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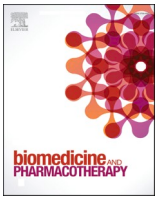

Review

Peppermint essential oil: its phytochemistry, biological activity, pharmacological effect and application

Hui Zhao [a,b,1], Shan Ren [a,1], Han Yang [a], Shun Tang [a], Chenyang Guo [b], Maolun Liu [a], Qiu Tao [a], Tianqi Ming [a], Haibo Xu [a,*]

[a] State Key Laboratory of Southwestern Chinese Medicine Resources, Department of Pharmacology, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China

[b] Department of Pharmacology, School of Pharmacy, Henan University of Chinese Medicine, Zhengzhou 450000, China

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ABSTRACT

Mentha (also known as *peppermint*), a genus of plants in the taxonomic family Lamiaceae (mint family), is widely distributed throughout temperate regions of the world. *Mentha* contains various constituents that are classified as *peppermint* essential oil (PEO) and non-essential components. PEO, consisting mainly of menthol, menthone, neomenthol and iso-menthone, is a mixture of volatile metabolites with anti-inflammatory, antibacterial, antiviral, scolicidal, immunomodulatory, antitumor, neuroprotective, antifatigue and antioxidant activities. Mounting evidence indicates that PEO may pharmacologically protect gastrointestinal, liver, kidney, skin, respiratory, brain and nervous systems, and exert hypoglycemic and hypolipidemic effects. Clinically, PEO is used for gastrointestinal and dermatological diseases, postoperative adjuvant therapy and other fields. This review aims to address the advances in the extraction and isolation of PEO, its biological activities, pharmacological effects, toxicity and applications, with an emphasis on the efficacy of PEO on burn wounds and psoriasis, providing a comprehensive foundation for research, development and application of PEO in future.

1. Introduction

Mentha (also known as *peppermint*) is a genus of plants in the taxonomic family Lamiaceae (mint family), and it is widely distributed across the temperate regions of the world. As a medicinal herb in folk medicine, aromatic *Mentha* is commonly applied in the medication for flu, headache, red eyes, fever and sore throat [1], and it is also used as a flavoring agent and functional tea. It is reported that the extracts of Iranian *Mentha* species show strong antioxidant and scolicidal activities, suggesting the development of anti-parasitic drugs from *Mentha* [2].

Mentha contains a variety of ingredients that are classified as *peppermint* essential oil (PEO) and non-essential components including steroids, flavonoids, triterpenoids, phenolic acids, etc. The PEO, consisting mainly of menthol, menthone, neomenthol and iso-menthone, is a mixture of biologically active secondary metabolites with anti-inflammatory, antibacterial, antiviral, scolicidal, immunomodulatory, antitumor, neuroprotective, antifatigue and antioxidant activities. Cumulative evidence reveals that PEO may pharmacologically protect gastrointestinal, liver, kidney, skin, respiratory, brain and nervous systems, and exert hypoglycemic and hypolipidemic effects. Clinically, PEO

Abbreviations: C-section, cesarean section; DSPI, dexamethasone sodium phosphate injection; ERK, extracellular regulated MAP kinase; FD, functional dyspepsia; GABA, γ-aminobutyric acid; GC, gas chromatography; H&E, hematoxylin and eosin; HSV, herpes simplex virus; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; IPSC, inhibitory postsynaptic current; JAK2, Janus kinase 2; MEBO, moisture burn ointment; MITF, microphthalmia-associated transcription factor; MS, mass spectrometry; NF-κB, nuclear factor kappa B; NMR, nuclear magnetic resonance; PAG, periaqueductal gray; PAMP, pathogen-associated molecular pattern; PEO, *peppermint* essential oil; PGE2, prostaglandin E2; PK-PD, pharmacokinetics and pharmacodynamics; PM, particulate matter; PUPPP, pruritic urticarial papules and plaques of pregnancy; ROS, Reactive oxygen species; RSV, respiratory syncytial virus; SC, stratum corneum; SPF, sun protection factor; STAT3, signal transducer and activator of transcription 3; TCR, T-cell receptor; TDDS, transdermal drug delivery system; TGF-β, transforming growth factor beta; TRP, tyrosinase-related protein; TRPM8, transient receptor potential melastatin 8; TRPV1, transient receptor potential cation channel subfamily V member 1.

* Correspondence to: State Key Laboratory of Southwestern Chinese Medicine Resources, Department of Pharmacology, School of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China.

E-mail address: haibo.xu@cdutcm.edu.cn (H. Xu).

[1] These authors contributed equally to this work.

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is used for gastrointestinal and dermatological diseases, in addition to other postoperative adjuvant therapy [3]. Due to the phytochemical property of the oil, the extraction and isolation of PEO are quite tricky but important for the research and development of PEO. Transdermal absorption following topical administration of PEO is critical, which influences the effectiveness of PEO applications in clinical settings, particularly in dermatologic disease, such as burn wounds and psoriasis.

Herein, we sought to systemically review the updated information on the extraction and isolation of PEO, its biological activities, pharmacological effects, toxicity and application, with a focus on the efficacy of PEO on burn wounds and psoriasis, providing a comprehensive foundation for in-depth research, intensive development, and broad application of PEO in future.

2. Extraction and isolation

PEO is a complex mixture of biologically active secondary metabolites, such as menthol, menthone, neomenthol, iso-menthone, and so forth. Since the phytochemical property of PEO is quite specific and is associated with the biological activities, pharmacological effects and applications of PEO, of importance are the extraction of *Mentha* and isolation of PEO, which are illustrated in Fig. 1.

2.1. Extraction of Mentha

Mentha is grown from rhizomes and harvested at the onset of flowering for maximum yields of high-quality essential oils. The extraction of bioactive components from plants is always a challenge, several different methods can be used to extract essential oils with specific qualities of active compounds from plant materials. Some of the techniques for extracting active compounds from essential oil are steam distillation, hydrogenation distillation, microwave-assisted extraction, supercritical fluid extraction, ultrasonic-assisted extraction and countercurrent extraction [4].

The recovery rate and chemical composition of essential oil components in plants, especially medicinal plants, are dependent on the original plant raw materials and the extraction technology used [5]. After being harvested, *Mentha* is sun-dried, followed by being ground in a blender. The average particle size and the range of particle size were determined after sieving, and the moisture content in the plant material was determined gravimetrically by drying at 105 ℃ until constant weight is reached. Adjusting the average particle size of *Mentha* to

