistillation which is the most common extraction technique. Recently, optimized condition necessary for essential oil extraction was determined and also characterized the extracted eugenol using Gas Chromatography (GC) and thermal techniques such as DSC for compare to standard eugenol [12].

Eugenol is an allyl chain-substituted guaiacol. Systemic chemical name of eugenol is 4-allyl -2 methoxy phenol. Thus, Eugenol is a member of the ally benzene class of chemical compounds. Chemical structure of eugenol presented in Fig.1. It is a clear, colorless or pale yellow oily liquid extracted from certain essential oils such as clove oil, nutmeg oil etc. The substance has a strong aromatic odor and a pungent spicy taste. Upon exposure to air, it darkens and thickens.

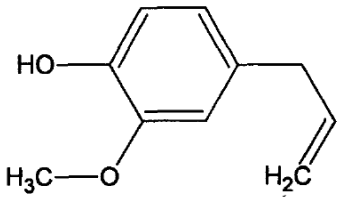

Fig.1 Chemical structure of Eugenol

Eugenol and its derivative such as isoeugenol are biosynthesized from the lignin precursor ferulic acid or coniferyl alcohol. The methylation of eugenol to methyl eugenol and isoeugenol to methyl isoeugenol is catalyzed by an S-adenosylmethionine dependent O-methyl transferase [13].

Traditional and modern day uses

As eugenol is the active principle of essential oils obtained from the traditional herbs such as *Eugenia aromatica* (clove), *Cinnamomum zeylanicum* (cinnamon) and *Ocimum sanctum* (tulsi). Traditional medical practitioners in India have been widely used these medicinal plants for management of various disease conditions from ancient time such as bronchial asthma, chronic fever, cold, cough, malaria, dysentery, convulsions, diabetes, diarrhea, arthritis, emetic syndrome, skin diseases, and in treatment of gastric, hepatic, cardiovascular and immunological disorders. However, not much is known about the mode of action of these plants, and a rational approach to this traditional medical practice with modern system of medicine is also not available. Therefore, it is necessary to link the traditional uses of the above plants to the modern day uses of eugenol, an active principle of the essential oil obtained from the plants. For example use of clove oil in dental pain is linked to the analgesic and anti-inflammatory action of eugenol. In ayurvedic traditional system uses of the *Ocimum sanctum* in immunological disorders may be associated with the antioxidant action of eugenol. Clove and *Ocimum sanctum* (tulsi) is also widely used in topical preparation due to antimicrobial activity of eugenol and other essential oil component. Tulsi is also considered to be an adaptogen, balancing different process and helpful for adapting to stress [14]. Anti-stress activity of eugenol was studied and proved by Sen et al., [6]. Further, clove and cinnamon are well known traditional food preservative which can be linked to the antimicrobial and insect repellant activity of eugenol.

In last decade, many studies have been performed to evaluate the pharmacological properties of eugenol on the basis of traditional knowledge. Ancient Chinese pharmacopoeias revealed that most herbal remedies indicated for Alzheimer Disease (AD or symptoms reminiscent of AD) contain the botanical *Rhizoma acori graminei* (RAG) which is rich in eugenol. In Iranian folk medicine, buds of clove have been used as an antiepileptic remedy [15].

On the basis of the above facts, scientist investigated the therapeutic potential activities of Eugenol for the management of other neurodegenerative and psychiatric disorders. Thus, traditional knowledge of using of the plant combined with the modern science will pave the way for discovering the rationale behind the activity and further assist to reveal the other pharmacological properties and mechanism of action of specific phytochemical.

