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Original article

The effect of *Elettaria cardamomum* extract on anxiety-like behavior in a rat model of post-traumatic stress disorder

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ABSTRACT

Post-traumatic stress disorder (PTSD) is a debilitating psychiatric condition which develops in 6–8% of the general population. Current standard pharmacological treatments for PTSD cannot be widely used due to having various side effects. Nowadays, various pharmacological properties have been related to *Elettaria cardamomum* L. (family of Zingiberaceae). The present study aims to evaluate the efficacy of *E. cardamomum* methanolic extract on anxiety-like behavior in a rat model of PTSD. Adult male Wistar rats (200–250 gr) were used in this study. The rats underwent single prolonged stress (SPS) or control and intraperitoneally received either saline or different dosages (200, 400, and 800 mg/kg) of *E. cardamomum* methanolic extract before and after stress sessions. Moreover, open field, elevated plus-maze, and rotarod tests were used to evaluate locomotion and anxiety-like behavior in the rats. Findings demonstrated that *E. Cardamomum* methanolic extract, particularly at the dose of 400 mg/kg, significantly ($P < 0.05$) improved anxiety-like behavior in a rat model of PTSD, as examined by the open field, elevated plus-maze, and rotarod tests. Administration of *E. cardamomum* methanolic extract after stress might help to prevent the formation of anxiety-like behavior in the animals. However, further studies are requiredto clarify the exact mechanisms involved.

1. Introduction

Post-traumatic stress disorder (PTSD) is a psychiatric illness with a life-time prevalence of 5–8% in the general population [1]. It is one of the most debilitating conditions which significantly reduces the quality of life in the patients [2]. The disease is characterized by three main symptoms, including recurring flashbacks and nightmares, hyperarousal, and numbing [3]. PTSD is an anxiety-disorder which could be classified as acute (acute stress disorder) or chronic (PTSD) [3]. Several treatment modalities have been proposed to prevent and treat anxiety in PTSD patients. Selective serotonin reuptake inhibitor (SSRI) and serotonin-norepinephrine reuptake inhibitor (SNRI) agents such as fluoxetine and paroxetine have been approved for the treatment of PTSD symptoms [4]. Despite their effectiveness against the PTSD symptoms, their use are limited because of various side effects, which highlights the need for further research on therapeutic modalities [5].

During recent decades plant-derived extracts and compounds were considered as a valuable source in order to cure a wide variety of diseases such as psychiatric disorders. This rich source has some advantages including few side effects, low cost and high availability.

The cardamom (*Elettaria cardamomum* L. (Maton) from Zingiberaceae family) is one of the most important spice crops cultivated widely in India and other tropical regions worldwide. *E. cardamomum* is called “Queen of Spices” in India and “Hel” in Iran thatused as a flavor agent (spice) in a variety of foodstuffs [6]. In the

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folk medicine, different parts of *E. Cardamomum* have been used in the treatment of gastrointestinal disorders and also used as stomachic, resolvent, retentive, digestive, antiemetic, carminative and anti-putrefactive during embalmment [7,8]. In recent years and in modern medicine various pharmacological properties such as antimicrobial, anti-inflammatory, analgesic, anti-depression, anticonvulsant and antispasmodic activities have been attributed to this plant [9–11].

There are much evidence about *E. cardamomum* beneficial effects against many complications and diseases as mentioned above but there is no study about *E. cardamomum* effect on PTSD. Considering *E. cardamomum* constituents such as quercetin, kaempferol and rutin of which quercetin has effects on CNS function we decide to evaluate *E. cardamomum* methanolic extract effects on anxiety-like behavior in rat model of PTSD [12–14].

2. Materials and methods

2.1. Plant materials

Dry *E. cardamomum* seeds were purchased from the market. The plant seeds were identified by a botanist at the Botany Department of Shahid Bahonar University, Kerman, Iran. A voucher specimen of the plant materials was deposited at the Herbarium of Department of Pharmacognosy of School of Pharmacy, Kerman University of Medical Sciences, Iran (KF1375).

