



# The Antidepressant-Like Effects of Menthol Via Dopaminergic System Using Forced Swimming Test in a Mice Model

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## Abstract

**Background and purpose.** Antioxidants are known to have protective and beneficial effects in the depression. Menthol is an antioxidant compounds in some plants. On the basis of our knowledge, no scientific study has investigated the antidepressant-like effects of menthol. Therefore, present study was thus conducted to evaluate the antidepressant-like effects of menthol by forced swimming test (FST) through dopaminergic system in a mice model.

**Materials and Methods.** The mice were intra-peritoneally (i.p.) administrated with menthol (10–30 mg/kg) 1 h before the FST. For evaluating the involvement of the dopaminergic system in the antidepressant activity of menthol, SCH 23390 (a dopamine D1 receptor antagonist), sulpiride (a dopamine D2 receptor antagonist) and haloperidol (non-selective dopamine receptor antagonist) were administered 15 min before menthol administration (10 mg/kg).

**Results.** Intra-peritoneally administration of menthol reduced immobility time and increased swimming and climbing times. It did not also cause significant changes in spontaneous locomotor function in the open-field test (OFT). In addition, pre-treatment of the mice with SCH23390, sulpiride, haloperidol blocked the anti-immobility effect of menthol in the FST.

**Conclusion.** Administration of menthol exerted antidepressant-like effects through dopaminergic (D1 and D2) system.

**Keywords.** Animal model, Depression, Dopaminergic system Forced swimming test



## Introduction

Major depression is a complex psychiatric disorder and almost 20% of the people are faced with it during their lifetime [1]. It is related with signs such as mood, interest, feeling, hope, appetite, sleep, performance and social relationships [1]. It was reported that typical antidepressants encourage synaptic action in the monoamines including dopamine (DA) through blocking the neuronal reuptake of these monoamines [2]. Serra et al. [3] suggested that the involvement of dopaminergic presynaptic receptors in the antidepressants activity a long back, it was not mentioned as one of the accepted hypothesis of mental depression. The impaired antioxidant system is associated with Reactive Oxygen Species (ROS) [4]. An increase in the production of ROS in depression is associated with increased levels of malondialdehyde and arachidonic acid [5]. It was reported an increase in oxidative stress in the animals submitted to stress [5]. It is accepted to use antioxidant agents for treatment of the depression [6]. Menthol is a strong antioxidant and improves the antioxidant and lipid profile in animals [7]. Abbasi-Maleki et al. [8] have reported that crude extract of *Mentha piperita* showed antidepressant-like effect through the monoaminergic system due to its compound such as menthol. As far as we know, no scientific study is conducted to evaluate the antidepressant-like effects of menthol by forced swimming test (FST) and through dopaminergic system using a mice model. The FST is broadly used in pharmacological models for evaluating the antidepressant effects [8]. The study was thus performed for evaluating the antidepressant-like effects of menthol via dopaminergic system in the FST model.

## Materials and methods

### Animals

Male Swiss albino mice with weight of  $22 \pm 2$  g and 6 weeks of age were used in this study. The animals

were maintained in cages ( $n=4$ ) with a lighting cycle 12-h light/12-h dark cycle, ambient temperature of  $23-25^\circ\text{C}$  and humidity of  $55 \pm 15\%$ . The feed and water were given ad libitum. The commercial pellet food was bought from Javaneh Khorasan, Iran.

### Materials

Menthol, SCH 23390 and sulpiride were purchased from Sigma-Aldrich, St. Louis, MO, USA. Only SCH 23390 was subcutaneously (s.c.) administered, but other agents and menthol were intra-peritoneally (i.p.) administered in a constant volume of 10 ml/kg. The FST was conducted 1 h after the single injection of the chemicals and menthol.

### Acute toxicity test

We used the Lorke's method for investigating toxicity test [9]. Briefly, after 24 h of fasting, a total number of twenty NMRI mice were grouped into 5 groups ( $n=4$ ) and intra-peritoneally treated with vehicle (normal saline with 10% Tween-80; 10 ml/kg) and menthol (10, 15, 20, and 30 mg/kg). The animals were monitored for behavioral and toxicity signs and also mortality for 24 h.

### The FST

The FST as a pharmacological test is used for evaluating the antidepressant activity of the agents. We conducted the FST as reported by Porsolt et al. [10]. 28 NMRI mice were grouped into seven groups as follows: Group I= vehicle (with 10% Tween 80; 10ml/kg, i.p.); Groups II-V= menthol (10, 15, 20, and 30 mg/kg; i.p., respectively); and Groups VI-VII= fluoxetine (20 mg/kg, i.p.) and imipramine (30 mg/kg, i.p.). The immobility, swimming and climbing were evaluated as stated by previous studies [8] for the rest four minutes during a six-minute period. We considered decreased immobility time and increased climbing or swimming times as behavioral profiles consistent with antidepressant-like activity [10].

