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Original Article

Nigella Sativa Hydro-Alcoholic Extract Attenuates Postpartum Depression Through Increase of Gamma Amino Butyric Acid-A Levels

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ABSTRACT

Background: Postpartum depression (PPD) is one type of major depression that has harmful effects on mother, infant and family relationships. Therefore, this study investigated the protective effects of hydro-alcoholic extract of *Nigella sativa* on PPD in mice.

Methods: In this experimental study, adult female mice were randomly divided into 6 groups (n=10): control, PPD, *Nigella sativa* 200, bicuculline, muscimol and fluoxetine. In all animals except for the control group, PPD was induced by progesterone withdrawal. In groups of *Nigella sativa* 200, bicuculline, muscimol and fluoxetine, mice received 200 mg/kg *Nigella sativa*, 1 mg/kg bicuculline + 200 mg/kg *Nigella sativa*, 0.5 mg/kg muscimol and 15 mg/kg fluoxetine, respectively. Then, after 1 hr, the forced swimming test and open field test were examined.

Results: PPD caused significant increases in the immobility times in the forced swimming test ($P < 0.05$). Administration of *Nigella sativa*, muscimol and fluoxetine attenuated depression-related behaviors compared with the PPD group (all $P < 0.05$). However, combined administration bicuculline with *Nigella sativa* prevented antidepressant effects of this extract. Moreover, there were no significant differences in the crossing number in the open field test of all groups.

Conclusions: Administration of *Nigella sativa* hydro-alcoholic extracts can be beneficial to the improvement in PPD and exerts possibly these protective effects partially through increase of gamma amino butyric acid (GABA)-A levels.

Key words: Bicuculline, Fluoxetine, Forced swimming test, muscimol, Open field test, Progesterone withdrawal

1. Introduction

Postpartum depression (PPD), one type of major depression, is the most common complication of childbirth that negatively affects the mother (*e.g.*, sadness, loss of appetite, anorexia, alcohol and illicit drug use, irregular sleep, disturbance in concentration, loss of interest or pleasure in activities and thoughts of suicide) [1]. Moreover, maternal depression has adverse consequences on the whole family and results in long-term negative outcomes on children such as poor cognitive functioning, behavioral inhibition, emotional maladjustment and violent behavior [2]. PPD usually starts between the fourth until eighth week after delivery and its prevalence is thought to be approximately 10-20% [3].

The exact molecular mechanism underlying PPD is not fully understood. However, various studies have shown that progesterone levels after delivery decrease leading to disturbing function of some neurotransmitters that has beneficial effects on mood [4]. One of these neurotransmitters is gamma amino butyric acid (GABA). It has been reported that GABA levels are inversely correlated with depression scores in women at risk for developing PPD [5].

Anti-depressant drugs have been indicated to be clinically effective. Nevertheless, these drugs also contribute to a variety of adverse events. On the other hand, women with PPD may choose psychotherapy as their first treatment, but high cost and side effects prevent its widespread use [6]. Thus, it seems essential to explore new and novel therapeutic strategies for PPD.

Many years ago, plants were used as a basis of remedies in the history of humankind. Recently, their application has risen dramatically for several ailments due to their easy accessibility and low cost. It is also believed that natural remedies have less side effects in comparison with synthetic medicines. Among various medicinal plants, *Nigella sativa* is considered as one of the most treasured nutrient-rich plants that is mostly distributed over Southern Europe, North Africa and Asia Minor [7]. This plant has been shown to have various therapeutic activities including anticancer, antihypertension, and antidiabetic [8]. Moreover, it is suggested that *Nigella sativa* also protects nervous system and reduces depression [9].

Considering the antidepressant effects of *Nigella sativa*, the present study examined this property of the plant in PPD induced by progesterone withdrawal in mice and evaluated whether *Nigella sativa* acts as antidepressant agent through modulation of GABA-A levels.

2. Material and Methods

2.1. Animals

Adult female mice (NMRI) (6-8 weeks) were prepared from Department of Physiology, Tehran University of Medical Sciences. Before using, mice were allowed to adapt to the laboratory environment for one week. Animals were maintained under controlled environmental conditions (20 ± 2 °C and 12 hr light-12 hr dark cycle) with free access to food and water. All procedures were carried out in accordance with the Guidelines on Animal Care and Use at Tehran University of Medical Sciences.