2.2. Preparing methanolic extract

The dried seeds of the plant (100 g) were grinded and extracted by percolation method and through using methanol (80%) for 72 h at room temperature. The solvents were removed in a rotary evaporator, and after filtering, the extracts were concentrated to dryness and stored at −20 °C until testing begins [15].

2.3. Phytochemical analysis of E. cardamomum

The *E. cardamomum* seed methanolic extract was screened for saponins, alkaloids, flavonoids, and tannins [16]. A TLC method (mobile phase composition with 46.15% chloroform, 30.77% ethyl acetate, 15.38% methanol, and 7.69% formic acid:) was used for separation and detection of different compounds in the extract. Quercetin concentration in the extract was determined by HPLC/UV detector through using different concentrations of standard quercetin [11].

2.4. Animals

Healthy adult rats of Wistar strain weighing around 200–250 g were used in the present study. The animals were housed in clean polypropylene cages and maintained in a well-ventilated temperature controlled animal house with constant 12 h light/dark schedule. The animals were fed with standard rat pellet diet and clean drinking water was made available *ad libitum*. All the procedures were performed from 12:00 to 16:00. Maximum effort was made to minimize pain for the animals.

2.5. Ethical statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (Publication No. 85-23, revised 1985). The protocol was approved by the Committee on the Ethics of Animal Experiments at Kerman University of Medical Sciences (Permit Number: KNRC/92-7). Moreover, all efforts were made to minimize suffering of the rats.

2.6. Experimental design

The rats were divided into two main groups. The first main group was single prolonged stress (SPS) group I containing 96 rats, which was subdivided into four subgroups; subgroup Ia (24 PTSD rats treated with saline as vehicle), subgroup Ib (24 PTSD rats treated with 200 mg/kg of the extract), subgroup Ic (24 PTSD rats treated with 400 mg/kg of the extract), and subgroup Id (24 PTSD rats treated with 800 mg/kg of the extract). Each subgroup was also subdivided into three further subgroups (n = 8 rats): (i) pre-stress (received saline or extract 30 min before establishment of PTSD), (ii) post-stress (received saline or extract 30 min after establishment of PTSD), and (iii) pre-test (received saline or extract 30 min before behavioral test). The second main group was control group II containing 96 rats, which was subdivided into four subgroups; subgroup Ia (24 non-PTSD treated with saline as vehicle), subgroup Ib (24 non-PTSD rats treated with 200 mg/kg of the extract), subgroup Ic (24 non-PTSD rats treated with 400 mg/kg of the extract) and subgroup Id (24 non-PTSD rats treated with 800 mg/kg of the extract).

2.6.1. Stress procedure

The rats were brought to the laboratory 24 h before commencement of stress procedure to get habituated to the environment. They were individually caged one day prior to the stress session. On the stress day, the rats were brought to the laboratory. The SPS procedure was performed on them as previously described elsewhere [17]. Three consecutive stressors, including restraint stress (2 h), swimming stress (20 min) in a cylindrical tank (with 40 cm diameter, 50 cm height, 35 cm water height, and 26 °C water temperature), and loss of consciousness with Diethyl Ether were administered to the rats after a 15 min rest [18]. Seven days post PTSD, the rats were again brought to the laboratory and undergone behavioral tests after one hour habituation.

2.6.2. Open field test

The rats were brought to the testing environment after 1 h acclimation period. The rats were then put in the middle of an open field. The open field apparatus was a square arena [90 × 90 × 30 [H] cm] which was made of Plexiglas, and its floor was divided into 16 squares so the field was divided into central and peripheral squares. The vertical and horizontal activities of the rats were recorded during a five min period and then analyzed using an EthoVision software [version 7.1], a video tracking software for automation of the behavioral paradigms [Noldus Information Technology, the Netherlands]. Parameters including total distance moved in cm (TDM), number of grooming and rearing (as a measure of vertical activity), and the time spent in periphery and center were recorded for each rat. At the end of each test, the rats were removed from the chamber and the field was cleaned with a damp cloth [19].