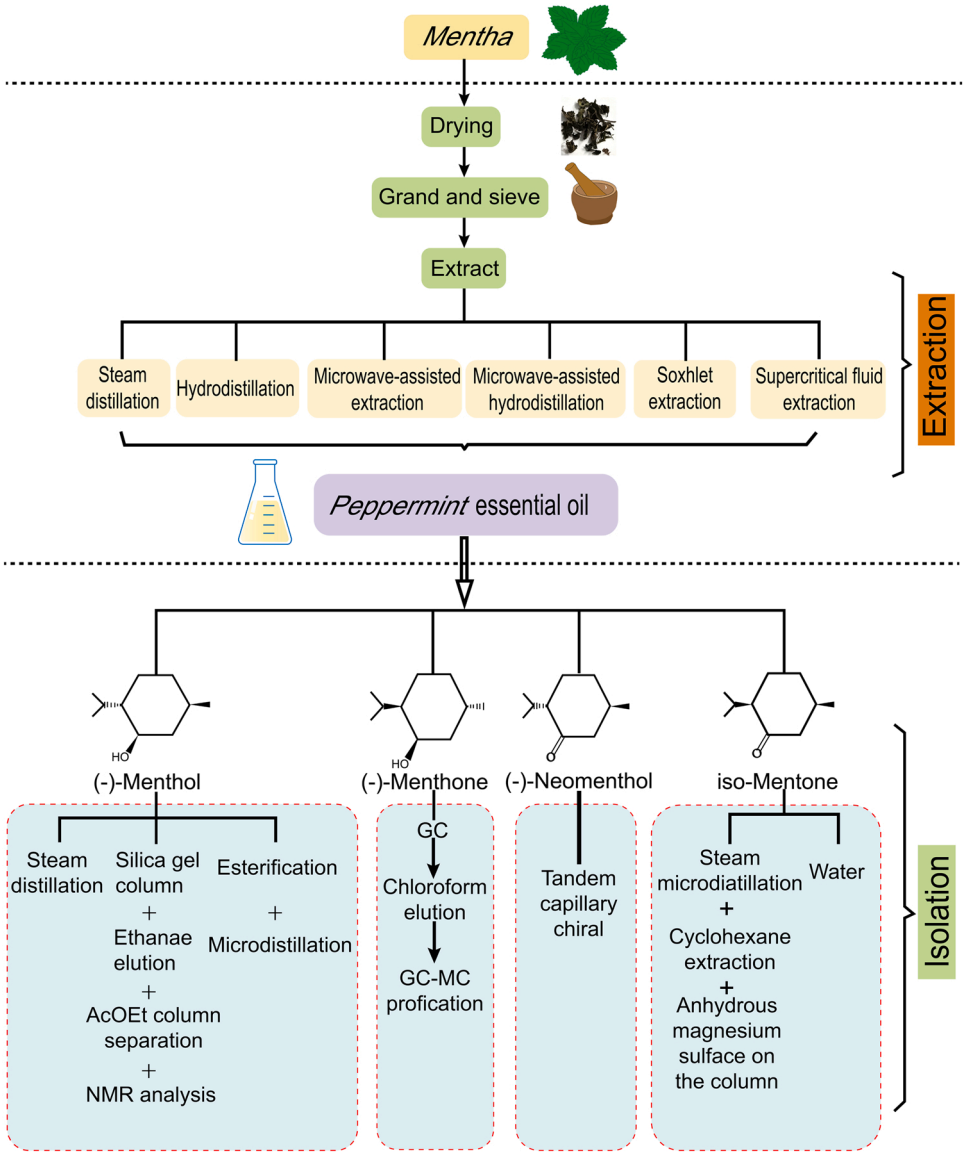

Fig. 1. Extraction and isolation process of the main components of *peppermint* essential oil (PEO).

0.224 mm and adjusting the moisture content to 8.77% can reduce mass transfer limitations and improve extraction [6]. The resultants are then collected in barrels in preparation for steam distillation. Hydrodistillation and ethanol solid-liquid extraction are usually used to extract essential oil components, and further rectification with fractional distillation technology can improve the aromatic odor of the oil [7].

The main chemical components in *Mentha* are identified as menthol, menthone, neomenthol, iso-menthone. There are three common extraction methods for the essential oil components, including hydrodistillation, steam distillation and microwave distillation [8]. Standard Soxhlet and supercritical fluid extraction, microwave-assisted extraction and ultrasonic-assisted extraction are the more classic methods for extracting essential oils from *Mentha*. Adjusting different power settings can get a higher yield of PEO. Conventional hydrodistillation, a traditional method proven to separate the essential oil components of *Mentha*, is a possible alternative method. Also, microwave-assisted hydrodistillation may be used to separate PEO [9]. Hydrodistillation and steam distillation are better in terms of extraction yield and quantification of major compounds, but microwave-assisted distillation can shorten the extraction time from 180 min to 30 min.

2.2. Isolation of PEO components

Twenty-six compounds have been identified in PEO. Among them, menthol, menthone, neomenthol, and iso-menthone are the chemical components currently considered to be important and effective in PEO. Purification of these chemical constituents is very critical for pharmacological study. In gas chromatography (GC), the range of different stationary phases, the thickness of different film, and the length of the column are factors to influence component isolation. Mass spectrometry (MS) increases discriminative power to separate compounds, and overlapping components may require spectroscopy to aid MS characterization. In all cases, it is self-evident that complete separation of the components simplifies the downstream identification process. The search for technology with improved separation capability continues, increasingly in the field of multidimensional gas chromatography [10].

2.2.1. Isolation of menthol

Menthol (also known as menthol camphor) is a cyclic monoterpene alcohol that is a major component of *Mentha*. Menthol works with menthone, iso-menthone, and other compounds to confer plants a cool minty scent. Menthol is biosynthesized in plants through an 8-step pathway of secondary metabolism. PEO contains high concentrations of menthol and menthone, and the former is usually obtained by steam distillation of *Mentha* [11]. Researchers have separated the essential oil components on a silica gel column through gradient elution with ethane followed by ethane/AcOEt chromatography, identified menthol components by high-performance thin-layer chromatography, and separated menthol by nuclear magnetic resonance (NMR) analysis [12]. Menthol can also be isolated by vacuum distillation using a micro distillation apparatus after the esterification reaction [13].

2.2.2. Isolation of menthone

A mixture containing menthone can be obtained by injection of PEO into the precap-GC system. By repeating the above operation and eluting with chloroform, menthone can be isolated. Additionally, following separation and purification of PEO with the Varian 3300 Gas Chromatograph, menthone can be further isolated using HP-InnoWax capillary column or HP-5 capillary column in a GC-MS system [14].

2.2.3. Isolation of neomenthol

Neomenthol, a stereoisomer of menthol, is a cyclic monoterpene. Menthone can be reduced to the epimer alcohols e.g. iso-menthol and neomenthol. In leaf discs of flowering *Mentha*, part of menthol was converted to menthyl acetate, while most menthol and neomenthol were converted to neomenthyl β-D-glucoside. In flowering *Mentha*, the major monoterpene component 1-menthone is converted to iso-menthol. A study of menthone metabolism in *Mentha* leaf discs confirms that menthone is converted to menthol and menthyl acetate, and that a significant portion of 1-menthol can be converted to d-neomenthyl-β-D-glucoside [15].

2.2.4. Isolation of iso-menthone

Menthone and iso-menthone are the main compounds in *Mentha* leaves, and they are highly synthesized during the filling of the epidermal oil glands in the rapidly growing young *Mentha*. Concomitantly, a small amount of neomenthyl acetate and menthol glycosides are produced, resulting in the specific metabolic profile of the epimeric reduction products in PEO. After steam micro-distillation and simultaneous cyclohexane extraction for 2 h, the iso-menthone component can be obtained by drying on anhydrous magnesium sulfate and concentrating [16]. Reportedly, iso-menthone may be gotten directly by water distillation [17].

3. Biological activities of PEO

PEO is mainly metabolized in the body by its reaction with glucuronic acid and then eliminated in urine or feces. A growing body of evidence reveals that PEO has anti-inflammatory, antibacterial, antiviral, scolicidal, immunomodulatory, antitumor, neuroprotective, antifatigue and antioxidant effects, which are depicted in Fig. 2.

3.1. Anti-inflammatory activity

Menthol is an agonist of the transient receptor potential melastatin 8 (TRPM8) channel. In irritable bowel syndrome (IBS), menthol can activate TRPM8 channel to inhibit the chemical and mechanosensory responses of nociceptive TRP channels and diminish the release of pro-inflammatory mediators from nerve endings [18]. PEO can regulate IBS by inhibiting the expression of pro-inflammatory cytokines and up-regulating the levels of anti-inflammatory cytokines. Oral administration of PEO can prevent xylene-induced intestinal inflammation in mice and acetic acid-induced colitis in rats [19]. The gastroprotective effect of menthol through anti-inflammatory activity is mainly attributable to mucus secretion, which is related to prostaglandin E2 (PGE2) production and K^+-ATP channel activation and antisecretory effect [20].

