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Menthol – Pharmacology of an Important Naturally Medicinal "Cool"

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Abstract: Menthol, a natural product of the peppermint plant *Mentha x piperita* (Lamiaceae), is a monoterpene which is widely used as a natural product in cosmetics, a flavoring agent, and as an intermediate in the production of other compounds. Various extracts from peppermint contain menthol as a major active constituent and have been used for centuries as traditional medicines for a number of ailments including infections, insomnia, and irritable bowel syndrome as well as an insect repellent. Menthol's characteristic cooling sensation is due, in part, to the activation of sensory neurons generally termed transient receptor potential (TRP) channels, in particular transient receptor potential melastatin family member 8 (TRPM8) and transient receptor potential subfamily A, member 1 (TRPA1). Menthol acts upon TRPM8 receptors by rapidly increasing intracellular calcium and mobilizing calcium flux through the channels to induce cold response signals at the application site. Aside from its cold-inducing sensation capabilities, menthol exhibits cytotoxic effects in cancer cells, induces reduction in malignant cell growth, and engages in synergistic excitation of GABA receptors and sodium ion channels resulting in analgesia. Notwithstanding its plethora of benefits, menthol's cold-sensitivity response mechanism has been shown to inhibit mucosal recognition of nicotine and cigarette toxins common in mentholated cigarette brands thus potentially leading to toxic effects. Menthol may prove a valuable lead structure for the synthesis of drugs that target multiple receptors involved with a number of pharmacological effects.

Keywords: CMR1, Menthol, *Mentha x piperita*, nicotine, TRPA1, TRPM8.

INTRODUCTION

Menthol, a natural product of the peppermint plant *Mentha x piperita (Lamiaceae),* is a monoterpene which may be extracted from the plant or its constituent parts using specific extraction techniques for semi-volatile substances [1]. Among various natural medicinal products, the terpenes is a class of plant-derived compounds characterized by one or more isoprene units joined in regular patterns and with characteristic odors, flavors, and often high volatility which may provide them with both central and peripheral pharmacological effects [2]. Menthol is one of the most widely used natural products for use as a supplement, in cosmetics, as a flavor, and as an intermediate in the production of other compounds. Menthol's characteristic "cool" sensation provides for numerous beneficial as well as detrimental effects on the patients' body systems and has been utilized for centuries in traditional medicines [3].

This review aims to coalesce the various medicinal uses of menthol so that all of its various currently known attributes may be analyzed and its medicinal potential be considered with sufficient context. This review will first identify menthol's chemical constitution and biological occurrence. Next, the paper will set forth menthol's primary pharmacological actions as a cooling agent and analgesic as investigated by *in vitro*, pre-clinical, and clinical studies. Finally, the various medicinal uses of menthol which take advantage of its pharmacological action will be discussed.

CHEMISTRY AND BIOLOGICAL ORIGIN OF MENTHOL

Chemical Constitution of Menthol

According to the Organization for Economic Cooperation and Development (OECD), all isomers of menthol are similar in physico-chemical, toxicological, ecotoxicological, and environmental properties [4]. However, the focus of this review is on the naturally occurring l- or (–)-Menthol, which chemically can be described as cyclohexanol-5-methyl-2-(1-methylethyl) according to IUPAC nomenclature, and in other literature is referred to as (–)-Menthol (1R, 3R, 4S)-(–)) (Fig. **1**).

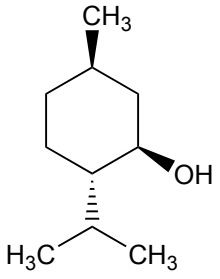

Fig. (1). Structure of (-)-Menthol.

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The natural menthol isomer, *l*-menthol, is formed via a reduction reaction within the epidermis of peppermint leaves whereby the natural precursor, *l*-menthone undergoes reduction at C-1 of the cyclic hydrocarbon (Fig. **2**) [5, 6].

The vast majority of natural menthol extracted from the peppermint plant is either the *l* or (–)-menthol stereoisomer and it is this isomer that contains many of the well-studied medicinal effects which have been reported in the literature [7, 8]. While menthol is acetylated in *Mentha* plants for storage purposes [5], menthol is metabolized in humans by the human liver cytochrome P450 enzyme system (CYP) and specifically in human liver microsomes by CYP 2A6 via alternating hydroxylation and oxidation reactions followed by glucuronidation to the water-soluble and inactive glucuronide [9].