2.6.3. Elevated plus-maze

The elevated plus-maze comprised a black wooden apparatus with arms having equal dimensions. Two of its arms were enclosed by walls (30 × 15 × 5 cm) and arranged in line with 2 opposite open arms (30 × 5 cm). The maze was elevated 50 cm above the floor. The rats were then placed at the center of the maze, facing the open arms. Two 100 W lamps brightly illuminated the arena. The rats were allowed to explore the maze, and their behavior was monitored for 10 min using the EthoVision software [version 7.1] [Noldus Information Technology, the Netherlands]. After each test, the apparatus was cleaned with 70% ethanol to eliminate the

remaining odors. Subsequently, the time spent in the open arms, the number of entries into the open arms, and the total number of entries into the arms was recorded [20,21].

2.6.4. Rotarod test

To analyze the effects of *E. cardamomum* on motor coordination and balance skills, we used the accelerating rotating rod [Hugo Sachs Electronik, Germany]. The rotarod speed which used was from 10 to 60 rpm. There were three trials up to 300 s followed by 30 min inter trial rest. We recorded the duration which each rat was able to maintain its balance walking on top of the moving rod [22].

2.7. Statistical analysis

The obtained results are expressed as the mean ± SEM. Data analysis was carried out by using SPSS statistical package version 17.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA with Tukey's post-hoc test was used to assess differences between experimental groups. In addition, $p < 0.05$ was considered statistically significant.

3. Results

3.1. Phytochemical analysis of E. cardamomum

Preliminary phytochemical screening of *E. cardamomum* showed that the methanolic extract of seeds contains tannins and flavonoids. However, the tests for alkaloids and saponins were negative. Moreover, larger amounts of kaempferol, rutin, and quercetin were observed using the TLC method. According to our previous experiments, the precise concentration of quercetin in the extract was 0.51 μg/ml [11].

3.2. Open field test

As shown in Fig. 1, in pre-stress subgroups, anxiety-like behaviors such as grooming, rearing, time spent in perimeter, and time spent in center were significantly ($P < 0.05$) altered in SPS group receiving the *E. cardamomum* extract, particularly at the 400 mg/kg dose, compared to those in the control groups, which indicated improvement of anxiety-like behaviors in this group. However, in the pre-stress subgroups, no alternation was observed