## The investigation of the dopaminergic system of menthol antidepressant-like effects in the FST

To evaluate the potential involvement of the dopaminergic system in the antidepressant-like activity of menthol, the mice, were pre-treated with SCH 23390 (0.05 mg/kg, s.c., a dopamine D1 receptor antagonist), sulpiride (50 mg/kg, i.p., a dopamine D2 receptor antagonist) and haloperidol (0.2 mg/kg, i.p., *non-selective dopamine* receptor antagonist) 15 min before the i.p. administration of menthol (20 mg/kg, i.p.) or vehicle (10 ml/kg, i.p.). The animals were exposed to FST 1 h after the treatment [8].

### Statistical analysis

We reported data as mean  $\pm$  S.D. ( $n=4$ ) and the data were analyzed by the one-way ANOVA for dose-response curves and time-course curves and two-way for assessing of the mechanism of action followed by Tukey's post-hoc test when appropriate.

## Results

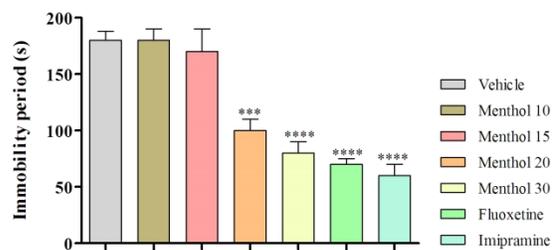
### Acute toxicity test

There were no any mortality and toxicity signs in the mice studied with doses used (10–30 mg/kg, i.p.).

### Immobility behavior

Our findings for one-way ANOVA in Figure 1 showed a significant reduction in immobility time ( $P<0.001$ ) and a significant increase in swimming time ( $P<0.001$ ) in the mice treated with menthol compared with the vehicle group. There were significant differences in the immobility time caused by menthol compared to that produced by treatment of imipramine and fluoxetine. The one-way ANOVA did not also show significant differences between the mice treated with menthol (20 and 30 mg/kg, i.p.) and fluoxetine in swimming time ( $P>0.05$ ) and also between the animals administrated with menthol (30 mg/kg, i.p.) and imipramine in climbing time

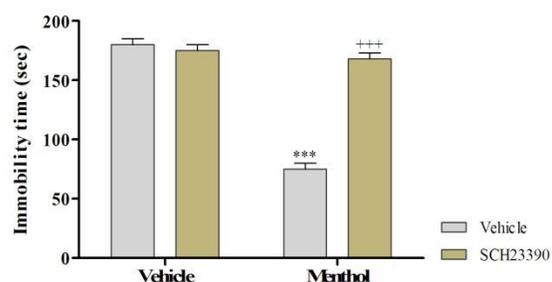
( $P>0.05$ ). The best response was observed in dose of the 20 and 30-mg/kg dose, this dose was thus used 20-mg/kg dose for the subsequent tests.

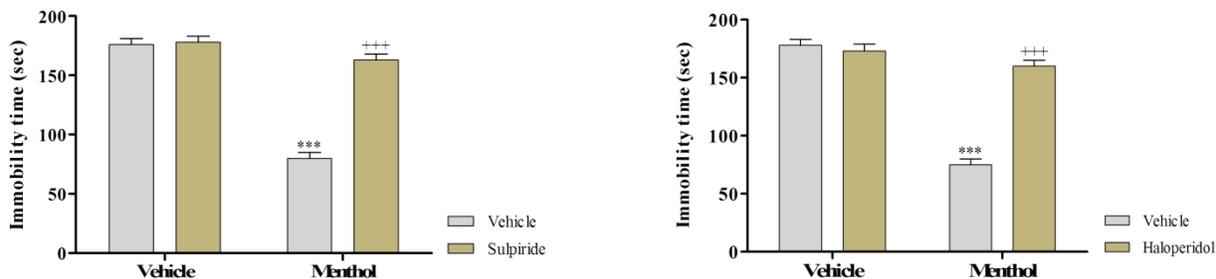


**Figure 1** The effect of the i.p. administration of the menthol in the FST. Values are presented as mean  $\pm$ SD. \*\*\*\* and \*\*\* superscripts show significant differences between the vehicle (control) and other groups at  $P<0.0001$  and  $P<0.001$ , respectively.

### Involvement of the dopaminergic system in the antidepressant-like effect of menthol in the FST

Pre-treatment of the animals with SCH 23390 (a dopamine D1 receptor antagonist), sulpiride (a dopamine D2 receptor antagonist) and haloperidol (*non-selective dopamine* receptor antagonist) significantly prevented the antidepressant-like effect of menthol (Figure 2). Our findings for dopamine receptor antagonist were as follows. For the SCH 23390 pre-treatment ( $P<0.001$ ), menthol treatment ( $P<0.001$ ) and SCH 23390 pre-treatment  $\times$  menthol interaction ( $P<0.001$ ), for sulpiride pre-treatment ( $P<0.001$ ), menthol treatment ( $P<0.001$ ) and sulpiride pre-treatment  $\times$  menthol interaction ( $P<0.001$ ) and for haloperidol pre-treatment ( $P<0.001$ ), menthol treatment ( $P<0.001$ ) and haloperidol pre-treatment  $\times$  menthol interaction ( $P<0.001$ ).