Pharmacological and therapeutic actions of eugenol

Anti-oxidant and Pro-oxidant action

Oxidative stress, the consequence of an imbalance of prooxidants and antioxidants in the organism, is rapidly gaining recognition as a key phenomenon in chronic diseases such as cancer, arteriosclerosis, diabetes, and immune deficiency. Phenolic groups play an important role in antioxidative activity [16]. The strong antioxidant activity of eugenol is due to the presence of the phenolic group. Electron spin resonance (ESR) spin trapping has shown that allyl group in the structure of eugenol is responsible for scavenging effect [17]. Further, eugenol compounds have a methoxyphenolic structure similar to capsaicinoids. Eugenol interferes with initiation as well as propagation of lipid peroxidation and it is attributed to free radical scavenging effect of eugenol [18]. Malonaldehyde formation from cod liver oil by 88 % was reduced at 160 μg/ml of Eugenol [19]. The inhibitory activity of clove extract and eugenol on hexanal oxidation at 50 mg/ml of dose was comparable to that of α-tocopherol or BHT [19]. Iron-mediated lipid peroxidation and copper dependent LDL oxidation was also inhibited by eugenol compounds [20].

Reduction of non-enzymatic peroxidation in liver mitochondria by eugenol was also reported by Nagababu and Lakshmaiah, [18]. One study has shown that eugenol has double role i.e. it is both prooxidant and antioxidant. Thus, ingestion of these compounds may help to prevent in vivo oxidative damage, such as lipid peroxidation, which is associated with many diseases, including cancer, arteriosclerosis, diabetes, and immune deficiency. It is the prooxidant activity of eugenol that causes cytotoxicity[21].

Analgesic and anti-inflammatory action

Eugenol is widely used as analgesic in dentistry. Analgesic activity of eugenol can be attributed to its ion channel blocking properties. Eugenol blocks Ca^{++} currents in trigeminal ganglion (TG) neurons including dental afferent neurons and this inhibition was independent of TRPV1 receptor activation [22]. Eugenol also blocks Na^{+} and K^{+} channels in TG neurons in the same manner as Ca^{++} channels [23-24]. So, it can be assumed that analgesic effect of eugenol is due to inhibition of Ca^{++} dependent presynaptic release of neurotransmitters as well as inhibition of PGE_2 and interleukin 1β synthesis. In addition to this, eugenol appears to have an agonist effect on γ-aminobutyric acid (GABA) and an antagonist effect on NMDA (N-methyl-D-aspartate) glutamate receptors which played important role in pain transmission [25-26]. Eugenol has also been reported to inhibit Nitric Oxide (NO) production in LPS stimulated RAW 164.7 cell lines [27] and to block the release of interleukin 1-β, tumor necrosis factor α and prostaglandin E_2 from LPS stimulated macrophages showing its ability to act as an anti-inflammatory agent [28].

Leukotrienes (LTs) are biologically active 5-lipoxygenase (5-LO) products of arachidonic acid (AA) metabolism and are implicated in inflammation and allergic manifestations. LTs have been implicated as mediators in a diversity of diseases including asthma and a number of other inflammatory pathologies such as rheumatoid arthritis, inflammatory bowel disease, psoriasis and glomerulonephritis. Eugenol significantly inhibits 5-LO enzyme activity by non-competitive mechanism in human Polymorphonuclear leukocytes (PMNL) cells. The formation of peptidoleukotriene LTC4 in PMNL cells activated with AA and calcium ionophore A23187 was also inhibited by eugenol. The inhibitory mechanism of 5-LO studied by substrate dependent enzyme kinetics showed that eugenol at different concentrations did not change the Km value of 5-LO, but decreased the maximum velocity (Vmax) of the enzyme at different concentrations of the substrate AA. These results showed that eugenol may not bind to the active site of the enzyme, but may combine at a different site of the enzyme or may scavenge the lipid peroxyl radicals formed during oxidation of AA and thus block the formation of 5-HPETE as evidenced by decreased Vmax during enzyme kinetic experiments. Thus, the eugenol may modulate 5-LO mediated cellular events and pathophysiological effects of LTs [28].