It is documented that PEO may effectively alleviate excessive inflammation and subsequent atopic dermatitis-like lesions by inhibiting the ERK-NF-κB pathway [21]. And, menthol can reduce inflammation and attenuate oxidative stress [21,22]. PEO inhibits carbachol-induced muscle contraction involving autonomic ganglia, and has anti-inflammatory and analgesic effects, especially in respiratory disease [23]. Also, PEO is found to have anti-gout and analgesic effects [24], and exert strong anti-inflammatory activity in the croton oil-induced mouse ear edema, by inhibition of NO and PGE2 production [25].

3.2. Antibacterial activity

Mounting evidence demonstrates that PEO possesses powerful antibacterial activity [26]. *Staphylococcus aureus* belongs to one of the superbugs of the *Staphylococcus* genus, becoming a troublesome bacterial strain in contemporary invasive medication [27]. PEO exhibits inhibitory effects on human pathogenic bacteria *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtilis*, *Escherichia coli*, *Neisseria gonorrhoeae*, and *Pseudomonas aeruginosa* [28,29]. The analyses with broth microdilution and disc diffusion method show that PEO has a strong antibacterial effect on *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus* and *Escherichia coli* [30].

Accumulating evidence has established that PEO can suppress *Streptococcus* pneumonia, *Salmonella* enteritidis and *Salmonella typhi*,

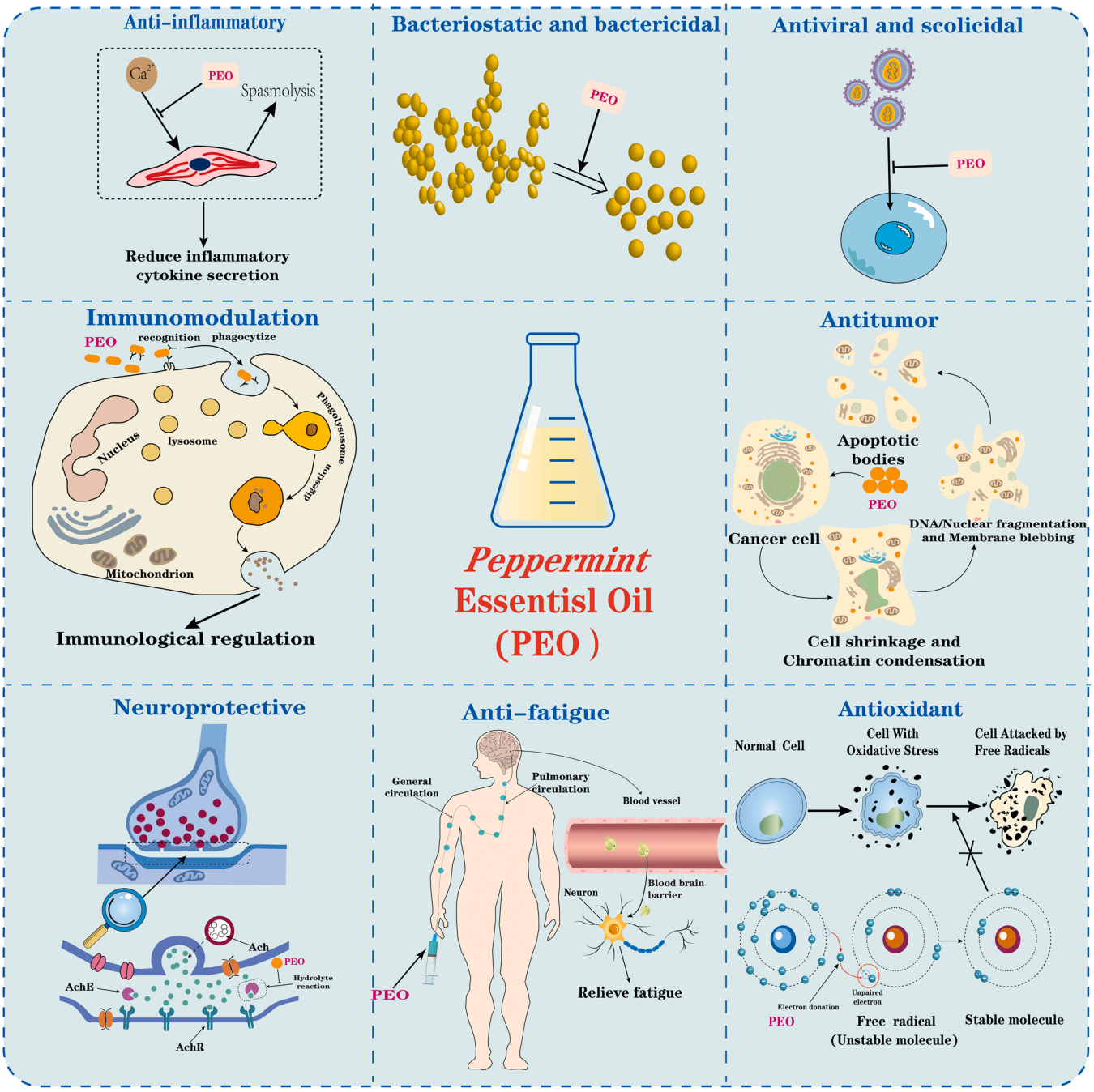

Fig. 2. Biological activities of *peppermint* essential oil (PEO).

especially against different skin molds and *Candida albicans* [31]. PEO can also inhibit the growth of fungal strains, such as *Aspergillus niger* [32], and *Vibrio* [33]. Moreover, PEO can reduce the movement of worms, and kill adult *Salmonella mansoni* and schistosomes [34]. The menthol in PEO is shown to weaken the quorum sensing activity of Gram-negative pathogens [35].

PEO can be effective against *Chlamydia trachomatis* in several ways. First, PEO inhibits *Chlamydia trachomatis* and prevents its entry into host cells. Secondly, PEO inhibits the replication of *Chlamydia trachomatis*, thereby reducing infectivity and thus the progression of infection. The combination of PEO and erythromycin can decrease the replication of *Chlamydia trachomatis* and reduce the dose of antibiotics [36]. PEO can decrease the number of *Clostridium nucleatum*, plankton and biofilm-embedded cells, indicative of an effective therapeutic agent for oral disease [37]. In addition, PEO can alleviate abdominal pain in IBS-like rats by modulating fungal biota [38].

Studies have shown that PEO can synergize with other agents to produce synergistic antibacterial effects against Gram-positive and Gram-negative microorganisms [39]. When PEO is used in combination with gentamicin, the dosage of gentamicin required to kill bacterial strains is significantly reduced [40].

It is documented that PEO has a strong bactericidal effect on *Escherichia coli* and can destroy the integrity of bacterial cell membrane [41]. Besides, PEO can hamper the formation of bacterial biofilm, and reduce the activity of biofilm and bacterial adhesion [42].

3.3. Antiviral and scolicidal activity

Virus particle fusion experiments showed that the entry of HIV-1 into cells was severely suppressed after the particles were treated with PEO, and the replication efficiency of the virus could be inhibited at the early stage of virus infection. Studies have revealed that PEO can rapidly mitigate the infectivity of HIV-1 virions at a concentration non-cytotoxic to the host. The human respiratory syncytial virus (RSV) is a syncytial virus that causes respiratory infection. It is demonstrated that PEO exhibits strong activity against RSV [43].

