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Mini Review

Methyl Eugenol: Potential to Inhibit Oxidative Stress, Address Related Diseases, and Its Toxicological Effects

Mozaniel Santana de Oliveira[1,2*], Ravendra Kumar[3], Suraj Mali[4] and Eloisa Helena de Aguiar Andrade[1,2]

[1]Programa de Pós-Graduação em Ciências Biológicas - Botânica Tropical, Universidade Federal Rural da Amazônia, and Museu Paraense Emílio Goeldi, Belém, PA, Brazil; [2]Laboratorio Adolpho Ducke, Coordenação de Botânica, Museu Paraense Emilio Goeldi, Belém, PA, Brazil; [3]Department of Chemistry, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India; [4]School of Pharmacy, D.Y. Patil University, Navi Mumbai, Maharashtra, India

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Abstract

This study investigates the potential of methyl eugenol (ME), a compound found in the essential oils of various plants, to inhibit oxidative stress and its impact on diseases associated with this process. ME has been shown to possess antioxidant properties and antiproliferative activity in several cancers. It also demonstrates neuroprotective potential in conditions such as Alzheimer's disease and ischemic brain injury. The mechanism of action involves the activation of the nuclear factor erythroid 2-related factor 2, which facilitates the transcription of antioxidant genes and modulation of pathways such as AMP-activated protein kinase/glycogen synthase kinase 3 beta, thereby reducing the production of reactive oxygen species and pro-inflammatory cytokines. However, research has identified potential toxicological risks associated with ME, including hepatotoxicity and changes in the gut microbiota. These findings highlight the need for caution when considering prolonged exposure to this compound.

Introduction

The potential role of natural products in alleviating disease is an important area of research, particularly with respect to compounds such as methyl eugenol (ME). It is imperative that studies address the limitations of previous research and elucidate the potential contributions of such compounds to therapeutic advances.[1,2] Oxidative stress, defined as an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses, represents a significant health risk. ROS have the potential to damage critical cellular components, including DNA, proteins, and lipids, ultimately leading to genetic mutations and cellular dysfunction.[3–5] Additionally, ROS can activate various signaling pathways that promote cell proliferation and survival, complicating the overall relationship between oxidative stress and cancer, which remains incompletely understood and warrants further investigation.

Keywords: Natural compounds; Phenylpropanoid; Anticancer; Neuroprotection; Essential oil; Methyl eugenol.

***Correspondence to:** Mozaniel Santana de Oliveira, Programa de Pós-Graduação em Ciências Biológicas - Botânica Tropical, Universidade Federal Rural da Amazônia, and Museu Paraense Emílio Goeldi, Av. Perimetral, 1901, Terra Firme, Belém 66077-830, PA, Brazil. ORCID: https://orcid.org/0000-0002-4076-2443. Tel: +55-91-988647823, E-mail: mozaniel.oliveira@yahoo.com.br; mozanieloliveira@museu-goledi.br

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Alzheimer's disease (AD), a progressive neurodegenerative disorder characterized by memory loss, cognitive decline, and behavioral changes, has been implicated in oxidative stress as a contributing factor in its development and progression. The increased production of ROS in the brain, combined with decreased antioxidant defenses, can lead to neuronal oxidative damage. This process facilitates the accumulation of toxic protein aggregates, including beta-amyloid plaques and tau tangles, which are characteristic of AD pathology.[6,7]

Natural compounds represent a promising avenue for mitigating oxidative damage, with ME emerging as a compound of particular interest. ME is a naturally occurring organic compound with the chemical formula $C_{11}H_{14}O_2$, produced by the secondary metabolism of aromatic and medicinal plants. It is a phenylpropene derivative and is abundant in the essential oils of several plant genera and families, such as Poaceae, Cupressaceae, Euphorbiaceae, Apiaceae, Lamiaceae, Zingiberaceae, and Myrtaceae. ME is associated with numerous potential biological activities that may be of importance for human health.[8–12] The objective of this review was to explore these activities with an emphasis on their implications for the pharmaceutical industry, illustrating the compound's prospective role in addressing oxidative stress-related conditions.