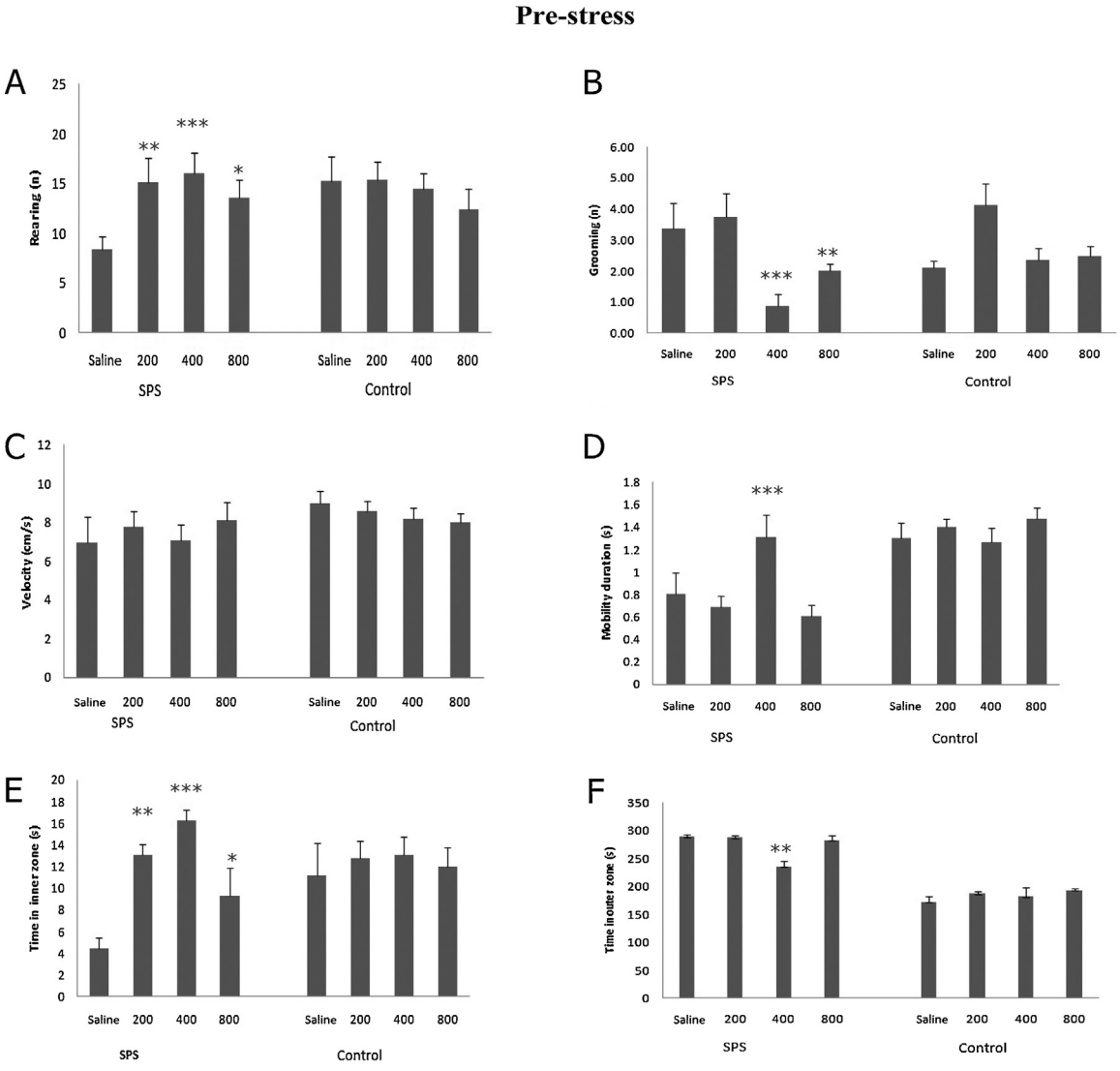

Fig. 1. Effect of various doses of *E. cardamomum* methanolic extract on locomotion and anxiety-like behaviors using with open field test in the pre-stress subgroups. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ in comparison to the control groups.

