

Orginal Article

Effect of fruit essential oil on ephedrine induced manic like behavior: evidence from a new protocol

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Abstract

Introduction: Estimating ephedrine's effect on animal's locomotor activity and evaluation of cumin fruit essential oil (FEO) effect and mechanism of ephedrine-induced hyper-locomotion.

Methods: A new protocol developed for evaluation of manic behavior induced by ephedrine in a dose and time dependent manner. Following the suppressive effects of cumin FEO on acquisition and expression of ephedrine induced manic behavior was examined. Furthermore, the effect of L-NAME on expression of manic behavior and effect of bicuculline on suppressive effects of cumin FEO was evaluated.

Results: Ephedrine at the dose of 100 mg/kg (i.p.) in a five days protocol significantly increased the locomotor activity in mice. In addition, cumin FEO at the specific dose of 2% significantly suppressed the acquisition and expression of hyperactivity induced by ephedrine. L-NAME at doses of 30 ($p<0.01$) and 40 mg/kg ($p<0.05$) showed similar effects as cumin FEO at the dose of 2%. Bicuculline (as a GABAA antagonist) at the specific dose of 2 mg/kg ($p<0.01$) could significantly reverse the suppressive effects of cumin at the dose of 2%.

Conclusion: It could be concluded that cumin can suppress the manic behavior induced by ephedrine through the both previously suggested mechanisms of nitric oxide syntheses pathway and GABAergic system.

Declaration of Interest: None.

Keywords: Cuminum cyminum fruit, Ephedrine, Manic disorder.

Introduction

Bipolar disorder is a psychiatric disorder characterized by intermittent periods of mania and depression (1). As many other psychiatric disorders (e.g. PTSD, depression) there are well-established animal model protocols in order to evaluate the effect of a wide range of drugs on behavioral changes following this disorder. Amphetamine (AMPH) was used for induction of acute mania (2,3). AMPH-like psycho-stimulants promote acute and long-term activity in monoaminergic neurons (4). Long-term administration of AMPH leads to changes in dopaminergic and serotonergic systems activity in brain (2,3).

Ephedrine is a psycho-stimulant drug with wide range of effects on the nervous system. It has been suggested that ephedrine like amphetamine might act on adrenoceptors and dopamine receptors (5). Previously, considering stimulant properties of ephedrine it was used for treatment of narcolepsy. On the other hand, psychosis from therapeutic

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doses of ephedrine and amphetamine has been observed in narcoleptic patients (6). In a more recent study, Meng et al. (1999) showed IV injection of ephedrine could increase locomotor activity in mice. They proposed that this effect could be justified by similar structure of ephedrine and methamphetamine (7).

*Cuminum cyminum*L. (Apiaceae) is an annual herbaceous plant with 15-50 cm stalk growing in Iran and many other xerothermic regions of Middle East and South America. The plant has narrow feathery leaves, small white or pink flower and fruits, which each one contains a small rhombus green seeds. In Iranian folk medicine it is used for treatment of diarrhea, toothache and epilepsy (8,9). Previously Janahmadi et al. (2006) showed that cumin fruit essential oil (FEO) can inhibit the seizure induced by Pentylenetetrazol (PTZ) in F1 neurons of sub-esophageal ganglia in *Helix aspersa* (Iranian garden snail). In addition, before the above study Sayyah et al. (2002) studied the anticonvulsant effects of cumin FEO agonist seizure induced by PTZ and maximal electroshock (MES). They showed that cumin could suppress the seizure and reduce mortality induced by MES and PTZ. In a more recent study Haghparast et al. (2008) showed that *cumin* FEO significantly can attenuate the expression of morphine tolerance and dependence in dose-dependent manner while this effect was less on the development (acquisition) of morphine tolerance and dependence in morphine-dependent mice. In addition, in another set of experiments Khatibi et al. (2008) showed that the cumin FEO can significantly attenuate both acquisition and expression of morphine induced conditioned place preference in male albino mice. Two major mechanisms were proposed to be involved in cumin FEO effect on animals behavior: GABA mechanism and NOS pathway (8,10).

In the current study we investigated whether ephedrine could induce hyperlocomotor activity following long-term i.p. administration and the suppressive effects of *cumin* FEO on the hyperlocomotor activity induced by ephedrine was evaluated. In the follow up study two proposed mechanisms about the effect of cumin FEO, which are the GABA mechanism and NO mechanism, were tested.

Methods

Fruits of *cuminum cyminum* were obtained from a local market. M. Kamalinejad authenticated the plant at the department of pharmacognosy, faculty of pharmacy, Shahid Beheshti University, M.C., Tehran, Iran. A voucher specimen (no. C-1456) was deposited in the herbarium of this department. The fruits were subjected to hydrodistillation for 4 h by using a Clevenger apparatus and produced 3% (v/w) yield.