While *d*- or (+)-menthol lacks the medicinal qualities and effects of *l*- or (–)-menthol [7], it bears mentioning that this stereoisomer of menthol has been extracted from Japanese fungi, *Phomopsis amygdali* F6a and Niigata 2 [10]. Unlike the biosynthesis of *Mentha l*-menthol, *Phomopsis* menthol originates from a geranyl pyrophosphate which undergoes cyclization and double-bond isomerization followed by a hydroxylation of terpenyl cations and a hydrogenation-oxidation reaction by fungal enzymes [10]. Contrary to the reduction which yields a majority of *l*-menthol from *l*-menthone in peppermint, fungal generation of *d*-menthol from geranyl pyrophosphate does not appear to take place in compartments in the fungus.

PHARMACOLOGICAL ACTIONS OF MENTHOL

Effects on Transient Receptor Potential Channels

TRPM8 and Membrane Calcium Flux

Menthol has well-known topical cooling, analgesic and antiseptic properties due in part to its mediated activation of a melastatin of the transient receptor potential superfamily of ion channels [11]. It is known that mammalian temperature sensitivity is governed by a family of sensory neurons generally termed transient receptor potential (TRP) channels, among which two provide neuronal responses to cold temperatures – transient receptor potential melastatin family member 8 (TRPM8) and transient receptor potential subfamily A, member 1 (TRPA1) [12]. Normally, TRPM8 permits the channeling of charged ions, usually calcium or potassium ions, to flow through cellular membranes to which it is attached when temperatures drop at or below 26 ± 2°C [13]. When temperatures fall below this region, the TRPM8 channel allows for membrane currents to increase at the peripheral nerve endings of cold-specific non-nociceptive afferents (A delta fibers) resulting in cold perception [13, 14]. At those same temperatures, an associated intracellular increase in calcium ions is observed across the calcium permeable TRPM8 channel [13]. The literature shows that menthol acts within the presynaptic regions where TRPM8 channels are prevalent and the somatic sensory synapses connect primary afferent fibers and dorsal horn neurons in the spinal cord to the central nervous system (CNS) [15]. Upon application of menthol to test cultures of dorsal root ganglia (DRGs) a rapid increase in intracellular calcium at the presynaptic terminals from intracellular calcium stores was observed [15]. The flux of calcium that is caused by menthol activity within the presynaptic sites acts as a mediator for release of glutamate within the somatosensory synapses [15]. Thus, the mobilization of intracellular calcium by menthol corresponds to the modulation of the synaptic transmissions from the local TRPM8 channels disposed on the membranes of DRG presynaptic terminals [15]. This relates to the signaling of a cold response when temperatures drop below 24 °C.

TRPA1 and Membrane Calcium Flux

Similar to the actions of menthol at the TRPM8 receptor, electrophysiological and binding experiments with human and mouse TRPA1 receptors also indicate that menthol modulates this receptor to increase intracellular calcium. In contrast, non-mammalian TRPA1 receptors are insensitive to menthol. However, the modulation appears to be bimodal with activation of the human TRPA1 receptor at low concentrations but inhibition of the receptor at high concentrations [16]. In addition, menthol interacts distinctly different with the TRPA1 receptor than the TRPM8 receptor