The free virus is very sensitive to PEO. Inhibition of herpes simplex virus (HSV) by PEO appears to occur before or during viral entry to the host cells. PEO reduces viral infectivity, possibly by direct interactions with viral envelope and glycoprotein. Additionally, PEO may interfere with the later stage of HSV-1 life cycle, resulting in the suppression of viral replication [44].

It is reported that Iranian *Mentha* species possess strong scolicidal activity against protoscolices of hydatid cysts. Particularly, 200 mg/ml methanol extract of *Mentha aquatica* exhibits the highest scolicidal activity (99.54%) after 30 min of exposure time, suggesting that antiparasitic agents may be developed from *Mentha* [2].

3.4. Immunomodulatory activity

Phagocytes, especially macrophages, are the first-line effector of innate immune system, by eliminating pathogenic microorganisms that invade the host. Activation of macrophages is associated with the recognition of pathogen-associated molecular pattern (PAMP). In a study in vitro, PEO is found to modulate immune activity through phagocytosis [45].

Also, PEO can inhibit airway epithelial hyperplasia, collagen deposition and goblet cell activation in asthmatic mice, by decreasing IL-6 level via regulation of phosphorylation of Janus kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3) [46].

3.5. Antitumor activity

Under healthy conditions, cell renewal and proliferation are balanced by cell death, e.g. apoptosis. This balance is disrupted during tumor development to promote cancer cell proliferation. PEO exhibits weak to moderate antiproliferative properties and may impede the growth of neuroblastoma by down-regulation of the expressions of some oncogenes, including epidermal growth factor receptor [47].

It is evidenced that PEO significantly inhibits the proliferation of colorectal cancer cells SW480, by inducing apoptosis and arresting cell cycle at G1/G0 and G2/M phases. In combination with sage *(Salvia officinalis* L.), the inhibitory effect of PEO on cancer cells was drastically heightened [48]. Another report indicates that PEO may suppress the activity of Topoisomerase I to inhibit gene expression in cancer cells [49].

3.6. Neuroprotective effect

The menthol in PEO enhances phasic and tonic γ-aminobutyric acid (GABA) A receptor-mediated currents in neurons located in all subregions of the periaqueductal gray (PAG). The effect of menthol on tonic current appears to be mediated by extrasynaptic GABAA receptors lacking the delta subunit. Menthol-induced enhancement of GABAergic inhibition within the PAG has the potential to modulate the analgesic and anxiolytic functions of this brain region. Menthol prolongs the decay time of spontaneous inhibitory postsynaptic currents (IPSC) in PAG neurons, but does not affect IPSC dynamics in hippocampal CA1 pyramidal neurons [50]. In addition, menthol exhibits concentration-dependent GABAA and nicotinic receptor-binding properties and significant suppression of acetylcholinesterase, thereby potentially increasing the synaptic availability of acetylcholine. PEO can cause an increase in Ca^{2+} concentration and prolongation of the depolarization response, neuroprotective property under oxidative stress in CAD cells [51]. Also, menthol has a significant effect on subpopulations of sensory ganglion neurons. Menthol stimulates the sensation of cooling and enhances the response of isolated sensory neurons to cool temperature. The findings suggest that menthol can repress cold-sensing trigeminal neurons, with little effect on oral trigeminal thermal sensors [52].

PEO may affect the enteric nervous system to interfere with gastrointestinal neuromotor function, because PEO acts as a smooth muscle relaxant that reverses acetylcholine-induced contraction through calcium channel blockade and antagonizes serotonin-induced contraction by directly inhibiting contractility, thereby inducing circular smooth muscle relaxation in the colon [53]. In a rat model of inflammatory myalgia, topical stimulation of PEO, which can be applied to the skin over the inflamed muscle, inhibits nociceptive neurons in the skin and alleviates muscle pain by activating skin nociceptors [54]. The nasal-brain pathway is a potential route for drug delivery as it bypasses the blood-brain barrier, and nasal administration of PEO can increase the bioavailability for the treatment of neurodegenerative disease [55]. Besides, PEO can relieve bronchospasm, by stimulating the production of nitric oxide and regulating the opening of K^+ channel [23].

3.7. Antifatigue activity

Physical fatigue is defined as the inability to maintain voluntary activities and is associated with physical decline. Exercise produces an excessive accumulation of blood lactate and blood urea nitrogen, which can lead to metabolic disturbance and ultimately fatigue. Peripheral injection of PEO promotes mouse walking behavior. Menthol may increase cellular energy metabolism by stimulating the central nervous system, as menthol can stimulate the adrenal cortex to enhance energy, and lower blood lactate level [56]. Also, PEO may increase lung capacity in healthy subjects, to provide more oxygen to the brain and effectively eliminate fatigue [57].

In addition, PEO increases body alertness and improves mental refreshment [58]. PEO can modulate the olfactory pathway of the brain, relieve anxiety, diminish pain and impulse, and promote sleep quality, contributing to its antifatigue activity [59].

3.8. Antioxidant activity

Reactive oxygen species (ROS), such as superoxide, hydroxyl and peroxy radicals, play a vital role in the pathogenesis of various diseases, including neurodegenerative disease, cancer, cardiovascular disease, inflammatory afflictions, etc [60,61]. *Mentha* species and PEO possess free radical-scavenging activity and antioxidant function [2]. Neutrophil infiltration, free radical formation, and increased oxidative stress are established pathogenic factors in inflammatory bowel disease (IBD). Menthol in PEO reduces oxidative stress in colon tissue, and decreases malondialdehyde level and the end product of lipid peroxidation [62,63].

Besides, PEO lessens melanin synthesis in B16-F10 cells by attenuating the expression of microphthalmia-associated transcription factor (MITF), tyrosinase-related protein (TRP)− 1, TRP-2 and tyrosinase, attributable to its antioxidant activity [64].

4. Pharmacological effects of PEO

It has become a widely held view that PEO harbors multiple pharmacological effects, such as protection of gastrointestinal, liver, kidney, skin, respiratory, brain and nervous systems, and hypoglycemic and hypolipidemic effects. Mechanistically understanding these pharmacological effects is beneficial to the application of PEO in clinical settings.

4.1. Gastrointestinal protection

Mentha is primarily used for upper digestive tract malfunctions such as cramps, indigestion [65], and nausea in most patients [66]. Numerous lines of evidence demonstrate that PEO exerts effective actions against gastrointestinal disorders, as PEO ameliorates stomach and intestinal movement [67], and alleviates satiety and functional dyspepsia [68].

It is reported that PEO relieves gastrointestinal spasms and abdominal pain. Mechanistically, serving as a smooth muscle calcium channel antagonizer, PEO may attenuate the contractility of gastrointestinal smooth muscle by blocking calcium influx through endomysial L-type calcium channel [69]. On the other hand, menthol in PEO is an agonist of transient receptor potential TRPM8 channel that is expressed on colonic primary afferent neurons. Activation of TRPM8 channel by PEO inhibits chemo- and mechanosensory response of pro-nociceptive TRP channel [18], suggesting activation of TRP channel by PEO is associated with its efficacy for functional gastrointestinal disease [70].

Moreover, PEO reinforces gastric emptying, abates IBS [71], and relieves dysphagia and chest pain due to esophageal dyskinesia [72]. Mechanistically, PEO may inhibit the displacement of [^{3}H]GR65630 by [^{14}C]guanidine through the 5-HT3 binding site, and act on the influx of 5-HT3 receptor ion channel and serotonin-induced ileum shrink [73]. Another study implies that menthol can cause a sustained reduction in the release of acetylcholine in neural mechanism involving alpha-adrenergic receptor [74].