Animals Two hundred and sixteen adult male albino Wistar mice (Pasteur Institute, Tehran) weighing 18-30 g were used in these experiments. They were kept 8-10 per cage (45×30×15 cm) at a room controlled temperature (23 ±1ºC) and maintained on a 12-h light/dark cycle (light on 07:00 h) with free access to the standard rodent breeding diet and tap water. Each animal was used only once and killed immediately after the experiment. All experiments were executed in accordance with the guide for the care and use of laboratory animals (National Institutes of Health Publication No. 80-23, revised 1996) and were approved by the research and ethics committee of Shahid Beheshti University of Medical Sciences.

Locomotion testing apparatus A square (50*50×40) Plexiglas chamber was chosen

inorder to measure the of animal's locomotor activity. Animals were placed individually in the center of chamber and moved distance for each animal was recorded separately.

Locomotion tracking apparatus. Animal displacement, the total distance moved (distance traveled) during 5 min, was recorded on the test day by ethovision software (Version 3.1), a video tracking system for automation of behavioral experiments (Nodes Information Technology, the Netherlands), using a 3CCD camera (Panasonic, Japan) placed two meters above the square chamber.

In this experiment 40 mice displacement during 5 minute was recorded by ethovision software and after that they received either saline (10 ml/kg; i.p.) or ephedrine (30,50,100 mg/kg;10 ml/kg; i.p.). This protocol was repeated seven days and the animal's displacement was recorded every morning before injection. Finally, the best day and dose of ephedrine for induction of hyperactivity was selected for further experiments.

Dose-response effect of cumin FEO on expression of hyperactivity induced by ephedrine after 4 days of administration of ephedrine (100 mg/kg;10 ml/kg; i.p.; one injection each day) 48 mice received cumin FEO (0.1,0.5,1,2%;5 ml/kg, i.p.) 60 min before behavioral testing on fifth day.

In this experiment 48 mice received cumin FEO (0.1,0.5,1,2%; 5 ml/kg, i.p.) 60 min before each injection of ephedrine (100 mg/kg;10 ml/kg; i.p.) during four days of protocol.

In this phase 40 mice received either saline or bicuculline (0.5,1,2 mg/kg, i.p.) 30 min before injection of cumin FEO (2%,5 ml/kg, i.p.) 60 min before testing on 5th after 4 days of administration of ephedrine (100 mg/kg;10 ml/kg; i.p.), one injection in each day. Separate groups of animals received the above mentioned and 90 min later their locomotor activity was recorded during 5 minute and their moved distance were compared to intact mice. *Effect of L-NAME on expression of hyperactivity induced by ephedrine.*

In this phase 40 mice received either saline or *L-NAME* (30, 40, 50 mg/kg, ip) 60 min before testing on 5th after 4 days of administration of ephedrine (100 mg/kg; 10 ml/kg; i.p.), one injection in each day.

Results

Dose/time-response effect of ephedrine on locomotor activity

Our findings reveal that during 7 days of described protocol, animals, which received ephedrine 100 mg/kg showed higher scores of locomotor activity (Fig 1).

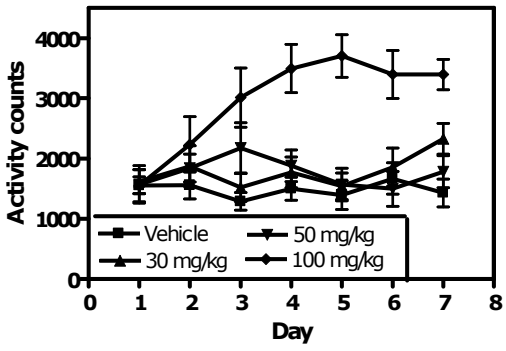

Fig 1. Locomotor activity after receiving different doses of ephedrine

One-way ANOVA showed there is a significant difference between day one measure of locomotor activity and day 2 in two different doses of 50 mg/kg ($P<0.01$) and 100 mg/kg ($P<0.01$). However, further analysis showed there is no significant difference between locomotor activity of day 2 and 3 in 50 mg/kg group ($P>0.05$). On the other hand, in 100 mg/kg group animals showed higher locomotor activity in days 3 ($P<0.01$), 4 ($P<0.05$), and 5 ($P<0.01$), but not in

days 6 (P>0.05), and 7 (P>0.05) in comparison with their previous days.

Considering observed data, it had been concluded that the dose of 100 mg/kg is the most appropriate dose for evaluating the effect of ephedrine on locomotor activity. While the 5th day of measurement showed significantly different data from all other days at this dose.