Antimicrobial action

Antimicrobial properties of essential oils have been recognized and used since ancient times for food preservation as well as for medicinal purpose. Several reports have shown the antimicrobial effect of eugenol against several species of bacteria and fungi. Antimicrobial activity of clove oil in *in-vitro* has been already reviewed [10]. Antimicrobial activity against a large number of multi-resistant *Staphylococcus epidermidis* isolated from dialysis biomaterial was also reported [10]. Therefore, main focus of the present review article to review *in-vivo* studies and activity of eugenol against the resistant microorganisms.
Eugenol and similar compounds such as cinnamaldehyde were also found to inhibit the growth of 10 different multi drug resistant pathogenic bacteria such as *E. coli*, *Staphylococcus*, *Proteus*, *Klebsiella*, *Enterobacter*, *H. pylori* and *Pseudomonas* isolated from human subjects [29-31].

The antifungal effect of essential oils (EO) has been noted in several studies [32-33]. Specific anticandidal activity of some essential oil is also well established [29, 34-35]. The phenolic major components of EO have potent antifungal activity [36]. *In-vivo* antifungal activity of eugenol was investigated by using an experimental model of oral and vaginal candidiasis in immunosuppressed rats [37]. Eugenol has potential therapeutic implication for biofilm-associated candidal infections when these infections are refractory to the conventional antifungal agents[9]. *C. albicans* biofilms are notoriously resistant to antifungal agents of various types, including biocides, antibiotics and antiseptics [38]. Several theories have been proposed to explain the antimycotic resistance of *C. albicans* biofilms [39]. As yet, specific resistance factors remain inconclusive. Eugenol alters the morphogenesis of the envelope of *C. albicans* which is pivotal in mediating the initial physico–chemical interactions between the fungus and its environment, including the human host. The envelope of *C. albicans* also plays a critical role in the development of the spatially organized architecture seen in mature, highly structured C. albicans biofilms [40].

It was also reported that Eugenol significantly decreases the viability of the organism *H. pylori*, irrespective of the strain. Activity of these compounds at low pH levels may help them achieve their efficacy in an environment such as human stomach. Further, literature survey showed that topical application of eugenol to the gastric lining enhances the mucus secretion which has beneficial effect in treatment of peptic ulcer via increased mucus secretion[41]. These findings are encouraging in view of growing treatment failures and antibiotic resistance in the area of *H. pylori* management [31].

Antiviral Action

Benencia and Courreges (2000) demonstrated that eugenol possesses antiviral activities *in-vitro* and *in-vivo* against human herpes virus [42]. The effective concentration of eugenol and acyclovir for 50% inhibition of HSV-I were 25.6 µg/ml and 0.30 µg/ml, respectively while those of HSV-II were 16.2 µg/ml and 0.27 µg/ml, respectively. Eugenol also proved to be effective in *in-vivo* model of HSV-I infection. They also showed that eugenol treatment significantly delayed the development of herpetic keratitis in the cornea of HSV-I infected mice. Eugenol exerts inhibitory effects on lipid peroxidation and an effective antivirus replication of either RNA or DNA virus. Thus, eugenol has a mechanism of action for antiviral activity that disables the viral lipidic envelope[43-47]. On the other hand some studies have shown that eugenol induced glutathione S- transferase (GST) in rat liver in vivo which in turns could inhibits the replication of HSV-I[48-49]. Thus, antiviral activity of eugenol could be due to an indirect effect of its reported modulatory influences on cellular GST activities and acid soluble sulphydryl levels.