Occurrence and biosynthesis

ME is a compound found in a number of different plant families, with its presence and concentration varying widely both within

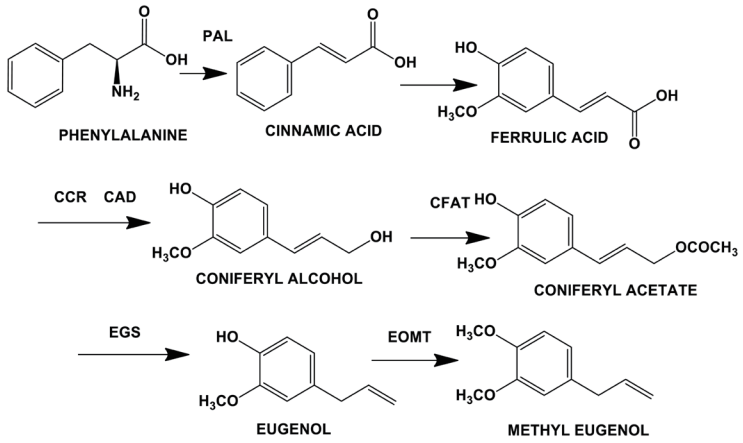

Fig. 1. Potential summary biosynthetic pathway of phenylpropanopid methyl eugenol (PAL). CAD, cinnamyl-alcohol dehydrogenase; CCR, cinnamoyl-CoA reductase; CFAT, coniferyl alcohol acetyltransferase; EGS, eugenol synthas; EMOT, eugenol O-methyltransferase. Adapted from Tan *et al*.[13]

each species and between families. The families with the highest occurrence of ME, in descending order, are as follows: Asteraceae (47 species), Apiaceae (44 species), Lamiaceae (38 species), Lauraceae (34 species), Aristolochiaceae (32 species), Rutaceae (23 species), Myrtaceae (20 species), Poaceae (12 species), Cupressaceae (10 species), Euphorbiaceae (10 species), and Zingiberaceae (10 species). A number of species have been found to contain high levels of ME, often exceeding 90%. These include *Croton malambo* (Euphorbiaceae), *Cinnamomum chordatum* (Lauraceae), *Melaleuca* species (Myrtaceae), *Pepper racemosa* (Myrtaceae), *Piper divaricatum* (Piperaceae), and *Clusena anisata* (Rutaceae). In addition, 68 species have been found to contain between 20% and 90% ME in their essential oils. These plant species represent a significant source of ME.[13]

The biosynthetic pathway of ME begins with the amino acid phenylalanine, which is converted to cinnamic acid. This conversion is catalyzed by the enzyme phenylalanine ammonia-lyase. Cinnamic acid is then converted to coumaric acid by the enzyme cinnamate 4-hydroxylase. The next step in the biosynthesis is the conversion of coumaric acid to caffeic acid, catalyzed by the enzyme coumarate 3-hydroxylase. Caffeic acid is further converted to ferulic acid by the action of caffeate O-methyltransferase. The final steps of biosynthesis involve the conversion of ferulic acid to eugenol and subsequent methylation to form ME. Ferulic acid is first converted to coniferyl alcohol by the enzymes cinnamoyl-CoA reductase and cinnamyl alcohol dehydrogenase. Coniferyl alcohol is then converted to eugenol by the enzyme coniferyl alcohol acetyltransferase. Finally, eugenol is methylated to produce ME, and this methylation reaction is catalyzed by eugenol O-methyltransferase.[13,14] In Figure 1, we can observe a summarized representation of the biosynthesis of ME.[13]