Acquired data in this phase revealed that injection of cumin FEO (0.1,0.5,1 and 2%), in comparison with vehicle group which received Tween-80 (0.5%), 60 min before testing on 5th day could significantly attenuate the hyperactivity induced by ephedrine (100 mg/kg) [f (5,47)=33.214, P<0.01]. Dunnett's multiple comparison tests showed that FEO only at the dose of 2% could significantly reverse the effect of ephedrine (Fig 2).

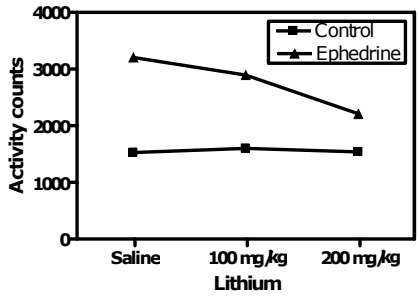

Fig 2. Effects of different doses of the fruit essential oil of *cuminum cyminum* on the expression of ephedrine-induced locomotor activity

Analysis data in this part revealed that bicuculline (0.5, 1, and 2 mg/kg), in comparison with saline, significantly reversed the suppressive effect of cumin FEO (2%) on hyperactivity induced by ephedrine (100 mg/kg) during the described protocol [P<0.01]. Dunnett's multiple comparison tests showed that bicuculline only at the dose of 2 mg/kg (p<0.01) could significantly reverse the suppressive effect of Cumin FEO (Fig 4).

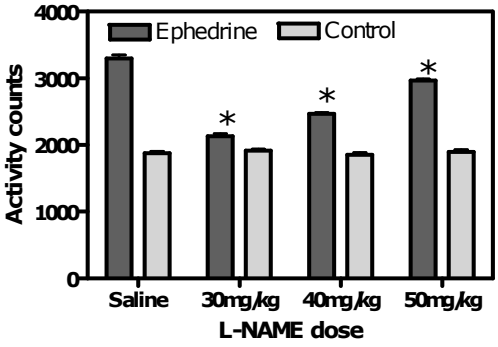

Fig 3. Effects of different doses of the fruit essential oil of *cuminum cyminum* on the acquisition of ephedrine-induced locomotor activity

Results revealed administration of cumin FEO (0.1, 0.5, 1 and 2%) 60 min before each injection of ephedrine (100 mg/kg) during the described protocol can significantly attenuate the hyperactivity induced by ephedrine [P<0.01] as compared to vehicle group that received Tween-80 (0.5%). Dunnett's multiple comparison tests showed that FEO only at the dose of 2% could significantly reverse the effect of ephedrine (Fig3).

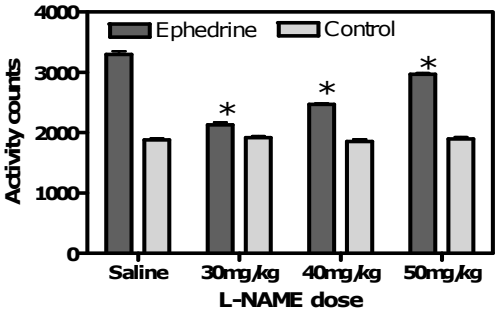

Fig 4. Effect of different doses of bicuculline on suppressive effect of cumin

Injection of above-mentioned doses of bicuculline to intact mice did not induce any changes into animal's locomotor activity (data are not presented).

Effect of L-NAME on expression of hyperactivity induced by ephedrine

Acquired data revealed that administration of L-NAME (30,40,50 mg/kg) on the test day instead of cumin FEO (2%) injection, in comparison with control group, which received, saline, significantly attenuates the hyperactivity induced by ephedrine (100 mg/kg) ($P<0.01$). Dunnett's multiple comparison tests showed that L-NAME at the doses of 30 ($p<0.01$) and 40 mg/kg ($p<0.05$) significantly suppressed the effects of ephedrine (Fig 5).

Sole administration of L-NAME (30,40,50 mg/kg) did not alter the animal locomotor activity.