Antileishmanial and Antihelmintic Action

Protozoan parasites of the genus Leishmania cause visceral, cutaneous, and mucosal diseases in humans, which are collectively referred to as leishmaniasis. A major emerging problem is coinfection of Leishmania with human immunodeficiency virus, especially because there is no effective treatment for these patients. The number and efficacy of drugs available for the treatment of human and animal trypanosomiasis, leishmaniasis, amoebiasis and malaria are limited. Extracts and biologically active compounds isolated from plant species commonly used in herbal medicine attract the attention of scientists to investigate as alternative medicine for treating the leishmaniasis due to the side effects and the resistance that pathogenic protozoan parasites develop against the conventional drugs [50-51]. Nakamura et al., [4] demonstrated in the study that 100% of *L. amazonensis* parasites were destroyed by both essential oil (at 100 mcg/ml) and eugenol (at 100 mcg/ml), after 60 min treatment, regardless of the developmental form of the parasites (promastigote or amastigote). Mouse macrophages were unaffected by the same conditions [4]. Nitric oxide production was dramatically stimulated when mouse peritoneal macrophages were treated with 150 mcg/ml essential oil, both before and after infection with *L. amazonensis*. Nitric oxide (NO) is the principal effector molecule mediating intracellular killing of Leishmania, although the mechanisms by which phagocytes kill microorganisms are still not completely understood. Undoubtedly, phagocyte-derived reactive oxygen species (ROS) and reactive nitrogen species (RNS) are important molecules in this process.

Antihelmintic action of eugenol was also investigated in-vitro by using microwell plate assay on *Caenorhabditis elegans* wild strain, N2 type. ED_{50} for eugenol was found to be 62.1µg/ml which is comparable to the standard compounds levamisole and p-Anisaldehyde. ED_{50} value is 3.2 µg/ml and 93.7 µg/ml for levamisole and p-Anisaldehyde, respectively[52].

Central and Neuroprotective action

Eugenol apart from the peripheral actions also acts at central level. In nervous system, eugenol is neuroprotective against excitotoxicity, ischemia and amyloid-β peptide, inhibits the conduction of action potential in sciatic nerves and improves neuronal and vascular complications in experimental diabetes [53-57]. Eugenol has been also reported to elongate bradykinin and kallikrein induced tranquilization[58]. Ancient Chinese pharmacopoeias revealed that most herbal remedies indicated for Alzheimer disease (AD or symptoms reminiscent of AD) contains the botanical Rhizoma *acori graminei* which is rich in eugenol[11]. Alzheimer's disease (AD) is a chronic and progressive neurodegenerative disorder which was first described in 1907 by Alois Alzheimer and is characterized by a progressive loss of neurons and synapses with the presence of large numbers of extracellular amyloid plaques. It severely affects cognitive function and other behavioral aspects such as executive function and language skills. Both extracellular and intracellular accumulation of Aβ initiates a cascade of events including synaptic and neuritic injury, microglial and astrocytic activation (inflammatory response), altered neuronal ionic homeostasis, oxidative damages, changes of kinases/phosphatases activities, formation of NFTs, and finally cell death. Visible pathological changes such as amyloid plaque formation and neurofibrillary tangles (NFTs) are a consequence of Aβ deposition. Eugenol protects neuronal cells from NMDA-induced excitotoxic and oxidative injury[54, 59]. Eugenol has also shown to have neuroprotective effect in hippocampal tissue due to its ability to induce brain-derived neurotrophic factor (BDNF) and inhibition of Amyloid beta peptide (Aβ) induced cell death by blocking abnormal Ca^{+2} influx caused by Aβ[53]. Eugenol has also got neuroprotective effects due to its ability to block excitotoxicity and antioxidant activity.

In US patent file no 61/134,593 (2008), combination of niacin and eugenol was reported for the treatment of a disorder and/ or a disease caused by excess production of amyloid beta peptide-42 (Aβ42), its deposition, accumulation, and plaque formation [60]. The eugenol molecule potentiate the activity of niacin and can used in the treatment of Alzheimer's Disease, dementia and mild cognitive impairment as well as other neurodegenerative diseases such as Parkinson's Disease and ischemic stroke.

Irie and Keung [53] also reported that eugenol is responsible for epileptic action of rhizome *acori graminei* (RAG), a medicinal herb which used in the East Asia for centuries [53]. It prompts the scientist to investigate therapeutic potential activities of Eugenol for the management of other neurodegenerative and psychiatric disorders such as depression. Depression is mood disorder the prevalence of which is about 10-20% of the general population and which causes common psychological problems. Various drugs are available for treating depression. However, recurrent and chronic depressions are still present at high prevalence. These problems of recurrent and chronic depression propel the scientist to discover new antidepressant drugs.