Inhibition of oxidative stress

Oxidative stress is a term used to describe an imbalance between the production of ROS and the ability of the body's antioxidant defense mechanisms to neutralize and remove these harmful molecules. ROS include free radicals, such as superoxide anion (O^{2-}), hydroxyl radical (OH), and non-radical molecules like hydrogen peroxide (H_2O_2). In this context, the antioxidant capacity of ME has the potential to inhibit the damage caused by these relative species.[15] Recent studies have demonstrated the ability of ME to inhibit the proliferation of diseases related to oxidative stress. For example, renal oxidative damage can be inhibited through the modulation of the AMP-activated protein kinase (AMPK)/glycogen synthase kinase 3 beta (GSK3β) axis to regulate the cytoplasmic-nuclear translocation of nuclear factor erythroid 2-related factor 2 (Nrf2), which may result in nuclear retention of Nrf2, thereby increasing transcription of target antioxidant genes that protect the kidney from oxidative damage.[16] Other authors also report the potential for free radical stabilization by ME.[17] In Figure 2, we present a schematic representation of the inhibition of oxidative stress by ME.[18]

In addition, ME has demonstrated a robust antioxidant capacity across various biological systems, primarily by modulating cellular responses to oxidative stress. One of the key mechanisms by which ME exerts its effects is through the activation of Nrf2, a central regulator of the antioxidant response. ME activates Nrf2 in a dose-dependent manner, facilitating its translocation to the nucleus, where it binds to antioxidant response elements in the promoter regions of several protective genes. This leads to the upregulation of genes involved in cellular antioxidant defense, such as glutamate cysteine ligase modifier subunit and glutathione S-transferase A1, which are essential for maintaining redox homeostasis and neutralizing ROS. In addition, ME stabilizes Nrf2 by preventing its degradation mediated by Keap1, allowing Nrf2 to accumulate in the cytoplasm and translocate to the nucleus. This process has been confirmed by studies showing enhanced nuclear retention of Nrf2 following ME treatment. ME also reduces intracellular ROS levels, protecting cells from oxidative damage induced by agents like H_2O_2. This protective effect has been observed in several cell lines, including HEK 293 and NIH 3T3 fibroblasts, where ME pretreatment significantly enhanced cellular resistance to H_2O_2-induced damage. Furthermore, ME activates the AMPK/GSK3β pathway, a molecular signaling cascade that modulates Nrf2 function. ME binds with high affinity to AMPK, promoting its activation, which leads to the phosphorylation of GSK3β and inhibition of its activity. This blocks Nrf2's nuclear export signal, facilitating prolonged retention of Nrf2 in the nucleus. The antioxidant effects of ME have also been demonstrated in ischemia/reperfusion injury models in the kidneys and intestines, where ME reduced oxidative stress markers like malondialdehyde and lactate dehydrogenase and modulated inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-6. Additionally,