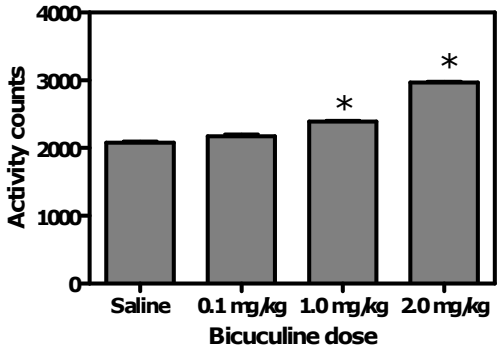

Fig 5. Effects of different doses of L-NAME on the expression of ephedrine-induced locomotor activity

Conclusion

In the current study we investigated whether ephedrine can be used for induction of hyperactivity as a criterion for bipolar disorder. Our results show that ephedrine at the dose of 100 mg/kg during a 5 day protocol can induce hyperactivity. In several previous researches, increase in locomotor activity during a specific period is considered as a criterion for manic behavior (Arban et al., 2005; Kita et al., 2000). In many of these researches amphetamine was used as a psycho-stimulant for induction of hyperactivity. Furthermore, Zarrindast (1981) showed that ephedrine affects adrenoceptors and dopamine receptors and this effect is similar to that of amphetamine. Considering similar effects of amphetamine and ephedrine on adrenoceptors and dopamine receptors and also hyperlocomotor activity induced by ephedrine and with due attention to the previous researches which hyperlocomotor activity induced by amphetamine was used as a criterion for induction of manic behavior, it could be concluded that the effect of ephedrine on induction of hyperlocomotor activity, during the described protocol could be considered as a criterion for manic behavior.

Furthermore, in another set of experiments we investigated whether and how cumin FEO can affect hyperactivity induced by ephedrine. Acquired data in this phase revealed that cumin FEO at the specific dose of 2% can significantly suppress the acquisition and expression of ephedrine induced hyperactivity. Previously it had been proposed that cumin FEO affects through two putative mechanisms: GABA transmission system and nitric oxide synthase pathway (8).

GABA is a neurotransmitter with inhibitory effects on central dopaminergic neurons (5). In addition, amphetamine and ephedrine affect central nervous system through dopamine receptors and this effect might lead to manic behavior in those animals, which are under the effects of these substances (5). On the other hand, previous researches revealed the anticonvulsant properties of cumin FEO (13,14). In a previous study Janahmadi et al. (2006) showed that administration of cumin FEO can inhibit the epileptic activity of F1 neurons of *Helix aspersa* (Iranian snail garden) induced by pentylentetrazol (PTZ). Also Sayyaah et al. (2002) previously indicated that the lethality and seizure induced by PTZ or maximal electroshock can be reduced by

i.p. injection of cumin FEO. They also showed that the seizure induced by PTZ could be prevented by administration of drugs enhancing the GABAreceptor mediated inhibitory transmission. In addition, previous studies showed that sodium valporate as an anticonvulsant mood stabilizer drug, which is useful in acute treatment of bipolar disorder (11,15). Since GABA is a neurotransmitter with inhibitory control over central dopaminergic neurons (5), and sodium valproate can enhance GABA transmission (16) in the central nervous system it could be concluded that this drug may attenuate locomotor activity through GABA related mechanisms (11). Overall, considering the antiepileptic properties of cumin FEO and also the anti-sensitization effects of anticonvulsant drugs that act through GABA transmission system (e.g. sodium valporate) on manic behavior, it could be concluded that cumin FEO inhibits the manic behavior induced by ephedrine through changes in GABAergic system and the results of 4th experiment provide enough evidence to support this hypothesis. In this experiment, bicuculline as a $GABA_A$ Antagonist reversed the suppressive effects of Cumin FEO on hyperactivity induced by ephedrine.

On the other hand, another explanation about justifying the mechanism for the Cumin FEO properties is the pathway of Nitric oxide (NO) production by the nitric oxide synthase (NOS) (8). In a previous study Eroglu and Caglayan (1997) showed that intravenous administration of Methylene blue (a direct inhibitor of nitric oxide synthase) results in antidepressant effects which suggest this hypothesis that NOS-NO pathway is involved in antidepressant action of Methylene blue. On the other hand, previous studies showed that there are major constituents in *Cuminum cyminum* green seeds, such as gamma-terpinene, thujadien, *para*cymene, d-glucopyrano sides and linolool (17,18), which have inhibitory effects on NOS in different cells and tissues. For example, in various studies β-dglucopyr-anoside, which are found in cumin seeds (18) demonstrated an inhibitory action on NOS (19, 20&21). Considering inhibitory effects of cumin FEO constituents on NOS and also previous investigation on effect of NOS inhibition using methylene blue on bipolar disorder (22) it could be concluded that cumin FEO takes action on locomotor activity following long-term administration of ephedrine through NOS inhibition. Results from the 5th experiment showed that L-NAME as a NOS inhibitor can significantly reduce the locomotor activity following long-term administration of ephedrine. Along with these findings it had been shown that solely administration of LNAME does not affect animals' locomotor activity. It emphasis that observed results cannot be linked to the effect of L-NAME on animals' locomotor activity. These findings provide supports for hypothesis that cumin takes action through NO-NOS pathway.

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