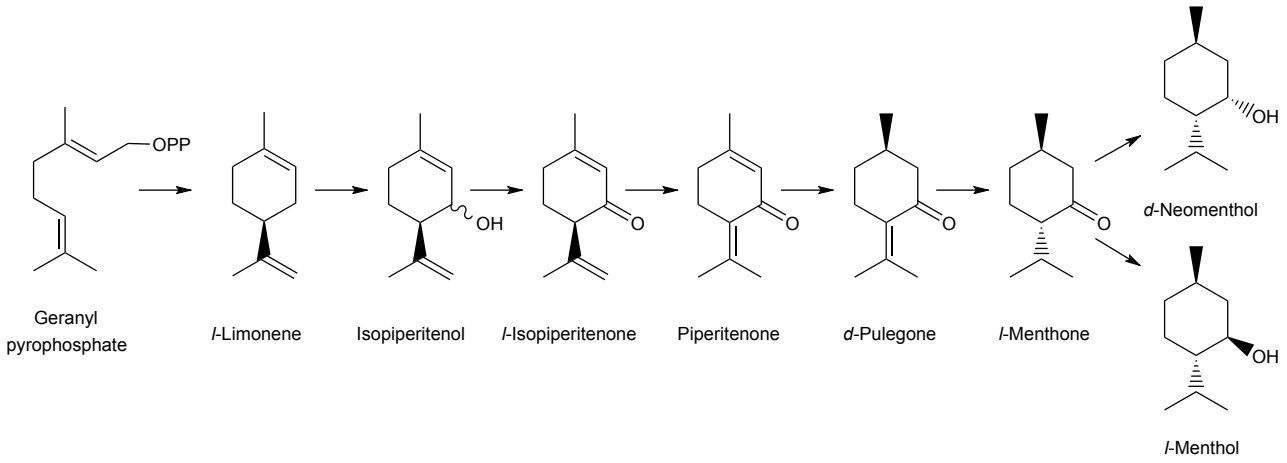

Fig. (2) Biosynthesis pathway for l-Menthone and l-Menthol.

through transmembrane region 5. While many reactive chemicals interact with TRPA1 through covalent but reversible binding to cysteine residues [17], menthol as a non-reactive chemical appears to act through hydrophobic interactions which may explain its bimodal effects on the receptor.

Pharmacological Action of Menthol as an Agonist within Somatosensory Channels

One article indicates that menthol acts on the TRPM8 channel through two cysteine residues comprising the channel's peptide structure, cysteine-929 (C929) and cysteine-940 (C940), both of which have been noted as essential docking points for menthol to take effect within the active site between transmembrane regions 5 and 6 of the TRPM8 channel [12]. However, more recent studies suggest that menthol's hydrogen bond with a tyrosine 745 (Y745) residue of TRPM8 is required for menthol's agonistic effects on the channel (Fig. **3**) [12, 18]. In docking studies, it has been elucidated that the interaction of the hydroxyl group of menthol with the tyrosine in position 745 as well as hydrophobic interactions of the methyl group with valine residues in positions 742 and 743 [19]. These interactions have been confirmed through docking station modeling which reported an EC_{50} of 4.1 μM, a Shapegauss score (indication of shape complementarity) of -113.15 kCal/mol, and a zapbind score (accounts for polar interaction through a combination of surface contact terms and Poisson-Boltzman energy approximations) of -10.87 kCal/mol [19]. These approximations match very well with observed potencies of menthol derivatives. It has been reported that the binding of menthol and other TRP channel ligands also takes place in the determinant in transmembrane region 2 and the carboxyl terminus of TRPM8 [12]. This constitutes two potential binding sites for menthol to TRPM8 receptors. It has been proposed that binding to the transmembrane region 2 and the carboxyl terminus refers to a preserved general binding site for modulators of the receptor while the specific interaction with cysteine residues between transmembrane regions 5 and 6 may point to a menthol-specific interaction with the receptor (Fig. **4**). This would explain the differences in responses to the cold-sensitizing agent ilicin and menthol [20].

A similar menthol "docking" takes place between menthol and mammalian TRPA1 in the transmembrane region 5 whereby menthol engages within the receptor potential channel at threonine-877 (T877) and serine-876 (S876) residues [16]. The concentration dependent response of menthol binding to TRPA1 was bell-shaped and did not interfere with the activation of the receptor by the known agonist mustard oil. Several other compounds were also evaluated for their activity on TRPA1 receptors and confirmed the essential binding domain to be preserved in transmembrane region 5 of the TRPA1 receptor. Antagonism of TRPA1 receptor agonists appears to occur in the form of covalent cysteine binding as observed for the electrophilic antagonist CMP1, direct competitive binding to the active residues T877 and S876 in the transmembrane region 5, or through a mechanism yet unknown.