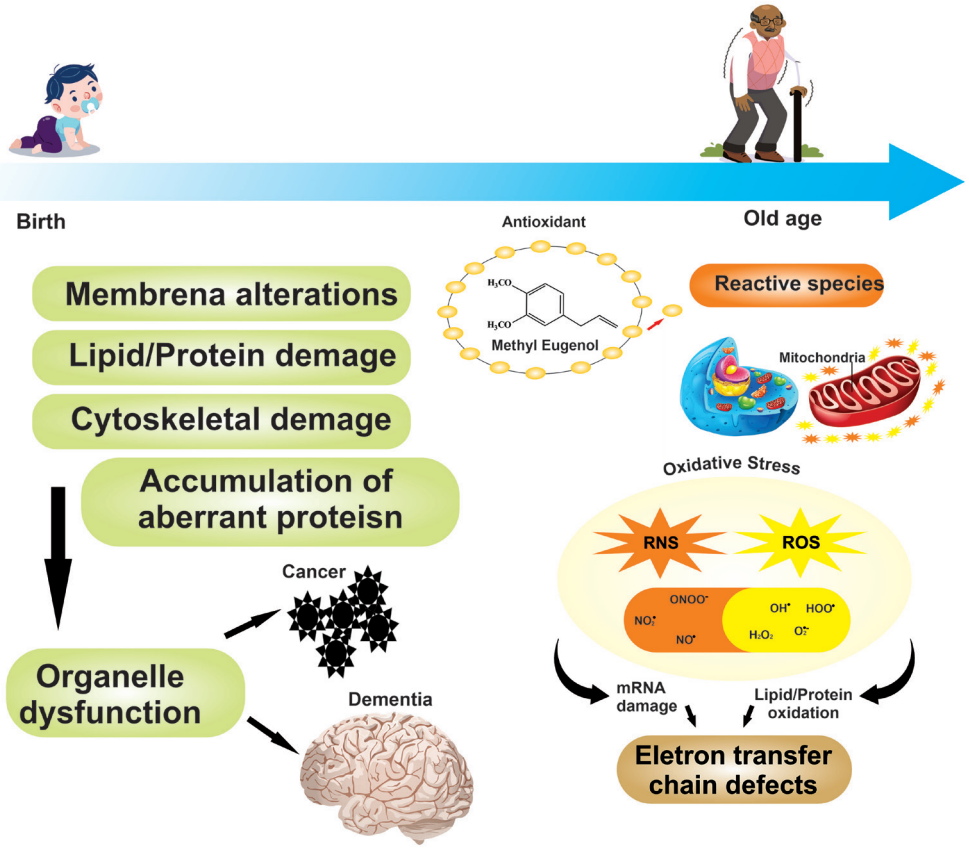

Fig. 2. Excessive reactive nitrogen species (RNS) and reactive oxygen species (ROS), such as H_2O_2 (hydrogen peroxide), HO^- (hydroxyl radical), HOO^- (hidroperoxil), NO^- (nitric oxide radical), NO^{2-} (nitrogen dioxide radical), O^{2-} (superoxide anion radical), and ONOO- (peroxynitrite anion), play a critical role in oxidative stress associated with aging and age-related diseases. The accumulation of ROS causes messenger RNA (mRNA) damage, as well as lipid and protein oxidation, reduces mitochondrial function, and increases oxidative stress, contributing to the development of diseases such as dementia and cancer. Adapted from Tan *et al*.[18]

ME protected against apoptosis and improved tissue integrity, providing significant protection against oxidative damage. In tumor promotion models, ME reduced TPA (12-O-tetradecanoylphorbol-13-acetate)-induced cell proliferation, demonstrating its ability to attenuate oxidative damage caused by TPA, including lipid peroxidation, while enhancing the activity of antioxidant enzymes such as catalase, glutathione reductase, and superoxide dismutase (SOD). In summary, ME exerts its protective effects against oxidative stress through a multifaceted approach, including activation and stabilization of Nrf2, its nuclear translocation, and modulation of essential redox signaling pathways, making it a promising therapeutic agent for conditions related to oxidative stress.[16,17,19–21]

Antiproliferative

Previous studies have reported the effectiveness of eugenol and its derivatives against several cancer types, including leukemia, gastric cancer, colon cancer, prostate cancer, breast cancer, lung cancer, colorectal cancer, skin cancer, and cervical cancer.[22] Eugenol has been found to inhibit tumor proliferation and formation, as well as genotoxic effects in different cancer cells.[23] One study found that ME exhibits significant anticancer activity against retinoblastoma RB355 cells by inducing autophagy and modulating the expression of the m-TOR/PI3K/Akt signaling pathway, which is considered a critical target for the development of anticancer systemic therapies.[24] In another study, the combination of ME with myricetin was found to synergistically enhance the inhibition of cancer cell growth by inducing strong apoptosis, upregulating caspase-3 activity, and arresting cells in the G0/G1 phase of the cell cycle in HeLa immortal cervical cell lines.[25] Several studies have also reported the effectiveness of methyl eugenol-rich essential oils against various cancer types. For example, treatment of *Ocimum tenuiflorum* essential oil significantly inhibited cell viability and metastasis in gastric cancer cells, leading to cell death.[26]