2.2. Dose response

At first, we aimed to select the antidepressant dose of *Nigella sativa*. Twenty-five animals were randomly assigned to two groups, *i.e.* control (n = 5) and PPD (n = 20). Following induction of PPD (progesterone injection for 5 days and progesterone withdrawal for 3 days), mice were further divided into four groups: PPD (n = 5), in which animals intraperitoneally received 2 ml normal saline once on the eighth day; *Nigella sativa* 100 (n = 5), in which mice were intraperitoneally given 100 mg/kg

Nigella sativa once on the eighth day; *Nigella sativa* 200 (n = 5), in which animals intraperitoneally received 200 mg/kg *Nigella sativa* once on the eighth day; and *Nigella sativa* 400 (n = 5), in which mice were intraperitoneally given 400 mg/kg *Nigella sativa* once on the eighth day. The three doses of *Nigella sativa* and the duration of treatment were selected from the previously published literature [10, 11]. One hr after treatment regimen, the animals were subjected to the forced swimming test.

2.3. Establishment of a murine model of PPD and treatment regimen

Mice were randomly divided into six groups (n=10): control, PPD, *Nigella sativa* 200, bicuculline (GABA-A antagonist), muscimol (GABA-A agonist) and fluoxetine (as a positive control). Except for animals in the control group, others were induced PPD according to the protocol by Hosseini [11]. Briefly, mice intraperitoneally received progesterone (Iran Hormone Pharmaceutical Company) (5 mg/kg in sesame oil) for 5 days. Then, progesterone injection was withdrawn for 3 days. In the *Nigella sativa* 200 group, animals were orally given hydro-alcoholic extract of *Nigella sativa* (200 mg/kg in normal saline) once on the eighth day. In the bicuculline group, mice intraperitoneally received bicuculline (Sigma-Aldrich, USA) (1 mg/kg in normal saline) [12] and hydro-alcoholic extract of *Nigella sativa* (200 mg/kg in normal saline) once on the eighth day. In the muscimol group, animals were intraperitoneally given muscimol (Sigma-Aldrich, USA) (0.5 mg/kg in normal saline) once on the eighth day [13]. In the fluoxetine group, mice orally received fluoxetine (Abourayhan Pharmaceutical Company) (15 mg/kg in normal saline) once on the eighth day [14](Figure 1].

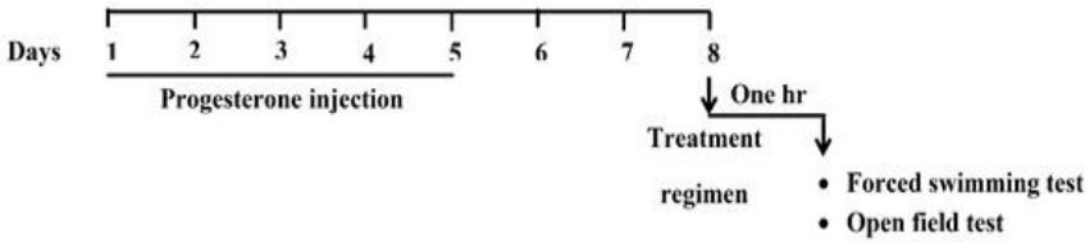

Figure 1. The experimental protocol

2.4. Extract preparation

Black seeds of *Nigella sativa* (400 g) were obtained from the Pharmacognosy Laboratory of Tehran University of Medical Sciences. The Herbarium School of Pharmacy identified the seeds with PMP-671 code in Herbarium. The seeds were crushed by milling to powder. Then, with 80% ethanol, it was poured into a sterile jar and placed on a shaker for 24 hr. After this step, the mixture was passed through a filter paper and concentrated by rotary evaporation. Finally, 105 ml *Nigella sativa* hydro-alcoholic extract (dark-colored oily liquid) was obtained.

2.5. Forced swimming test

One hr after treatment with regimens, the forced swimming test was used to evaluate depression-related behaviors [15]. An investigator blinded to the groups placed individually animals in an open cylindrical container (diameter 10 cm, height 25 cm) which was filled with water (23 ± 1°C) to 19 cm height. The

total time of the test were 6 min. In the first 2 min, mice were adapted to swim in water. The immobility times were recorded during the last 4 min. Each mouse was considered as immobile when it stopped struggling and only kept its head above water.