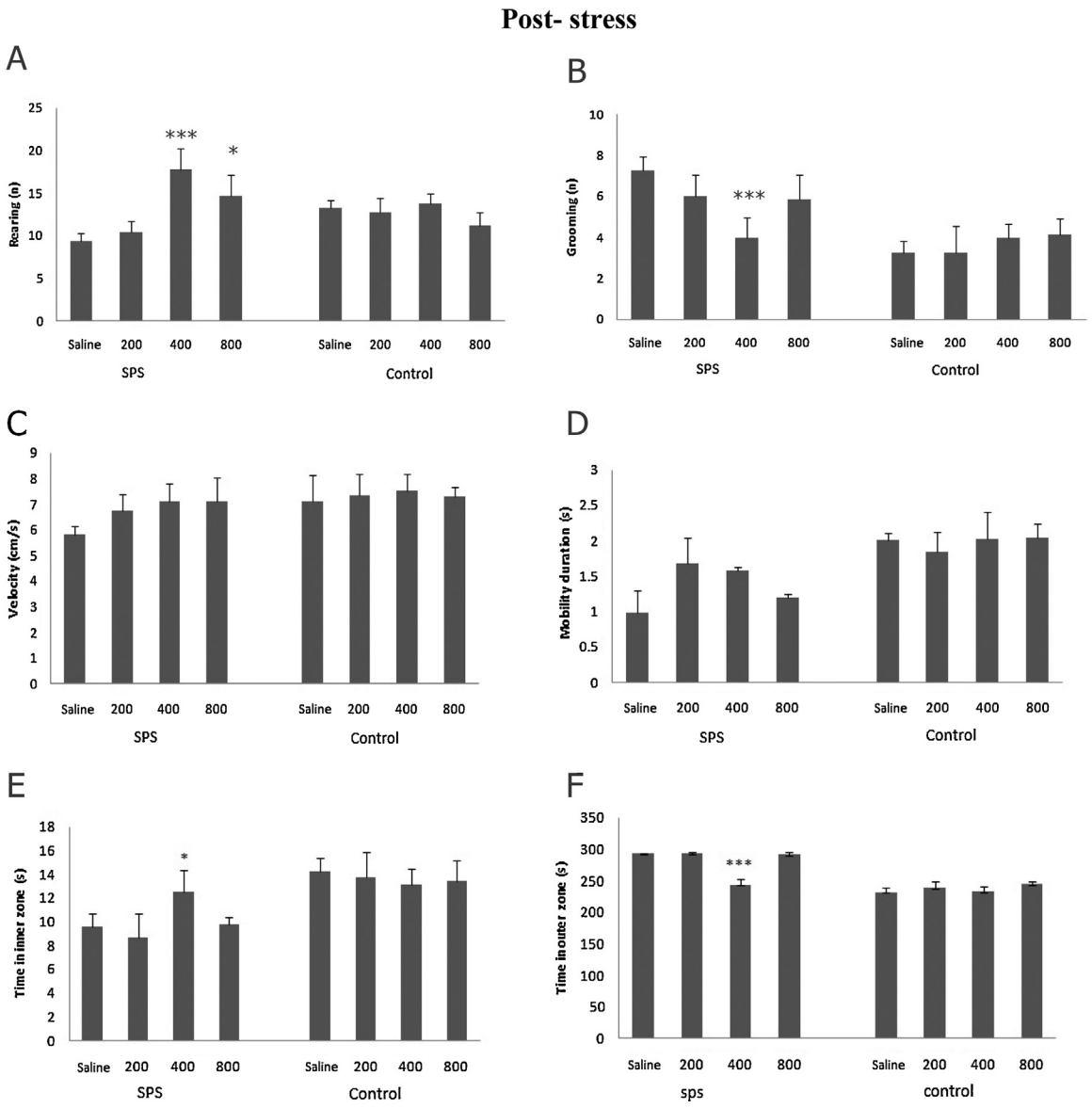

Fig. 2. Effect of various doses of *E. cardamomum* methanolic extract on locomotion and anxiety-like behaviors using with open field test in the post-stress subgroups. * $p < 0.05$, and *** $p < 0.001$ in comparison to the control groups.

in velocity duration (Fig. 1C). Further, mobility increased in the rats of SPS subgroups that received the *E. cardamomum* extract at the dose of 400 mg/kg ($P < 0.05$) (Fig. 1D). Similarly, in post-stress subgroups, the anxiety-like behaviors mentioned above were all significantly ($P < 0.05$) altered in the SPS group receiving the *E. cardamomum* extract, especially at the dose of 400 mg/kg, indicating improvement of anxiety-like behaviors in this subgroup. In the pre-stress subgroups, although velocity and mobility increased in the rats treated with the *E. cardamomum* extract, the difference was not significant compared to the rats in the control groups (Fig. 2).

3.3. Elevated plus-maze

As shown in Figs. 3 and 4, the rats in both the pre- and post-stress subgroups receiving the *E. cardamomum* extract showed a significant difference in terms of anxiety behaviors using elevated plus-maze in comparison with those in the control groups.

In the pre-stress subgroups, the rats treated with the *E. cardamomum* extract at the doses of 200 and 400 mg/kg had a significant ($P < 0.05$) increased time spent in the open arms compared with those in the other groups, implicating a reduced anxiety-like behavior in them (Fig. 4A). However, the rats in these subgroups treated with the *E. cardamomum* extract at the doses of 200 and 400 mg/kg showed a reduced time spent in the close arms in comparison with those in the control groups ($P < 0.05$) (Fig. 4B).