The anticancer potential of ME, a plant-derived phenylpropene, was investigated in a study conducted on retinoblastoma RB355 cells. ME demonstrated a dose-dependent cytotoxic effect, with an IC_{50} value of 50 μM, resulting in a reduction in cell viability in these cancer cells. Furthermore, the compound induced G2/M phase arrest in the cell cycle, indicating that ME may interfere with cell proliferation. Autophagy was also triggered in a dose-dependent manner, indicating that ME not only inhibits cell growth but may also initiate cell self-destruction processes. The study additionally examined the molecular mechanisms underlying ME's anticancer activity, with a particular focus on the mTOR/PI3K/Akt signaling pathway. Western blot analysis demonstrated that ME treatment resulted in a concentration-dependent downregulation of key proteins involved in this pathway, including mTOR, phospho-

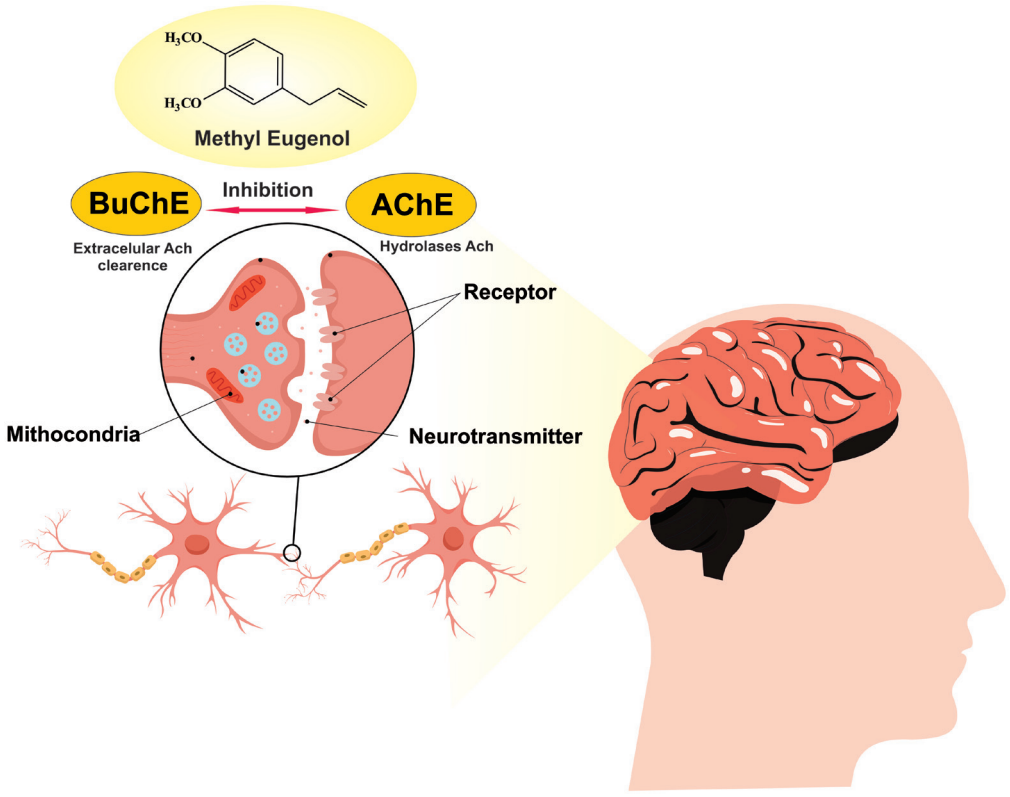

Fig. 3. Schematic representation of methyl eugenol as an inhibitor of the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in the cholinergic system.

rylated mTOR, PI3K, and Akt. This suggests that ME exerts its anticancer effects by targeting and modulating the mTOR/PI3K/Akt signaling pathway, which is essential for regulating cell growth, survival, and autophagy. In conclusion, these findings suggest that ME may have the potential as an effective anticancer agent against retinoblastoma. Further in-depth *in vivo* studies are warranted to explore its therapeutic potential.[24]