2.6. Open field test

In order to examine ambulatory behaviors, animals in all groups were subjected to the open field test [16]. The apparatus consisted of a wooden box (40 × 60 × 50 cm). The floor of the field was divided into 12 equal squares. Mice were placed in the center of the field and the crossing number was counted in a 5 min session by a blind investigator.

2.7. Statistical analysis

All results are expressed as the mean ± standard error of the mean (SEM). Differences between groups were calculated by one-way analysis of variance (ANOVA) followed by Tukey's post hoc. Statistical significance was considered as $P < 0.05$.

3. Results

3.1. Dose response

There were significant increases in the immobility times in the PPD group compared to the control group ($P < 0.05$) (Figure 2). There were no significant differences in the immobility times with administration of 100 mg/kg *Nigella sativa* hydro-alcoholic extract in comparison with the PPD group (Figure 2). However, administration of *Nigella sativa* hydro-alcoholic extracts from doses of 200 and 400 mg/kg significantly decreased the immobility times compared to the PPD group ($P < 0.05$) (Figure 2).

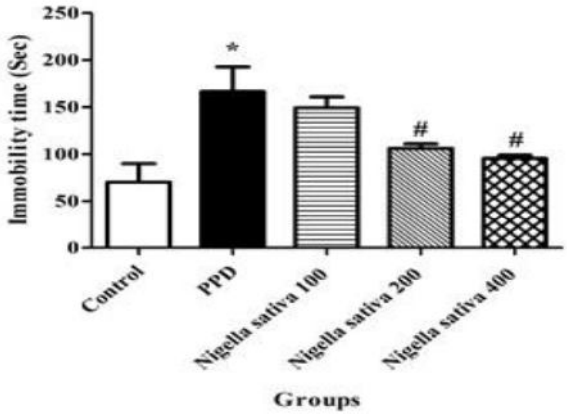

Figure 2. Changes in the immobility times in different groups. The data are expressed as mean ± SEM. * $P < 0.05$ versus the control group. # $P < 0.05$ versus the PPD group. PPD: postpartum depression

3.2. Effect of 200 mg/kg *Nigella sativa* hydro-alcoholic extract on the immobility times in the forced swimming test

There were significant increases in the immobility times in the PPD group compared to the control group ($P < 0.05$) (Figure 3). Administration of *Nigella sativa* hydro-alcoholic extract, muscimol and fluoxetine significantly decreased the immobility times in comparison with the PPD group ($P < 0.05$) (Figure 3). There were no significant differences in the immobility times between animals in the groups of PPD and bicuculline (Figure 3).

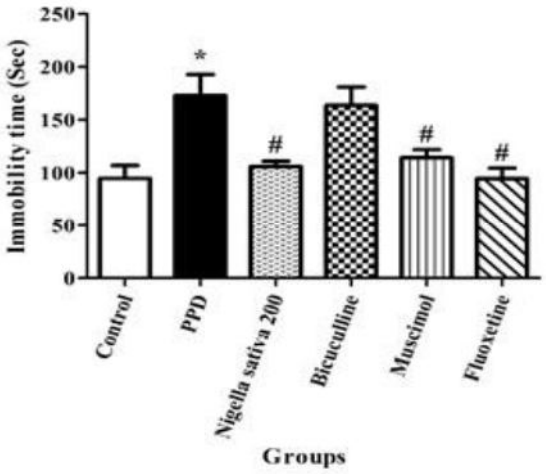

Figure 3. Changes in the immobility times in different groups. The data are expressed as mean ± SEM. * P < 0.05 versus the control group. # P < 0.05 versus the PPD group. PPD: postpartum depression

3.3. Effect of 200 mg/kg *Nigella sativa* hydro-alcoholic extract on the crossing number in the open field test

There were no significant differences in the crossing number between animals in the groups of control, PPD, *Nigella sativa* 200, bicuculline, muscimol and fluoxetine (Figure 4).