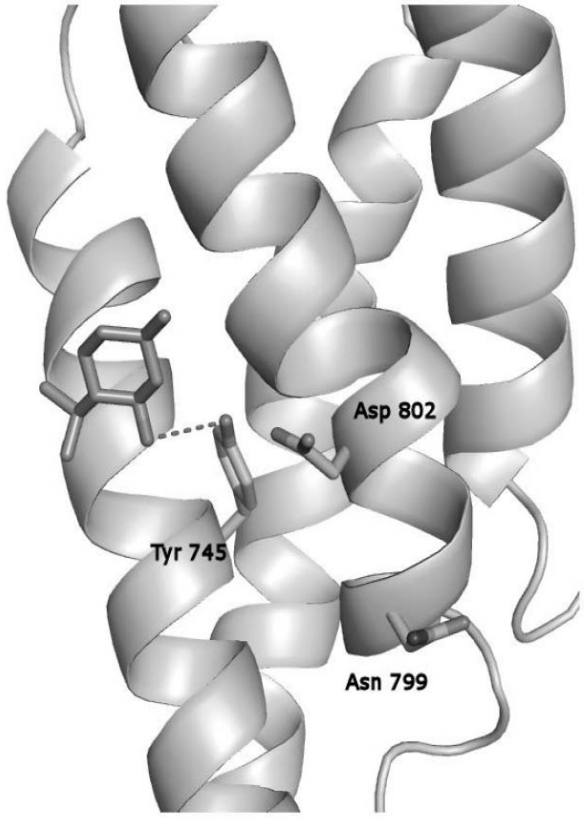

Fig. (3). Binding interaction of menthol with the loop between transmembrane regions 5 and 6 of the TRPM8 receptor. Specifically highlighted is the interaction with the tyrosine 745 residue in the loop. Modified from [18].

THERAPEUTIC EFFECTS OF MENTHOL

Menthol Effects via TRPM8 Channel

Through its binding in the TRPM8 membrane channel, menthol induces cooling sensations to inflamed areas of skin or muscle via its anti-nociceptive effects of capsaicin-sensitive fibers which desensitize peripheral neurons to signals from pain and agitation [11]. Topical applications of menthol to a pain site have been shown to produce similar sensational effects to application of half a kilogram of crushed ice, along with similar blood flow reductions and breathing [21]. The authors connected menthol's topical cooling capabilities and reduction in blood flow to the stimulation of TRPM8 thermosensitive neurons and the absorption rates of menthol through the epidermis layer. However, to explain cooling sensations felt outside the local application of menthol, the authors suggested further studies were required.

A clinical example of menthol's internal use of the TRPM8 channel may be further illustrated with menthol lozenges and their ability to cool sore throats and induce cooling sensations for elderly individuals to overcome swallowing reflexes due to dysphagia [22]. Menthol should not be given to children younger than 3 years of age because the triggering of cold sensation in toddlers can lead to