Neuroprotection

AD and Parkinson's disease are neurodegenerative disorders affecting the elderly. Alzheimer's causes cognitive decline, while Parkinson's affects the motor system due to dopamine cell loss.[27] There is no cure for these conditions, and treatments focus on symptom management. ROS have been implicated in neurodegeneration. Excessive ROS production can cause oxidative stress, leading to damage of cellular components and disruption of normal neuronal function. This correlation highlights the importance of antioxidant defenses in mitigating neurodegenerative processes.[28,29] Several studies assessing the neuroprotective properties of various essential oils have denoted their neuroprotective action.[28,29] A phenolic analog of eugenol, i.e., ME (4-allyl-1, 2-dimethoxybenzene), is a component of many essential oils, including, but not limited to, clove and anise, and possesses potent neuroprotective potentials. The literature includes several reports suggesting the acetylcholinesterase inhibitory potentials of the phenylpropanoids ME, eugenol, and β-elemene. However, their exact mechanisms have not been fully understood. In one study, Banpure and Chopade conducted molecular docking analysis of several phytoconstituents, including ME, against 11 different Alzheimer targets, such as acetylcholinesterase and butyrylcholinesterase, including 4TPK, 4AQD, 6EP4, 1H22, 4EY5, 2XQF, 6O4X, 6O4W, 4BDT, 6EQQ, and 1B41. ME demonstrated good binding affinities against these selected targets.[27] One study concluded that exposure to ME reduced tert-butyl hydroperoxide-induced cytotoxicity, decreased ROS production, and increased SOD and glutathione levels.[30] This was linked to the upregulation of glutamate-cysteine ligase catalytic/modifier, heme oxygenase-1, and NAD (P) H: quinone oxidoreductase, which relied on Nrf2 induction, inhibition of Keap1 expression, and enhanced antioxidant response element activity.[30] Meena *et al*.[31] discovered that the extract of *Ocimum sanctum* has inhibitory effects on α-synuclein aggregation, a target for preventing Parkinson's disease. They conducted molecular docking of the extract against the α-synuclein target (PDB: 1XQ8) and found a docking score of −7.2 Kcal/M^{-1}.[31] The ME from the extract was found to interact with Lys10, Ala17, Lys21, and Lys80 through hydrogen bonding.[31] In Figure 3, we present a summary of the neuroprotective potential of ME.

ME exerts its neuroprotective effects against ischemic injury through a multifaceted mechanism, primarily by modulating oxidative stress and inflammation. In an *in vivo* model of middle cerebral artery occlusion and reperfusion, ME was observed to significantly reduce cerebral infarction and edema, indicating its protective capacity.[32] This effect is largely attributed to ME's ability to scavenge ROS and enhance antioxidant defenses. The administration of ME resulted in a reduction in superoxide anion generation and a decrease in ROS levels in both ischemic brain tissue and cultured cells subjected to oxygen-glucose depriva-

tion and reoxygenation. Furthermore, increased activities of key antioxidant enzymes, such as SOD (Mn-SOD) and catalase, were observed, playing a critical role in mitigating oxidative damage. ME also demonstrated the capacity to inhibit nitric oxide production and downregulate the expression of inducible nitric oxide synthase in both brain tissue and glial cells, thereby alleviating oxidative damage. With regard to the inflammatory response, ME suppressed the production of pro-inflammatory cytokines, including IL-1β and TNF-α, which are commonly elevated in ischemic conditions. ME reduced the mRNA and protein expression of these cytokines in ischemic brain lesions and in immunostimulated glial cells. Concurrently, ME facilitated the expression of anti-inflammatory cytokines, including IL-10 and transforming growth factor beta, reinforcing its anti-inflammatory efficacy. Furthermore, ME reduced the activation of caspase-3, an executioner of apoptosis, thereby protecting neurons from cell death. In conclusion, the neuroprotective effects of ME can be attributed to a combination of direct free radical scavenging, upregulation of endogenous antioxidant enzymes, and modulation of inflammatory responses. This makes ME a promising therapeutic agent for ischemia-related brain injuries.[32]