Similarly, treatment with the *E. cardamomum* extract improved the anxiety-like behaviors in the post-stress subgroups as the rats treated with the extract at the dose of 400 mg/kghad a significant ($P < 0.05$) increased time spent in the open arms (Fig. 4A) and decreased time spent in the close arms (Fig. 4B) in comparison with those in the other groups.

3.4. Rotarod test

In both the pre- and post-stress subgroups, a significant reduction was observed in the time spent by the rats treated with the *E. cardamomum* extract at the dose of 800 mg/kg ($P < 0.05$) on revolving rod when compared to the control rats ($P < 0.05$) (Fig. 5).

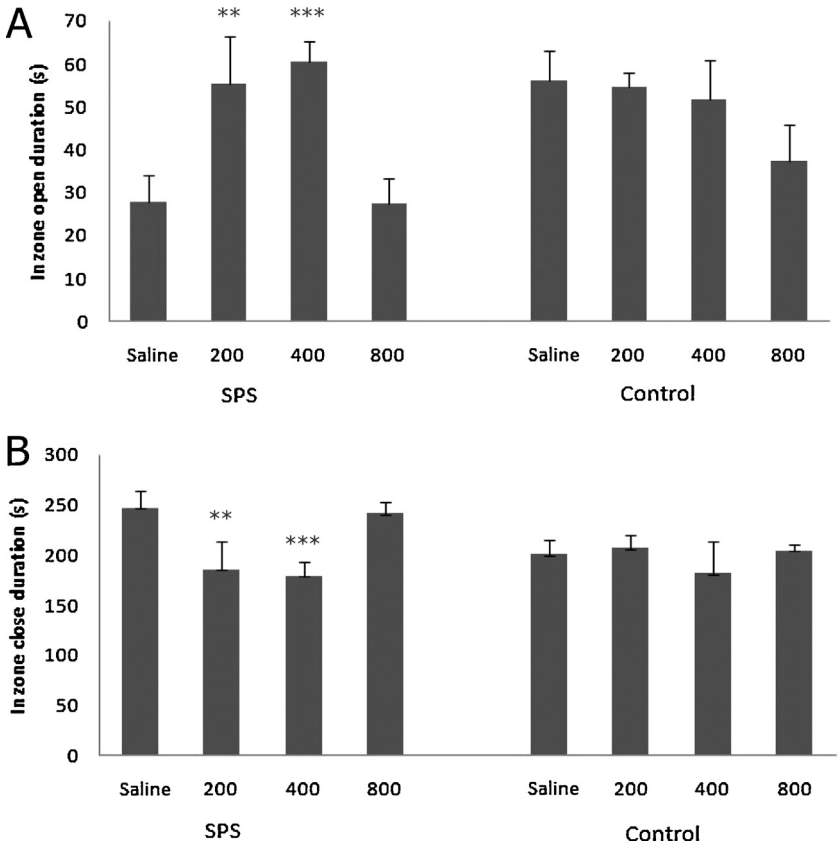

Fig. 3. Effect of various doses of *E. cardamomum* methanolic extract on anxiety-like behaviors using with elevated plus-maze test in the pre-stress subgroups. ** $p < 0.01$ and *** $p < 0.001$ in comparison to the control groups.

4. Discussion

Natural products such as plants crude extracts or their purified compounds provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity [23]. According to World Health Organization (WHO) more than 80% of the world population relies on the traditional medicine for their primary healthcare needs. Recently, study in the area of herbal psychopharmacology has demonstrated a variety of promising medicines that can provide benefit in the treatment of stress and anxiety disorders. Considering advantageous wide rang flavonoid effects such as cognitive, anxiolytic, anti-depressants and sedative effect and also presence of a large scale of flavonoids including quercetin in cardamom, we assessed *E. cardamomum* methanolic extract effects on PTSD rat model [11–14].

We demonstrated that *E. cardamomum* methanolic extract significantly improved anxiety-like behaviors in a rat model of PTSD examined by the open field, elevated plus-maze and rotarod tests. The open field test is used to determine the animal emotional state [24]. Our findings revealed that the *E. cardamomum* methanolic extract caused anxiolytic-like activity in the rats, especially at the dose of 400 mg/kg, as its effect showed significant alternation in their anxiety-like behaviors, including grooming, time spent in perimeter and time spent in center as compared to those of the rats in the control groups.