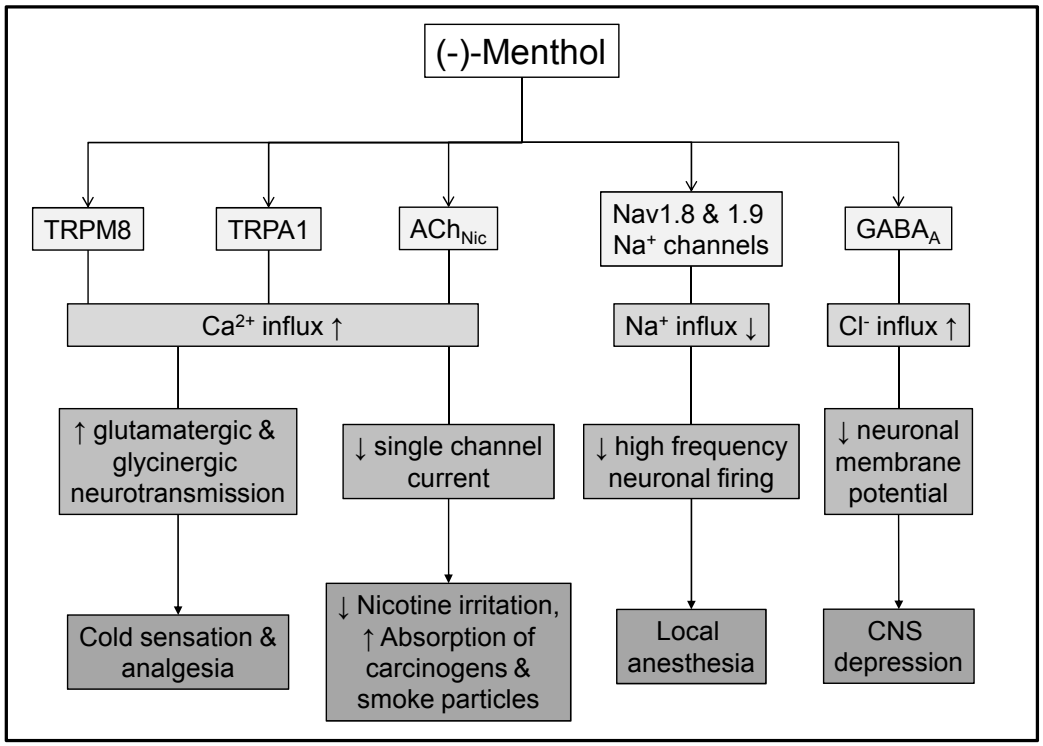

Fig. (4). Illustration of the TRPM8 ion channel pore with the 6 transmembrane regions. The highlighted amino acid residues indicate the potential binding sites for menthol at the TRP binding domain close to the carboxyl terminus, the transmembrane region 2, and the loop between transmembrane regions 5 and 6.

constriction of the airways (laryngospam) [23]. This menthol mediated activation of the TRPM8 channel also has been shown to exist in the trigeminal nerve in the olfactory epithelium and likewise causes cool sensations at the trigeminal interface that exists in the topical application to skin [3].

Another study notes that menthol can have "biphasic" effects on skin and mucosa because at low doses it provides a cooling sensation and at high doses irritation or burning [24]. The pathways involved in the experience of "cold pain" were researched by Wasner *et al.* [14], where menthol through action on the TRPM8 channel sensitized cold-specific A delta nerve fibers for prolonged periods of time, thereby leading to the cooling sensation on the skin. However, it was further observed that overexposure of menthol to a treatment site resulted in desensitization of the A delta fibers which are necessary to mediate vasoactive C nociceptors [14]. Thus, menthol's TRPM8 desensitization of A delta fibers diminishes the ability of the A delta fibers to counterbalance heat pain from C nociceptors, which results in the menthol induced cold hyperalgesia experienced with high doses [14]. Interestingly, the ability of menthol to activate TRPM8 was reported as being entirely resistant to pH effects whereas other noteworthy ligands and effectors of TRPM8, icilin and cold, respectively, were inhibited by pH due to the effects on Ca^{2+} activity on TRPM8 in more acidic solutions [25]. The pH may play an important role in cold sensation and hyperalgesia in inflammation, particularly in acidic pH environments due to histamine and inflammatory mediator release.

While at least one report indicates that menthol has the ability to increase Ca^{2+} concentrations independently of TRPM8 such as in the endoplasmic reticulum and Golgi bodies of HEK293 cells, cells which do not express detectable amounts of TRPM8 [24], other work has shown that TRPM8 was actually present in the endoplasmic reticulum and played a role in menthol-activated TRPM8-mediated Ca^{2+} release and storage depletion [26]. However, the literature seems to overwhelmingly suggest that the cooling effects of menthol are purely through TRPM8, which has been dubbed the Cold- and Menthol-sensitive Receptor 1 (CMR1) [15, 27, 28]. Menthol appears to have sensitivity-control capabilities due, in part, to its control of Na^+ and Ca^{2+} to and from neuroreceptors, e.g. C nociceptor fibers and TRPM8 [11].

While menthol's cooling effects may be primarily through TRPM8 binding, menthol's Ca^{2+} flux effects within cells may provide additional benefits as will be discussed later (Fig. **5**).