4.2. Liver and kidney protection

The liver plays a central role in maintaining systemic lipid homeostasis and is particularly vulnerable to ROS damage. In a rat model of liver injury caused by intraperitoneal injection of tetrachloromethane solution, PEO shows liver protection by enhancing the integrity of the plasma membrane, and elevating the capacity of repair and regeneration of hepatic cells, through anti-oxidative stress [75].

PEO is choleretic and it diminishes intrahepatic cholestasis. PEO can promote bile secretion, reduce total cholesterol and increase bile acid concentration, by potentially regulating the expression of some genes involved in bile acid synthesis [76]. Particularly, Menthol and menthone in PEO can increase bile flow, reduce the coupling of cholesterol secretion to bile salt secretion, modulate inflammatory processes, and suppress liver fibrosis [77].

Urolithiasis is the formation of stones in the urinary system. Urinary crystals and high concentration of calcium oxalate induce tubular epithelial inflammation, which are the major risk factors for urolithiasis. PEO may prevent and treat urolithiasis by crystal-inhibiting, antioxidant, anti-inflammatory, antispasmodic, and diuretic effects [78]. In addition, PEO can significantly prevent gentamicin-related renal injury, without interfering with the antibacterial efficacy of gentamicin [79].

4.3. Skin protection

PEO is widely used to treat a variety of dermatic disorders, including wounds, skin infection, inflammation, eczema, hives, psoriasis, scabies and insect bites [80]. The terpenes in PEO may act as solubilizers across the barrier of the epicuticle, and increase the drug distribution in the stratum corneum. It is well established that PEO alters skin permeability and promotes hair growth, by enhancing vascular formation in the dermal papilla of hair, which may help the early stage of hair growth, indicative of a promising approach for the treatment of hair loss with PEO [81]. It is reported that PEO has a very high sun protection factor (SPF) value, as PEO can form an even and long-lasting sunscreen on skin, and prevent skin from drying by sun and wind [82].

Chronic pruritus is defined as itching that persists for more than 6 weeks and is severe enough to interfere with lifestyle. Itching can be a sign of many skin conditions as well as other non-dermatological conditions. Topical PEO is effective in the treatment of chronic pruritus, as PEO is shown to relieve itching sensations by activating A-delta fibers and k-opioid receptors [83]. In addition, PEO can weaken pruritus of pregnancy (PG) caused by hormonal alteration [84].

It is documented that neomenthol in PEO exhibits inhibitory effects on human epidermoid cancer cells by arresting cell cycle at G2/M phase, inhibiting hyaluronidase activity and affecting tubulin polymerization, leading to suppression of tumor growth, metastasis and angiogenesis [85]. PEO is shown to reduce melanin synthesis in B16-F10 cells. In particular, beta-caryophyllene in PEO is reported to attenuate melanin formation by down-regulating the expression of MITF, TRP-1, TRP-2 and tyrosinase [64].

It is well known that topical administration of PEO produces a lasting cooling effect on the skin against facial neuralgia, mainly due to the spatial changes in cold receptor calcium channel and relaxation of pericranial muscle, giving rise to cutaneous blood flow to the forehead [86].

4.4. Respiratory protection

Fine particulate matter (PM) exposure is one of the risk factors to exacerbate airway inflammation and pulmonary destruction in asthma. In PM10-caused asthmatic mice, inhalation of PEO by nebulization mitigates respiratory epithelial hyperplasia, collagen deposition, and goblet cell activation in asthmatic mice, with lowered levels of IL-6 and pro-inflammatory T helper 2-specific cytokines, and down-regulation of phosphorylation of JAK2 and STAT3, suggesting PEO relieves asthma by inhibiting IL-6/JAK2/STAT3 axis activity [46].

Relaxant PEO may serve as an antispasmodic agent, as PEO and its component pulegone are both effective against acetylcholine- and KCl-elicited contraction of rat tracheal smooth muscle, without influencing the function of ganglia and NO. Further, PEO suppresses $CaCl_2$-evoked contraction in rat depolarized trachea, indicative of impeding extracellular calcium entry [87].

It is documented that menthol suppresses respiratory sensory irritation response to various cigarette smoke irritants, evidenced by that menthol, an agonist of TRPM8 ion channel in cold-sensitive sensory neurons, can abolish mouse irritation response to acrolein, an agonist of transient receptor potential ankyrin 1. Besides, menthol can lessen irritation response to acetic acid, cyclohexanone and transient receptor potential cation channel subfamily V member 1 (TRPV1). These findings indicate that menthol is efficiently absorbed in the respiratory tract, generating a local concentration sufficient for activating sensory TRP channels [88].

4.5. Hypoglycemic and hypolipidemic effects

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, polyuria, polydipsia and polyphagia, with potential complications including retinopathy, nephropathy, neuropathy, ketoacidosis, and so forth. PEO is shown to lower blood glucose, increase insulin and C-peptide levels, and improve pancreatic beta cell structure [89], indicating that PEO may serve as a hypoglycemic agent for diabetes mellitus and its complications [90].

In addition, PEO can effectively resist hyperlipidemia by improving lipid metabolism, reducing serum total cholesterol, triglyceride and low-density lipoprotein cholesterol, accompanied by a significant increase in high-density lipoprotein cholesterol level and a reduction in atherosclerosis index in hyperlipidemic rats. Also, PEO can raise hepatic glutathione level, improve liver function and antioxidant activity, and subsequently increase blood glucose uptake and utilization, which may underpin the hypolipidemic and hypoglycemic effects of PEO [91].

4.6. Brain and nervous system protection

PEO is reported to protect the brain and nervous system. Menthol and menthone in PEO are neuroactive substances. These compounds can

inhibit cholinesterase and bind to nicotine and GABAA receptors, resulting in increased activity of neurons. Continuous administration of PEO significantly facilitates the elimination of mental fatigue [91].

4.7. Transdermal absorption (osmotic absorption)

The skin is a large and effective barrier between the organism and the external environment, preventing the invasion of pathogens and resisting chemical and physical attacks. It is also the most accessible organ of the human body, making it an ideal site for topical administration. Therefore, transdermal drug delivery system (TDDS) has lots of advantages, such as fewer adverse reactions and bypassing the first-pass effect. The outermost stratum corneum (SC) of the skin is the main limitation of TDDS. SC is recognized as a barrier that protects underlying tissues from infection, dehydration, chemicals, and mechanical stress. SC is composed of 15–20 layers of keratinized epidermal cells without nuclei and organelles. The permeability of the SC barrier is attributed to the intercellular lipid matrix. The intercellular lipid domain is widely considered to be the primary pathway for the penetration of most drug molecules through stratum corneum.

The percutaneous administration of PEO can treat a variety of diseases, as PEO penetrates human skin, primarily by passive diffusion, and affects skin permeability of other bioactive substances that are locally irritating. PEO is an essential mixture of aromatic compounds with low molecular weights and various chemical structures, that can easily penetrate the skin to the bloodstream, but they are also easily excreted via urine and feces [92]. The active compounds of PEO can promote transdermal absorption, and the mechanism of action is mainly based on changing the structure of the stratum corneum barrier and interaction with intercellular stratum corneum lipids to increase the diffusivity of osmotic absorption. The menthol component in PEO may be used as a penetration enhancer and preferentially distributed to the intercellular space and intercellular lipid structure of stratum corneum. It is reported that PEO enhances the percutaneous flux of nicardipine hydrochloride by partially extracting lipids of stratum corneum [93].