The elevated plus-maze test is used to assess psychomotor performance and emotional aspects of rodents. The obtained results revealed that the *E. cardamomum* methanolic extract at the dose of 400 mg/kg had anxiolytic activity, since increases in open arm entry parameters [25].

The rotarod test used to evaluate the neurotoxicity of anticonvulsants and later used to report motor dysfunction produced by centrally acting drugs to determine possible changes in the motor coordination ability of the animal [26]. We showed that the *E. cardamomum* methanolic extract (800 mg/kg) significantly reduced the fall-off time from the rotating rod in the treated rats, implicating the skeletal muscle relaxant activity.

Reviews have demonstrated preclinical evidence of anxiolytic activity in various plant medicines including *Achillea millefolium*, *Coriandrum sativum*, *Stachys lavandulifolia*, *Magnolia spp.*, *Eschscholzia californica*, *Panax ginseng*, *Zizyphus jujuba*, *Tilia spp.* and some other plants [27,28]. Moreover, several human clinical trialsprovide preliminary positive evidence of anxiolytic activity for *Matricaria recutita*, *Ginkgo biloba*, *Passiflora incanata*, *Echium amoenum* and *Scutellaria lateriflora* in chronic uses (i.e. greater than one day); whereas acute anxiolytic activity was found for *Centella asiatica*, *Salvia spp.*, *Melissa officinalis*, *Passiflora incarnata* and *Citrus aurantium* [29].

Many studies about chemical constituents of plants extract suggest that plants containing flavonoids, alkaloids, phenolic acids, saponins and tannins have beneficial effects against a wide range of CNS disorders [30]. The phytochemical screening of the *E. cardamomum* methanolic extract showed the presence of tannins and flavonoids in this plant [11]. Therefore, the anxiolytic action of *E. cardamomum* could be attributed to phytoconstituents in this plant. Other studies also showed that plants which have high contents of flavonoids such as quercetin, kaempferol and rutin showed anti-depressant and anxiolytic effects. Quercetin is a flavonoid which have been found in *E. cardamomum*, tea, apples and etc [11,31]. It has been described before that quercetin has