Toxicological effects of ME

A critical review of the toxicological effects of ME has identified significant concerns, particularly regarding its use as an insecticide and in agricultural products. Although ME is an effective insecticide with an LC_{50} of 0.116 μg/mL air, its toxicity poses a risk to non-target organisms, including humans and other animals. Administration of ME to pigs has been shown to induce myorelaxant effects that affect bronchial contraction.[33] This suggests that at elevated concentrations, ME may impair respiratory function, particularly in exposed individuals such as agricultural workers who are continuously exposed to the substance. Toxic effects are of particular concern in mammals, as evidenced by a 28-day study in rats exposed to ME by inhalation. The study showed significant damage to red blood cells and liver enzymes such as alanine transaminase and aspartate transaminase, as well as increases in markers of oxidative stress, indicating severe damage to the liver and hematological system. These effects suggest that prolonged exposure to ME may compromise vital organs and lead to the development of hepatic and hematologic diseases, posing a significant risk to exposed workers. In addition, studies in mice have shown that ME induces hepatotoxicity, manifested by elevated liver enzymes and alterations in hepatic metabolism, particularly in the TCA cycle pathways and glutamate metabolism.[34] These findings suggest that ME may interfere with key biological processes essential for liver function. Furthermore, metabolomic analysis revealed alterations in metabolic pathways critical for liver function. This suggests that ME may disrupt these pathways, potentially leading to significant liver damage. A notable finding was the alteration in the gut microbiota of mice exposed to ME, which may exacerbate liver damage. This is because changes in intestinal bacterial populations affect amino acid metabolism and energy production, which can directly impact liver function. The exact relationship between changes in the gut microbiota and liver damage remains unclear. However, this finding suggests that the microbiome may act as a modulator of ME toxicity, potentially exacerbating its effects. This evidence is of concern because ME is a widely used chemical that persists in the environment and can be inhaled by workers, resulting in ongoing exposures that can lead to chronic damage, as observed in studies in rats and pigs. It is therefore imperative that the regulations governing the use of ME in agricultural products and fragrances be reviewed in light of these emerging toxicological data. In conclusion, although ME is an effective insecticide, it poses a significant hazard to human and animal health, particularly when exposure occurs over prolonged periods or at high doses. Hepatotoxicity, changes in the gut microbiota, and interference with hepatic metabolism are of paramount concern and require further investigation and reassessment of product safety and use regulations, accompanied by the implementation of stringent safeguards to mitigate public health risks.[33–37]

Conclusions

ME has been shown to be a promising compound with a number of beneficial biological activities, including antioxidant, anticancer, and neuroprotective properties. However, the toxic effects observed in *in vivo* studies underscore the need for regulations and more comprehensive investigations to ensure the safety of its use in therapeutic and agricultural applications. Further investigation of its properties and mechanisms may facilitate the development of novel therapeutic approaches while taking into account potential health risks. To facilitate the further development of ME in clinical and industrial applications, it is imperative that additional studies are conducted to gain a deeper understanding of its long-term effects and the underlying molecular mechanisms. Future investigations should prioritize the optimization of safe doses and the exploration of formulations that minimize the toxic effects of ME. In addition, the evaluation of the effects of ME on the microbiota and other physiological systems should be a key area of investigation. The development of derivatives or synergistic combinations with other compounds may extend its therapeutic potential. Clinical trials will be necessary to validate its efficacy and safety, which may lead to the use of ME in the treatment of diseases related to oxidative stress and neurodegenerative conditions.

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Conflict of interest

MSO has been an editorial board member of *Future Integrative Medicine* since October 2022. The other authors declare no conflict of interest.

Author contributions

Study concept and design (MSO, SM), data acquisition (RK, EHAA), data analysis and interpretation, manuscript writing (MSO, RK, SM, EHAA), critical review of the manuscript regarding important intellectual content (MSO, EHAA, SM), administrative, technical or material support, and study supervision (MSO). All authors contributed significantly to this study and approved the final version of the manuscript.

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