4.8. Transdermal medication for wound healing and psoriasis

Wound healing is a complex process involving epidermal regeneration, fibroblast proliferation, neovascularization and angiogenesis, etc [94,95]. Severe burn wounds often result in local and systemic activation of the innate immune system, leading to marked inflammatory response, immunosuppression and multiorgan damage. For instance, burn wound may raise IL-10 level and suppress Th1 response, which is implicated in increased susceptibility to infection [96]. The transforming growth factor beta (TGF-β), a proponent of dermal fibrosis, plays a diverse role in the burn wound. TGF-β may function as a wound-healing promoting factor and potentiate overhealing outcomes including hypertrophic scar and keloid in the later stage of the healing process [97].

Topical administration of PEO can restrain the infiltration of immune cells into the wound area and prevent tissue inflammation, which is attributed to the antibacterial effect of PEO [98]. Also, transdermal PEO increases the number of fibroblasts in the wound area, collagen regeneration and epithelialization process [99]. It is demonstrated that PEO promotes wound healing in a mouse model, by regulating the expression levels of TGF-β and IL-10 genes in mice. This research shows that PEO can be used to treat infected wounds [100]. Moreover, PEO promotes wound healing in mice with atopic dermatitis (AD). For intracellular mechanisms, PEO inhibits the expression of molecules in STAT1- and STAT3-dependent pathways, reducing epidermal thickness and mast cell infiltration [101]. In addition, menthol dose-dependently inhibits lymphocyte proliferation and forkhead box P3 expression. Attenuated IFN-γ expression coupled with T-bet downregulation indicates the inhibitory effect of menthol on Th1 cell differentiation, thus suggesting its possible therapeutic potential in inflammatory disease [102].

In our study, a mouse burn model was established by a pre-heated 20-gram weight on depilated skin at 2 cm × 2 cm, and then treated with PEO by transdermal medication. The outcome showed that PEO significantly lessened burn index in mice, which was further substantiated by histopathological analysis of hematoxylin and eosin (H&E) staining, suggesting that PEO ameliorated the histopathological features of burn in mice (Fig. 3).

Psoriasis is a chronic immune-mediated inflammatory disease characterized by uncontrolled keratinocyte proliferation, increased cutaneous T-cell infiltration and release of proinflammatory cytokines [103]. Accumulation of inflammatory cells in the dermis leads to hyperproliferation of epidermal keratinocytes [104]. In this scenario, Treg cells are activated to become effector T cells under the stimulation of TCR (T-cell receptor) or IL-2, and secrete cytokines such as IL-10 and TGF-β, thereby affecting the proliferation of psoriasis-related cells [105].

In our study, we found that transdermal medication of PEO significantly ameliorated the symptoms of psoriasis in mice, such as decreased itches and erythema level, increased skin elasticity and melanin level, and more body weight gain, accompanied by attenuated serum levels of IL-10 and TGF-β in mice with psoriasis. The above data for PEO were corroborated by histopathological analysis with H&E staining, indicating that PEO diminished the histopathological features of psoriasis in mice (Fig. 4).

Collectively, PEO transdermal medication exerts strong effects for wound healing and psoriasis by intricate mechanisms, particularly regulation of IL-10/TGF-β signaling axis, as illustrated in Fig. 5.

4.9. Toxicity

It is well documented that oral administration of PEO to rats at the dosages of 40 and 100 mg/kg body wt. per day for 28 days may cause histopathological change including cyst-like spaces scattered in the white matter of the cerebellum, but no encephalopathy-related clinical symptoms were observed [106]. In a subchronic toxicity study of PEO in rats, oral administration of PEO at the dosage of 100 mg/kg body wt. per day for 90 days elicits cyst-like spaces scattered in the white matter of the cerebellum in male and female rats, without other signs of encephalopathy. In male rats, nephropathy is also noted [107]. In a short-term toxicity study of menthone in rats, no adverse effect was obtained at the dosages lower than 200 mg/kg b.w./day. However, oral PEO at 400 and 800 mg/kg b.w./day for 28 days can diminish creatinine, raise alkaline phosphatase activity and bilirubin in rat plasma, and increase the weight indices of liver and spleen. Besides, cyst-like spaces in the white matter of the cerebellum were got histopathologically [108]. Overall, these findings suggest that PEO should be used in human patients with caution, particularly at high dosages.

5. Clinical and other applications of PEO

A great many lines of evidence reveal that PEO may be applied to the clinical practice for the management of multiple diseases and other fields, such as food industry, cosmetic industry, agricultural field, due to the diverse properties of PEO.

5.1. Clinical application

5.1.1. Application in gastrointestinal disease

IBS and functional dyspepsia (FD) are common functional gastrointestinal disorders with overlapping symptoms. PEO is effective for IBS-related symptoms that may exist in FD patients, by ameliorating gastroenterological function, improving digestion, and relieving abdominal pain and bloating.

PEO causes significant opening of the pyloric ring and affects the transporting activity of enterocytes in the intestinal lumen by inhibiting its glucose uptake. Besides, PEO plays a role in relaxing the smooth