Eugenol showed positive results in force swimming test (FST) and tail suspension test (TST) that are comparable to imipramine[8]. Further, results obtained by using Real time PCR (RT-PCR) together with FST and TST indicated that Metallothionein-III (MT-III- brain specific member of MTs) may be involved in antidepressive action pathway of eugenol but not of impiramine. Thus, eugenol appears to exhibit its antidepressive activity in a different manner from that of imipramine, which may provide an alternative treatment to patients who are resistant to typical antidepressants. Further, the effect of eugenol on the catalytic activity of monoamine oxidase (MAOs) was studied which indicated that eugenol inhibits MAO-A preferentially with a Ki = 26 μM (Tao et al., 2005). Inhibition of MAO-B was also observed but at much higher concentrations of Eugenol (Ki = 211 μM). Tao et al., [11] suggested a nexus between anti-depressant activity of eugenol and it's MAO-A inhibition.

In Iranian folk medicine, the buds of *Eugenia caryophyllata* (Myrtaceae) have been used as an antiepileptic remedy[15]. Eugenol has also been reported to have antiepileptic activity [7]. It has been shown that eugenol has these activities due to its ability to block synaptic transmission in neurons and ability to block long term potentiation [7].

Toxicity

There are studies from several decades ago that showed eugenol to be a contact allergen when used in dentistry [61-62]. Eugenol penetrates the dental pulp tissue and can enter the bloodstream. Subsequently, eugenol can induce chromosome aberrations (CAs) in human dental pulp cells, and suggested it was a potential mutagen [63]. In rat, Eugenol induced CAs, with significant increases (3.5% aberrant cells) at 2500 mM, demonstrating cytotoxicity at higher doses. Eugenol was also found to be cytotoxic to the human osteoblastic cell line U2OS in an *in vitro* study. The inhibition of growth and proliferation was dose-dependent [64]. Clove oil was highly cytotoxic to human skin cells at concentrations as low as 0.03 percent (v/v) with the effect attributable to eugenol [65]. Clove oil and eugenol were found to be spermicidal in an *in vitro* study [66].

The possible mechanism of cytotoxicity and carcinogenicity could be due to the metabolic reaction of eugenol. Eugenol would undergo three metabolic reactions, hydroxylation, epoxidation and O-demethylation. The reactive metabolite, 2,3-epoxyeugenol, and 10-hydroxyeugenol, after originating the quinone methide, can react with DNA, forming adducts that can contribute to the genotoxic activity (deleterious action on the genetic material) of eugenol [67-71].

Conclusion

Scientists have realized an immense potential in natural products from medicinal plants to serve as alternate source of combating infections in human beings which may also be of lower cost and lesser toxicity. Current review article indicated that Eugenol is a potential phytochemical possessing multifaceted therapeutic activities as described above. Prolonged use of many drugs in patients such as antimicrobial agents, antileishmaniatic agents, and antidepressant drug (imipramine) causes development of resistance to therapeutic agents in individuals. Thus, these agents are of no use to treat the disease in those individuals who are resistant to the drugs.

Especially, antibiotic has certain life span regarding its efficacy. Further, only limited drugs are available for treating diseases such as microbial infections, leishmaniasis and neurodegenerative disorders (e.g. Alzheimer's disease). Eugenol potentiates the effect of Niacin in treating Alzheimer's disease. Thus, eugenol has immense potential to use as drug and potentiate the therapeutic activity of other drugs. Also, eugenol can be a good alternative molecule for treating the disease where resistance to conventional drug has been developed. However, eugenol's toxicity has been studied in laboratory animals or in *in-vitro* conditions and as yet little or no information is available on human exposure and subsequent possible adverse health outcomes. Both toxicological and human exposure data are needed to make accurate risk evaluation.

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