Inhibition and Regulation of Menthol-TRPM8 Activity

Menthol's effects on the TRPM8 channel are not without limitation. Thebault *et al.* observed that exogenous phosphatidylinositol (PIP_2) had cold stimulation regulatory capabilities in TRPM8, including the ability to modulate transmission of menthol-activated currents [26]. This was confirmed by Sarria and Gu when they observed PIP_2 hydrolysis as a regulation means of menthol-induced desensitization [13]. Additionally, Sarria and Gu concluded

that protein kinase C (PKC) and Ca^{2+}/calmodulin-dependent kinases II (CaMKII) inhibitors accelerated adaptation of menthol responses in MS/CI neurons (a population of neurons representing non-nociceptive cold-sensing neurons or neurons that register noxious cold), thereby attenuating menthol responses in these neurons faster than in MS/CS (nociceptive cold-sensing neurons). It was noted that phorbol-12,13-dibutyrate (PDBu), a PKC activator, reduced menthol responsiveness in DRG neurons, but such effect could be avoided with introduction of staurosporine or BIM [13]. Menthol-stimulated influx of Ca^{2+} into human glioblastoma cells and activation of big potassium (BK or slo1) ion channels, which has been shown to cause increased cellular motility and reduction in glioblastoma size, is also capable of regulation by paxilline or tetraethylammonium (TEA) ions [29]. Wondergem and Barley recognized that menthol-stimulated increases in Ca^{2+} concentrations in endoplasmic reticulum stores of TRPM8 membrane channels of DBTRG cells are most likely from paxilline and TEA's effects on the BK ion channels at which the menthol-response is targeted. Thus, paxilline and TEA may not be considered direct regulators of menthol *per se*, but can inhibit the results of its Ca^{2+} influx into the target cells.

Menthol Effects Exclusive of TRPM8

Menthol has been shown to inhibit DNA topoisomerase I, II-α and –β, and to promote NF-κB expressions in human gastric cancer SNU-5 cells [30]. There is also evidence to suggest that menthol was able to induce human promyelocytic leukemia HL-60 cell death through its ability to release Ca^{2+} ions from the endoplasmic reticulum [31]. Additional research showed that (–)-menthol exhibits cytotoxic effects on WEHI-3 tumor cells in BALB/c mice and demonstrated a decrease in such cells in a concentration-related manner [32]. In combination with 1α,25-dihydroxyvitamin D_3 ($1\alpha,25(OH)_2D_3$), menthol has also been used to reduce the proliferation of prostate cancer cells [33]. Park *et al.* identified menthol's intracellular Ca^{2+} flux capabilities through pancreatic LNCaP cancer cells as providing positive enhancement to $1\alpha,25(OH)_2D_3$ antiproliferation effects on the expression of the bcl-2 gene, and together enhanced the anti-cancer efficacy of the regimen.

Another action of menthol is through inhibition of cardiac L-type Ca^{2+} channels with an emphasis on late current inhibition thus prolonging depolarization of cells [34]. The other TRPM8 agonist with cooling properties, icillin, did not significantly affect cardiac calcium channels.

In contrast to its cooling sensation effects, menthol has been proposed to exert its well-known analgesic effects through blockade of voltage-gated sodium channels that are also located in the DRG in close proximity to the TRPM8 receptors [11]. Interestingly, patch clamping studies revealed that menthol selectively inhibited high frequency firing of tetrodotoxin-resistant Nav1.8 and Nav1.9 sodium channels while not interfering with normal neuronal activity. This has also been shown in animal experiments where application of menthol caused heat analgesia as well as displayed a biphasic cold analgesic effect in rats [35]. The biphasic effect displayed an enhanced cold sensitivity at very low menthol concentrations (0.01-1%) whereas at high concentrations (10-40%) menthol increased the threshold for cold sensitivity. This may point to cooling action via different receptors – such as the voltage-gated sodium channels and the TRPM8 ion channel (Fig. **5**). It appears though that most research groups agree that the cooling sensation of menthol is primarily mediated through the TRPM8 receptor whereas the action on sodium ion channels may be more relevant to local analgesic effects.

When sufficient concentrations of menthol were applied to hippocampal neurons, a stunning synergy was discovered between menthol- and $GABA_AR_S$-evoked currents through the hippocampal membrane [36]. It was proposed by Zhang *et al.* that the hydroxyl group of (–)-menthol enhanced the membrane current of GABA such that $I_{GABA+Menthol}$ was larger than the sum of I_{GABA} and $I_{Menthol}$ with the net result of inhibiting neuronal excitability *in vitro* and network hyper-excitability *in vivo* of hippocampal neurons which has the effect of inhibiting seizure activity. This points to a synergistic rather than additive effect of menthol to enhance binding of GABA to its receptor similar to the action of the general anesthetic propofol [37]. It has been shown that menthol is able to suppress seizure activity in pentylenetetrazole induced seizures in mice indicating a potential CNS activity for the terpenoid [36].