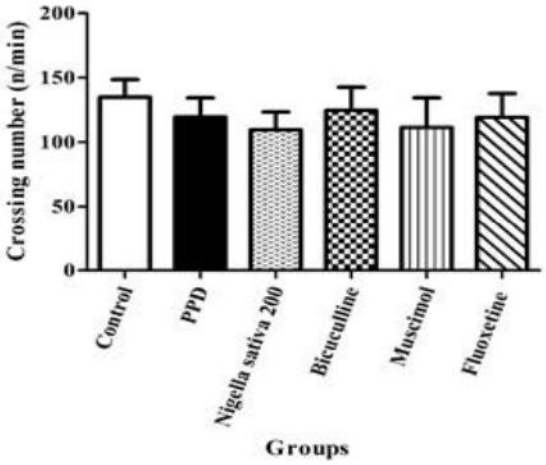

Figure 4. Changes in the crossing number in different groups. The data are expressed as mean ± SEM. PPD: postpartum depression

4. Discussion

This study demonstrated that mice with PPD showed considerable increases in depression-related behaviors. Similar to this result, another study found that in the PPD group, depression-related behaviors increased in comparison with the control group [17]. The present study used the forced swimming test as it is a valuable model for evaluating depression-related behaviors [15].

The underlying molecular mechanism of PPD is not fully elucidated. However, after delivery, the levels of some hormones, especially progesterone decreased [4]. This reduction of hormones levels may lead to changes in the functions of several neurotransmitters such as GABA-A [5]. Since GABA-A is considered as a

modulating agent of mood, it is reasonable to suppose that decreased GABA-A levels may cause this disorder.

In this study, the protective effects of *Nigella sativa* were examined on depression-related behaviors. The results demonstrated that administration of 200 mg/kg *Nigella sativa* hydro-alcoholic extract caused a significant reduction of depression-related behaviors which was similar to fluoxetine, a well-known drug with antidepressant effects. Our finding is in good agreement with another research that reported administration of 200 mg/kg *Nigella sativa* extract reduced depression-related behaviors [11].

Considering the important role of GABA-A in moods, we supposed that the antidepressant effects of *Nigella sativa* would be possibly partial through increase of GABA-A levels. Therefore, we used bicuculline, a GABA-A antagonist, in combination with 200 mg/kg *Nigella sativa* hydro-alcoholic extract. Interestingly, our study found that administration of bicuculline prevented these protective effects of *Nigella sativa*. Therefore, the result indicated just as muscimol, *Nigella sativa* has GABA-A agonistic property as both of them showed antidepressant effects. This observation is in accordance with study of El-Naggar *et al.*[18] that reported *Nigella sativa* significantly increased release of GABA in cultured neurons [18].

In the current study, we applied the open field test since it is a valuable method to assess animal's locomotor activity [16]. The finding of the present study showed that there were not any significant differences in the crossing number in the open field test between animals in all groups present. Thus, our results revealed that increases in the immobility times in the forced swimming test in the PPD group and decreases in these times in the *Nigella sativa* 200 group were due to changes in mood of animals not their locomotor system. Similarly, another study reported that induction of PPD in mice left no significant differences in the crossing number in the open field test between untreated and treated animals [17].

5. Conclusions

This study suggested that administration of 200 mg/kg *Nigella sativa* hydro-alcoholic extract can be effective in the improvement of PPD and exerts possibly these protective effects partially through increase of GABA-A levels.

Implications for policy makers

- Postpartum depression (PPD) is one type of major depression that negatively affects not only the mother, but also children and whole family.
- PPD should be considered as an important disorder because of relatively high prevalence (10-20%) among the mothers and its adverse effects on whole family.
- Similar to fluoxetine (a well-known antidepressant drug), *Nigella sativa* hydro-alcoholic extracts can be effective in PPD treatment as they have ability to mitigate depression-related behaviors.
- The application of *Nigella sativa* may be preferred because it does not have side effects similar to chemical drugs (*e.g.*, fluoxetine).

Implications for public

Postpartum depression (PPD) is one type of major depression that has relatively high prevalence (10-20%) among the mothers. PPD should be considered as an important disorder since it has harmful consequences on mother, infant and family relationships. In the clinics, anti-depressant drugs have been widely used for PPD treatment. However, these drugs are associated with a group of adverse effects. Thus, it seems

necessary to find new and novel therapeutic strategies for PPD. Using medical plants has been considered since many years ago and their application is recently increasing dramatically due to their easy accessibility and low cost. One of these medical plants is *Nigella sativa* that has various therapeutic properties. The findings of the present study revealed that *Nigella sativa* hydro-alcoholic extracts are able to attenuate depression-related behaviors suggesting that this plant can be effective in PPD treatment similar to fluoxetine, a well-known antidepressant drug.

Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contributions

Farzaneh Kianian and Tannaz Salehi equally contributed in the manuscript.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All authors declare that they embedded all data in the manuscript.

Ethics approval and consent to participate

The authors declared that they do not use human or animals in their research.

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