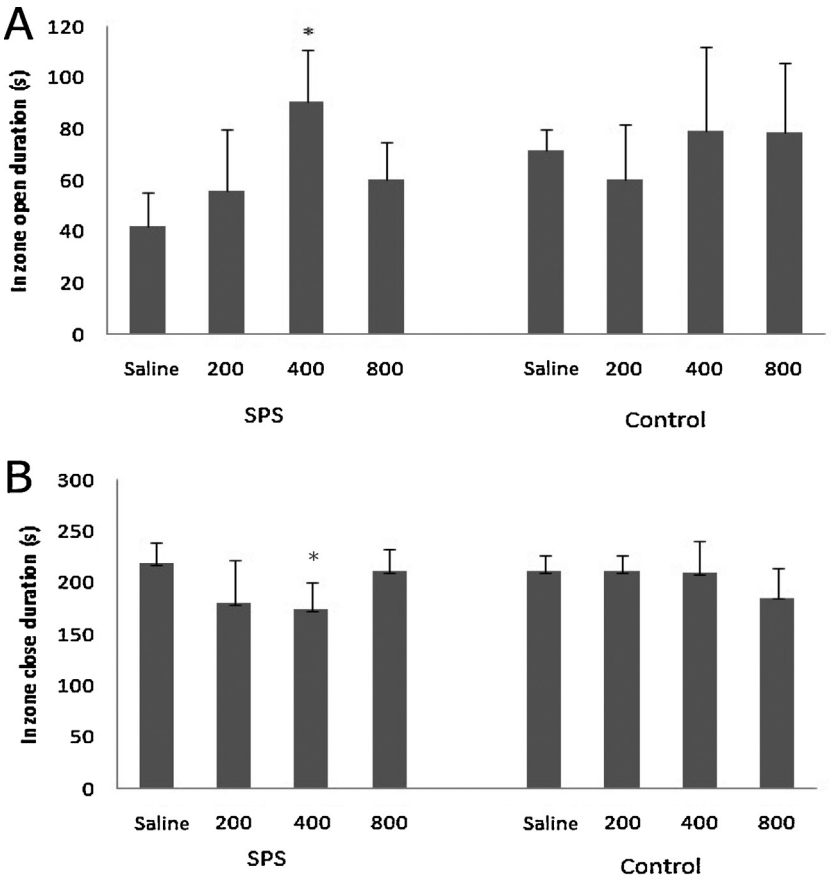

Fig. 4. Effect of various doses of *E. cardamomum* methanolic extract on anxiety-like behaviors using with elevated plus-maze test in the post-stress subgroups. * $p < 0.05$ in comparison to the control groups.

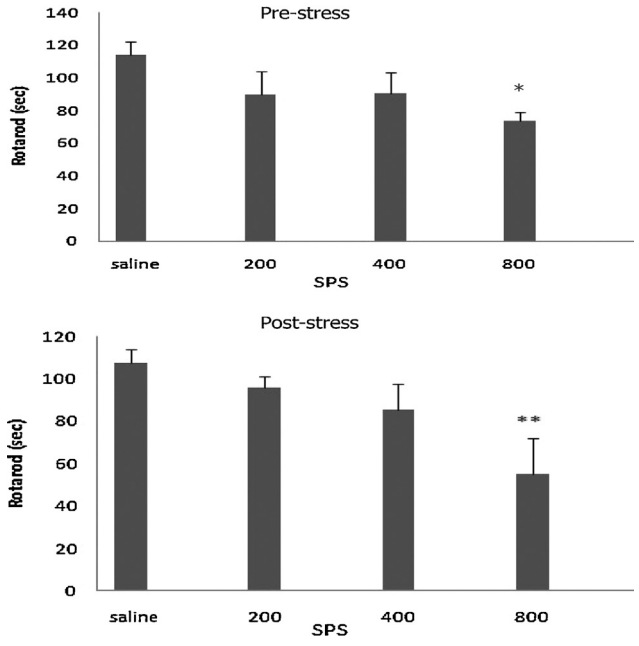

Fig. 5. Effect of various doses of *E. cardamomum* methanolic extract in rats in the pre and post-stress subgroups on rotarod performance. * $p < 0.05$, and ** $p < 0.01$ in comparison to the control groups.

neuroprotective effects and at doses 20–40 mg/kg showed anti-depressant and anti-anxiety effect [32]. In our previous study we showed that *E. cardamomum* is a good source of quercetin [11].

The exact mechanism of *E. cardamomum* is not identified. Previously it has been proved that $GABA_A$ receptor agonists are anxiolytic. Also, it showed that quercetin influenced corticotrophin releasing factor (CRF) induced anxiety through $GABA_A$ receptors [32]. Therefore we can assume that *E. cardamomum* effects which obtained in this study can be attributed to quercetin mediated effects via $GABA_A$ receptors which must be an attractive point for more evaluation. We assumed that these effects of *E. cardamomum* can be due to either quercetin or synergic effects of quercetin along with other flavonoid, but it needs more investigation to be clarified. Other study demonstrated that ethanol extract of *E. cardamomum* strongly inhibited acetylcholinesterase activity and enhanced cognition condition [33]. We also showed that methanol extract of *E. cardamomum* promotes cognition condition in rat model of PTSD. Finally, data about anxiolytic properties of *E. cardamomum* are poor and it needs to be more investigated.

5. Conclusion

The findings of the present investigation suggest that the methanolic extract of *E. cardamomum* possesses anxiolytic properties. We conclude that high flavonoids contents of *E. cardamomum* and especially quercetin is responsible for anxiolytic effects. However, further studies are warranted for elucidating the exact mechanism and bioactive compounds.

Conflict of interest

The authors declare no conflict of interest.

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