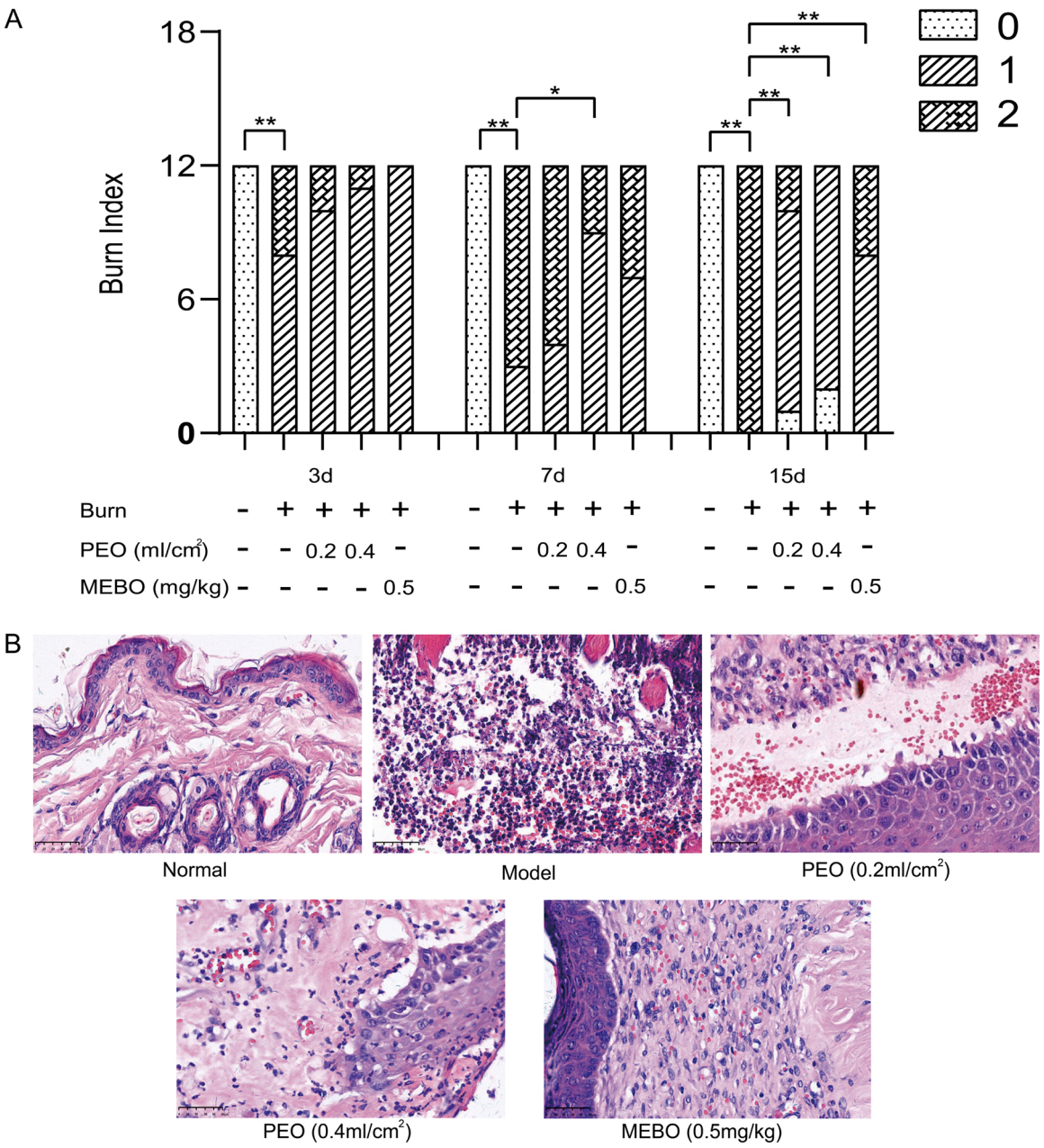

Fig. 3. Inhibitory effect of *peppermint* essential oil on burn wounds in mice by transdermal medication. After burning with a pre-heated 20-gram weight on depilated skin at 2 cm × 2 cm, mice were topically treated with PEO at dosages of 0.2 ml/cm^2 and 0.4 ml/cm^2, and commercially available MEBO (moisture burn ointment) at 0.5 mg/kg as a positive control, followed by assessment of the burn index and H&E staining of burned skin. (A) Topical PEO significantly decreased the burn index in mice with burn wounds. (B) H&E staining showed that PEO reduced the histopathological features of burn in mice. *P < 0.05 and * *P < 0.01 versus the Model group.

muscle of the gastrointestinal tract, which is helpful for the treatment of IBS. Menthol, one of the main constituents of PEO, has calcium antagonistic property comparable to potent calcium channel blocker, as menthol drastically lowers calcium influx [109,110].

PEO can relieve symptoms of indigestion, as PEO relaxes the lower esophageal sphincter and smooth muscle when applied to muscle strips in vitro. The T hysteresis was sharply reduced in healthy volunteers after ingestion of PEO, indicating that PEO can enhance gastric emptying, and the beta constant was also significantly decreased, suggesting that early gastric emptying was accelerated [111]. Also, PEO has antibacterial, anti-inflammatory, antioxidant, immunomodulatory, and anesthetic activities, all of which may potentiate the treatment of IBS [112].

In addition, PEO can relieve spasms in the intestinal wall, allowing for better visualization of the bowel with colonoscopy [113]. Finally, topical administration of PEO as aromatherapy can eliminate fever, relieve nausea and vomiting, and improve digestion [114].

5.1.2. *Application in dermatological disease*

One of intractable dermatoses is the pregnancy-specific skin condition, including PUPPP (pruritic urticarial papules and plaques of pregnancy), herpes gestationis and pruritic folliculitis of pregnancy, with itch as the most dominant symptom. It is well established that menthol in PEO can weaken itching caused by histamine through cooling the skin, by activation of A-delta fibers and κ-opioid receptors [84].

5.1.3. *Postoperative adjuvant therapy*

Inhaled essential oil through the nasal mucosa and lung may be absorbed systemically. After administration by inhalation, PEO is taken

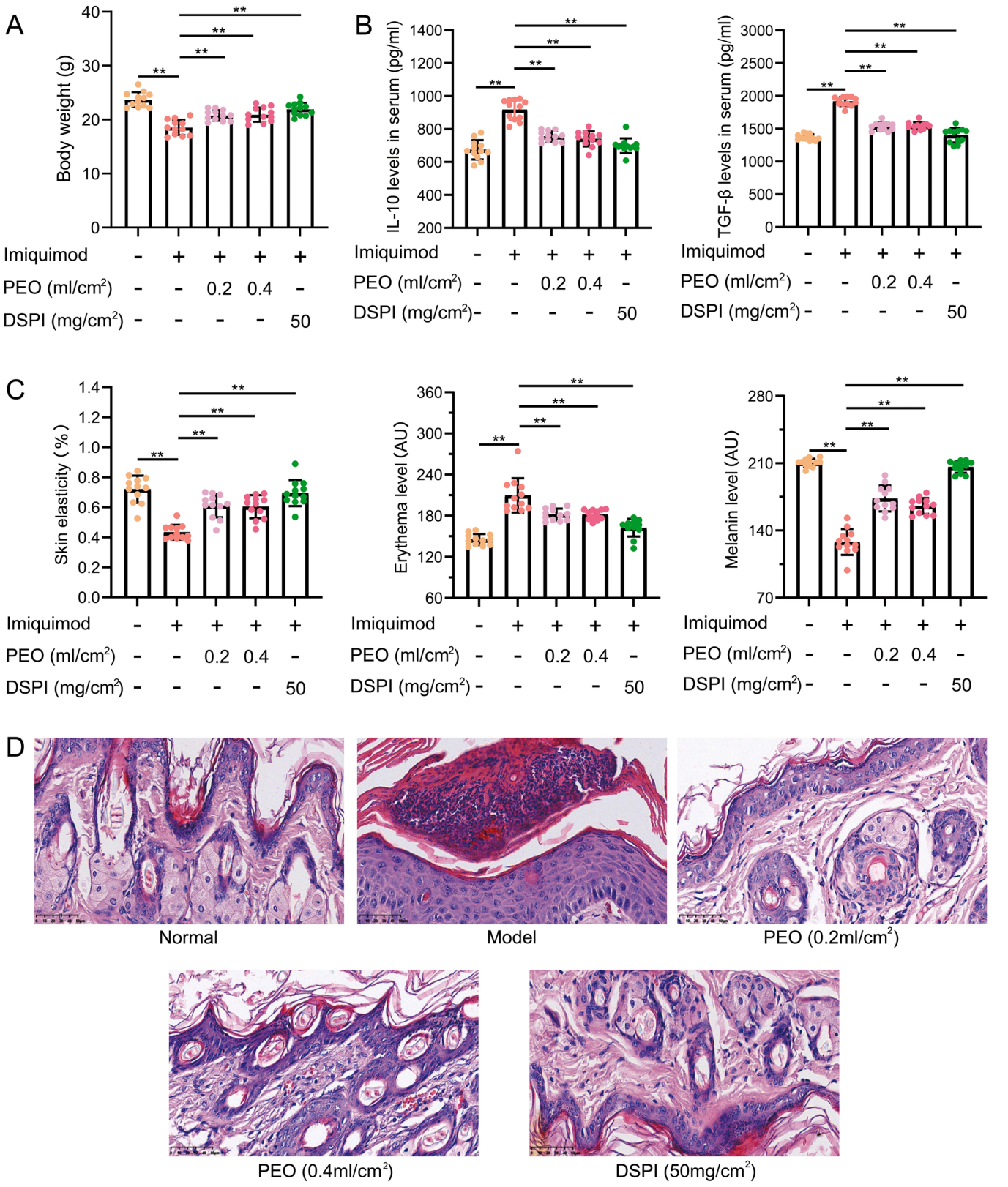

Fig. 4. Inhibitory effect of *peppermint* essential oil on psoriasis in mice by transdermal medication. After the establishment of psoriasis in mice by application of imiquimod cream on depilated skin at 4 cm × 6 cm, mice were topically administered PEO in doses of 0.2 ml/cm^2 and 0.4 ml/cm^2, with dexamethasone sodium phosphate injection (DSPI) at 50 mg/cm^2 as a positive control. (A) PEO increased the body weight of psoriatic mice. (B) ELISA analyses indicated that PEO reduced the serum levels of IL-10 and TGF-β in psoriatic mice. (C) Skin function analysis revealed that PEO prolonged skin elasticity, mitigated erythema level and heightened melanin level of psoriatic skin. (D) Histopathological analysis with H&E staining showed that PEO diminished histopathological features of psoriasis in mice. *P < 0.05 and * *P < 0.01 versus the Model group.