Toxic and Adverse Effects of Menthol

Among the numerous beneficial, medicinal purposes served by menthol, as discussed previously, menthol's analgesic and pain-inhibitory capabilities are put to use in ways that may exacerbate known harms to the organism. The exemplary case is the use of menthol in cigarettes and other tobacco products. The cooling effects of menthol are considered to be an enabling mechanism for continuous smoking, longer inhalation times of cigarette products, and increased exposure of endothelial cells and other tissues to carcinogenic by-products of cigarettes and nicotine [38].

Menthol has been found to reversibly inhibit nicotinic acetylcholine receptor proteins in the endothelium through allosteric modulation, which essentially reduces the regulatory function to signal nicotine irritation from cigarette smoke [39]. Hans *et al.* did find however, that menthol's allosteric modulation of acetylcholine receptor proteins only existed at the point where the receptor proteins were in a "closed" conformation and that upon opening, the menthol interaction site becomes obscured and its allosteric modification efficacy lowers (Fig. **5**). Consistent with its ability to increase Ca^{2+} influx through membranes (and therefore engage in heightened membrane permeability), the increased cell membrane permeability in the endothelium results in greater absorption of smoke toxins, including tar, carbon monoxide and free radicals [40].

The most obvious negative side effect of menthol usage in conjunction with tobacco products is the menthol inhibition of UGT2B10, the enzyme involved in the *N*-glucuronidation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), a nicotine breakdown product [41]. Menthol increases the *in vitro* absorption of the known tobacco carcinogen NNAL and inhibits the glucuronidation

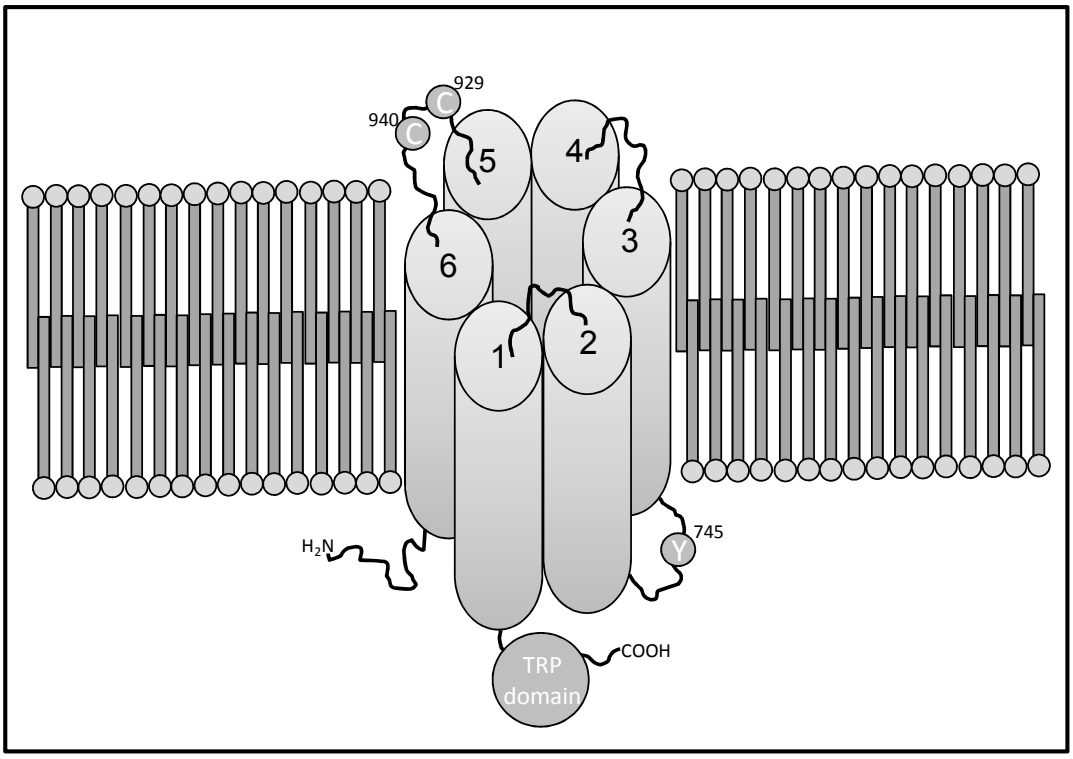

Fig. (5). Identified pharmacological effects of menthol on target receptors. TRPM8: transient receptor potential melastatin family member 8, TRPA1: transient receptor potential subfamily A, member 1, ACh_{Nic}: nicotinic acetylcholine receptor, $GABA_A$: γ-aminobutyric acid receptor A.