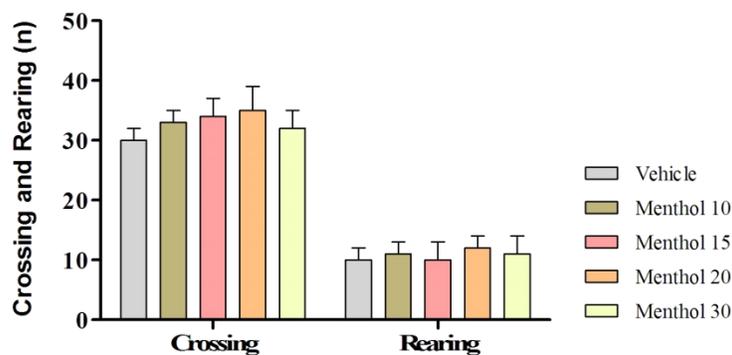




**Figure 2** The effect of the pretreatment of animals with SCH23390, sulpiride and haloperidol on the antidepressant-like effect created by the menthol in the FST. Values are presented as mean  $\pm$  S.D. \*\*\* shows significant differences among other groups and the (control) at  $P < 0.001$ . +++ shows differences among other groups and the extract group at  $P < 0.001$ .

### The OFT

It was not observed significant changes ( $P > 0.05$ ) in the crossing (Figure 3) and rearing (Figure 3) counts in the animals treated with menthol (10–30 mg/kg; i.p.) in comparison to the vehicle group.



**Figure 3** The effect of the menthol in OFT in the mice. Values are expressed as mean  $\pm$  S.D.

### Discussion

This study was conducted to evaluate the antidepressant-like effects of menthol by FST through dopaminergic system in mice model. In the current study, the results showed that menthol administered through i.p. route yields the antidepressant-like effect in the FST in mice. In addition, the locomotor activity was not altered. In addition, an involvement of dopaminergic (D1 and D2 receptors) systems in the antidepressant-like effect of menthol was observed. The FST is commonly used as a behavioral test and

also a model for evaluation of the depression in rodents [10]. The results showed that the effect of the menthol at dose of 30 mg/kg in the FST was similar to the response of fluoxetine and imipramine. It was reported that typical antidepressants encourage synaptic action in the monoamines including dopamine (DA) through blocking the neuronal reuptake of these monoamines [2]. It has been accepted the essential role of monoaminergic system in the pathophysiology and treatment of depression [2].

Traditional antidepressants decrease the depression signs by increasing the bioavailability of monoamines in the brain [11, 12]. The antidepressants have been used for the treatment of depression, because of increased synaptic levels of these monoamines [13]. It seems that menthol increases the levels of monoamines as is evidenced by increased dopaminergic levels. It has been reported that the antidepressant-like effects of active compounds such as menthone [14], cineole [15] and pulegone [16]. There is no any report available in the literature for antidepressant-like effects of menthol. Abbasi-Maleki *et al.* [8] attributed antidepressant-like effects of medicinal plants to their antioxidant properties. The results showed that menthol acts in the antidepressant-like effects through dopaminergic system. Antidepressant-like effects of the menthol were reversed through the pretreatment of animals with SCH23390, sulpiride, and haloperidol suggesting that menthol exerts antidepressant-like effects via the dopaminergic system. It has been accepted the role of the D1 and D2 receptors in the treatment of depression, because antagonists of these receptors reverse the antidepressant-like action of some antidepressants [17–19]. The dopamine directly promotes the factors diminished in people with depression such as motivation and attention [20]. It was reported that some dopamine receptor agonists have positive roles in the treatment of refractory and bipolar depression [21]. The results showed that dopamine D1 and D2 receptor antagonist reversed the antidepressant-like effect of the menthol showing that dopamine D1 and D2 receptors mediate the antidepressant-like effect of menthol. The results also showed that pretreatment of mice with haloperidol could reverse the antidepressant-like effect of the menthol, suggesting that dopamine receptors modulate in antidepressant-like response of the menthol.

## Conclusion

In summary, the administration of menthol exerted antidepressant-like effects through dopaminergic

(D1 and D2) system. We suggest using the menthol in commercial ointments for patients suffering the depression or depression like conditions.

## Ethical Considerations

### Compliance with ethical guidelines

Approval for this study was obtained from International Center for Intelligent Research.

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This study was supported by a grant from Georgian Center for Neuroscience Research (GCNR-2018-1841335).

### Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

### Conflict of interest

The authors declared no conflict of interest.

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