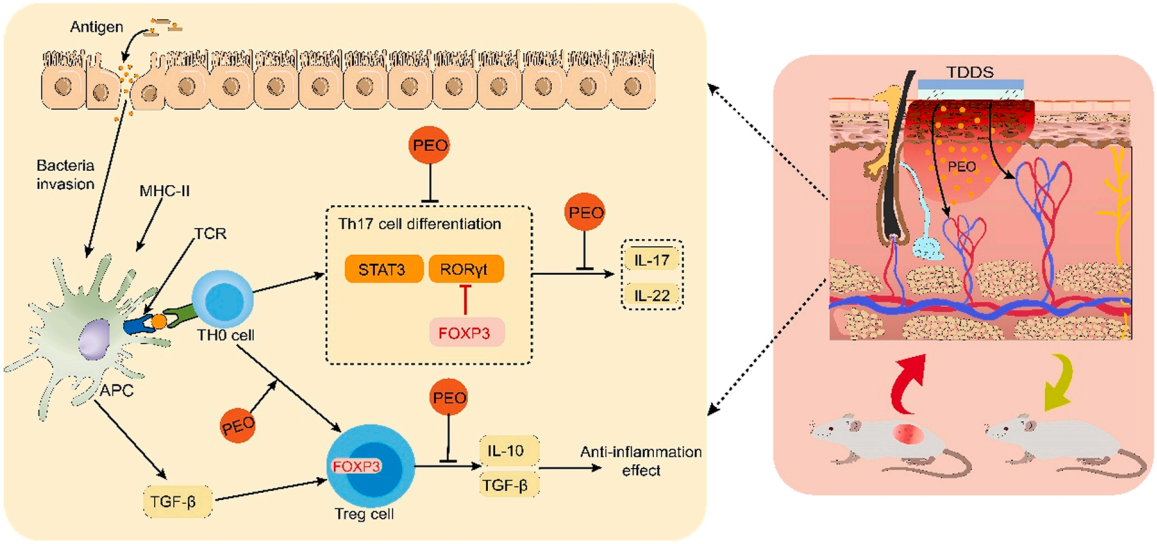

Fig. 5. Pharmacological effects of *peppermint* essential oil by transdermal absorption.

to blood circulation within several minutes [115], as lipophilic PEO is easily absorbed by the brain to exert efficacy quickly [116].

Postoperative patients are prone to nausea and vomiting, because vagus or sympathetic nerve stimulation, severe pain, visceral trauma, drugs and anesthesia can stimulate the receptors in medulla, leading to postoperative afflictions. For instance, after a cesarean section (C-section), women often experience nausea and discomfort. PEO aromatherapy may be an adjuvant treatment strategy for postoperative nausea and vomiting [117], as PEO can eliminate these C-section-caused disorders without causing sedation [118].

Postoperative urinary retention is a common complication of anesthesia, which elicits patients' agony and dissatisfaction due to intermittent catheterization, and leads to urethral trauma and potential infection. It is reported that ingested or topically inhaled PEO significantly reduces the need for intermittent catheterization, providing rapid intervention in urinary retention [119].

5.2. Other applications

Extensive data show that PEO as a dietary supplement or nutraceutical is beneficial for the management of functional gastrointestinal disorders [120], and IBS [121]. PEO-containing functional tea and topical liniment are also broadly employed for chronic functional digestive tract disorders [122], including IBS [123], by the inhibitory effect of PEO on visceral chemistry and mechanosensory [124].

In the food industry, PEO can be used as a condiment. Anti-oxidative PEO may be added to cheese to increase the content of TVFA and SN nitrogen, producing a more acceptable mint-flavored cheese with desirable multifunctional health effects [125]. The terpenes and their oxidized derivatives in PEO are inhibitory to the formation of mycotoxins. Therefore, PEO may serve as a seasoning material to restrain the growth of *Aspergillus flavus* and the production of *aflatoxin* in food [126].

In the cosmetic industry, PEO is widely utilized [127], owing to its strong antioxidant activity and tyrosinase inhibitory activity [128]. PEO can effectively improve the potency of sunscreen, attributed to the very high SPF (sun protection factor) value of PEO [82].

In the agricultural field, PEO is now added to the animal diet to exert anti-microbial and anti-inflammatory effects, so as to manage post-weaning diarrhea in livestock [129].

6. Conclusions and perspectives

PEO is a mixture of volatile secondary metabolites in *Mentha*, with a strong mint aroma and a cool, slightly bitter taste. It is well known that PEO is mainly composed of menthol, menthone, neomenthol, and iso-menthone, with strong anti-inflammatory, antibacterial, antiviral, scolicidal, immunomodulatory, antitumor, neuroprotective, antifatigue and antioxidant activities. Also, PEO shows pharmacological protection of gastrointestinal, liver, kidney, skin, respiratory, brain and nervous systems, and exerts hypoglycemic and hypolipidemic effects. Currently, PEO is broadly utilized for gastrointestinal and dermatological diseases, postoperative adjuvant therapy, nutraceuticals, the cosmetic industry, and other fields.

The extraction of *Mentha* and isolation of PEO are tricky but crucial for the downstream study of PEO, involving assessment of biological activities, determination of pharmacological effects and evaluation of applications. Nowadays, four types of constituents including menthol, menthone, neomenthol and iso-menthone have been isolated from PEO. However, to enforce the downstream study on PEO, some other novel bioactive ingredients in PEO should be identified with cutting-edge technologies, such as supercritical fluid extraction[130], membrane separation technology [131], and semi-bionic extraction technology [132].

To date, the biological activities and pharmacological effects of PEO have been extensively explored. However, the in-depth mechanisms underpinning these actions were intricate and still elusive. The underlying mechanisms are in great need of intense investigation with state-of-the-art techniques. In particular, the molecular targets and the involved signaling pathways on which PEO acts all merit clarification. In this case, some novel targets and their sequential cascades for PEO may be found out.

Transdermal absorption following topical administration of PEO is quite characteristic, which leads to convenient medication for both local disease and systemic disorders after absorption of PEO into blood circulation. To profile the onset and process of the action of PEO and probe the underlying mechanisms, PK-PD (pharmacokinetics and pharmacodynamics) study of PEO is urgently warranted [133].

Although *Mentha* and PEO have high therapeutic indices with few side effects, the toxicity of PEO exceeding a certain dose is still a matter of considerable public concern. Efforts should be made to clarify the toxic dosage under a particular circumstance and toxic mechanism, to ensure the safe application of PEO in a broad spectrum of fields.

In addition, most of the current research focus on the PEO mixture, with a few study on menthol. As PEO is a mixture of multiple constituents, attention should be drawn to other ingredients in the PEO, e.g. menthone, neomenthol and iso-menthone, which will facilitate

elucidation of the comprehensive action mechanisms of PEO in the foreseeable future.

Authors' contributions

HZ, SR and HBX conceived and designed the research project. HZ, SR, HY, ST ， CYG, MLL, QT and TQM carried out the study. HZ and SR drafted the manuscript. HBX revised the manuscript. All authors have read and approved the final manuscript for publication.

Declaration of Competing Interest

The authors report no declarations of interest.

Data availability

Data will be made available on request.

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