of nicotine by acting as an inhibitor substrate to UGT2B10 and other UDP-glucuronosyl transferases [41].

As a result of menthol's ability to "mask" the negative effects of nicotine intake, smokers of menthol cigarettes have been shown to decrease the amount of cigarette smoke inhaled, although the likely reason for such a finding is the increased permeability of lung endothelium resulting from menthol inhalation [42]. Further studies showed that in addition to the nicotine and smoke constituent interaction with the endothelium, menthol also increased electrophysiological brain activity, possibly leading to the positive association of menthol, which in the case of mentholated cigarettes, may induce use of menthol and nicotine combinations [43].

Clinical and Pharmaceutical Applications of Menthol

Menthol – either formulated as an isolated active ingredient or as part of a peppermint leaf extract – is being widely used in clinical and pharmaceutical practice both for ingestion and for topical application [3]. There are many topical formulations for menthol and peppermint extracts that are applied to the skin for treatment of local or joint pain [44, 45], for pruritus [46], and allergic dermatitis [47]. Internally, menthol has been used successfully as a treatment for digestive disorders, specifically irritable bowel syndrome [48], colonic spasms [49], and in the oral cavity as an antiseptic and for its local anesthetic properties [50, 51]. Menthol is sometimes added to topical and liquid formulations for its proposed antibacterial and antifungal properties in addition to its respective pharmacological actions such as the cooling effect or for gustatory and olfactory enhancements of the formulation [52]. One property of menthol that makes it a favorable formulation vehicle is the increase in dermal absorption for many hydrophilic compounds as a penetration enhancer [53, 54]. Menthol is extensively used as a topical agent for upper respiratory disorders by applying it to the chest and inhaling the vapors [55]. This application has been shown to shorten the duration of the common cold as well as to alleviate congestion and provide for improved sleep during the night. Although this use for menthol and peppermint preparations is very popular, it should be considered with caution in small children and patients with chronic respiratory disorders such as asthma and chronic obstructive lung disorder (COPD). In these populations, the inhalation of menthol may trigger an uncontrolled upper airway muscle reflex resulting in spasms and significant breathing difficulties [56]. Another important side effect of menthol and peppermint formulations is the potential for allergic reactions with the development of skin rashes [57] which can also cause worsening of asthma symptoms in vulnerable populations [58]. The reported data for these side effects, however, remains sparse and is currently limited to case reports.

CONCLUSION

Menthol-containing products have been used for many decades to provide topical analgesia and cooling sensations that alleviate localized pain. Many benefits may be attributed to the use of menthol as a topical pain inhibitor and cooling agent – possibly reducing the need for synthetic products (ibuprofen) and drugs that may be addictive and detrimental

to health (such as opioid analgetics). Though the primary application for menthol itself may be limited to its current use as a topical drug and as a penetration enhancer, the newly identified targets for menthol, especially the TRPM8, TRPA1, and voltage-gated sodium channels which are differentially modulated by the natural product, may be promising targets for new analgetic drugs (Fig. **5**).

COMPETING INTEREST STATEMENT

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

J.F. conceptualized the paper, J.F. and O.G. contributed equally to drafting and finalizing the manuscript.

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ABBREVIATIONS

OECD = Organization for Economic Cooperation and Development

TRPM8 = Transient receptor potential melastatin family member 8

TRPA1 = Transient receptor potential subfamily A, member 1

CMR1 = Cold- and Menthol-sensitive Receptor 1

PIP_2 = Phosphatidylinositol

PKC = Protein kinase C

CaMKII = Ca^{2+}/calmodulin-dependent kinases II

NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol

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