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Original article

Anti-Anxiety Effects of *Artemisia persica* in Male Rats

Najmeh Asgharzadeh, Zahra Lorigooini, Hossein Amini-Khoei, Aazam Ghaderian, Marzieh Mardani, Mohammad-Taghi Moradi, Mehrdad Shahrani*

Medical Plants Research Center, Basic Health Sciences Institue, Shahrekord University of Medical Sciences, Shahrekord, Iran.

Abstract

Background and objective: Anxiety is one of the most common diseases in human societies. Since *Artemisia persica* has a significant antioxidant capacity with phenolic compounds, and thesesubstances have anti-anxiety effect, the purpose of this research was to determine the Anti-anxiety like effects of Iranian *Artemisia persica* extract in male rats. **Methods**: *Artemisia persica* hydroalcoholic extract was prepared by maceration method. Animals were divided into 5 experimental groups. The first group was injected with normal saline. Groups 2 to 4 were injected with *Artemisia persica* extract at doses of 100, 200 and 400 mg/kg. The fifth group received 2.1 mg/kg of diazepam. The ability to maintain balance of the rates was measured using the rotarod device, anxiety was measured with elevated plus maze, and the motor activity was measured by the open field device. Antioxidant capacity and malondialdehyde levels were also measured in brain and serum tissues. **Results**: Intraperitoneal injection of doses of 100 and 200 mg/kg of the extract increased the number of entering and presence time in the open arm of the plus maze. Doses of 100 and 200 mg/kg of the extract showed significant increase in antioxidant capacity and significant reduction in malondialdehyde levels. In these experiments, the dose of 400 mg/kg showed less effect and in somecases reversed the effects. **Conclusion**: it seems that the anti-anxiety effect of *Artemisia persica* is dose dependent and increases by increasing the dose to 200 mg/kg; however, at higher dose (400 mg/kg) it shows pro-oxidant effects.

Keywords: anxiety; *Artemisia persica*; rat

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Introduction

Anxiety is associated with symptoms such as feeling tight in the chest and throat, problems in breathing, heart palpitations, mental distress, and sweating [1]. Studies have shown that various neurological and hormonal factors, such as gamma aminobutyric acid (GABA), serotonin, norepinephrine, dopamine, corticotropin releasing factor, cholecystokinin, neuropeptides, and various nerve centers such as amygdala and hippocampus, are involved in the development of anxiety disorders [1]. Dorsal hippocampus plays an important role in controlling anxiety and it is widely related to the septum, lucas serious, raphe nucleus, the hypothalamus, the amygdala, and the mid front of the hypothalamus [2].

GABAergic and serotonergic neurotransmitter system significantly affects the regulation of anxiety [3]. Studies have shown that the brain is one of the most vulnerable organs to oxidizing agents due to high oxygen consumption. Inside the cell, the normal products of oxygen metabolism are free radicals such as superoxide and hydroxyl. In addition, other molecules such as hydrogen peroxide and peroxynitrite are able to produce free radicals. Coexistence of intracellular active metabolites produces reactive

*Corresponding author: author: shahrani@skums.ac.ir

oxygen species (ROS) and reactive nitrogen species (RNS) [4]. Oxidative stress is caused by an imbalance between the production of RNS and ROS [5]. Free radicals attack RNS and ROS causeing damage to the cells by oxidizing membrane proteins and lipids as well as DNA [6]. Following lipid peroxidation by free radicals in the tissue, intermediate compositions such as malondialdehyde are formed in the cell which is the main characteristic of this process. Enzymes such as glutathione reductase and catalase naturally have antioxidant activity [7]. Brain is a high oxygen consuming member rich in lipid stores and produces high amounts of unsaturated fatty acids. Its antioxidant activity is low, so oxidative stress is the source of cellular damage in the brain [8]. Chemical drugs affecting neurotransmitters such as serotonin, norepinephrine and GABA are used to treat anxiety disorders. Commonly used drugs include serotonin reuptake inhibitors and norepinephrine (NSRIs), selective serotonin reuptake inhibitors (SSRIs), and benzodiazepines (BZDs) [9]. Currently, benzodiazepines that act with a boosting effect on GABA activity are the most widely used anti-anxiety drugs that cause complications such as indigestion, anorexia, palpitations, chills, dyspnea, and drug dependence [10].

Artemisia belongs to Asteraceae family and includes more than 400 species [11]. *Artemisia vulgaris* has shown anti-malarial [12], anti-spasm and bronchodilator [11] antihypertensive [13], liver protector [11], anti-bacterial [14], and anti-allergic properties [15].

Artemisia persica Boiss. grows in Iran. It is used in traditional medicine to treat infections and inflammations and has antibacterial and viral effects [16,17].

Artemisia persica has strong antioxidant effects, which is related to phenolic compounds including chlorogenic acid and gallic acid [18]. Chlorhenic acid is effective in the treatment of oxidative damage [19]. Research on several species of *Artemisia* has shown their effects on the central nervous system. For example, *Artemisia iwayomogi* has shown antidepressant properties in rat models [20]. Tarragon (*Artemisia dracunculus*) also had central analgesic effects [21]. The aim of this study is finding effective treatments for anxiety.

Materials and Methods

Ethical considerations

Ethical considerations based to Institutional Animal Care and Use Committee (IACUC). Laboratory rats had free access to water and food and were eventually killed post anesthesia, by rapid detachment of the head from the body and rapid amputation of the spinal cord The study protocol followed the Helsinki Protocol, and was approved by the Ethics Committee of the Shahrekord University of Medical Sciences (Approval no. : IR.SKUMS.REC.1396.194).

Extraction

Artemisia persica was collected from Lordegan in 2019 and identified and registered at the Herarium of Shahrekord University of Medical Sciences with Herbarium code 1008. The stems and leaves were dried and powdered in the shade. Plant powder was macerated with ethanol 70% (1:5). The hydroalcoholic extract was filtered after 72 h. The extract was then concentrated by vacuum distillation through a rotary device [22].

Animal study

Fifty male rats were kept in a room for animals (21 to 22 °C). The 12 h light cycle and the 12 h darkness cycle were accompanied by enough water and food. Animals were divided into 5 groups of 10 animals. Injections were performed one hour before behavioral and intraperitoneal testing. The first group was injected with normal saline. Groups 2 to 4 were injected with *Artemisia persica* extract at doses of 100, 200 and 400 mg/kg. The fifth group received 2.1 mg/kg of diazepam [23]. The ability to maintain balance was measured using the rotarod device [24], Anxiety like behavior was measured with elevated plus maze [25], and the motor activity was measure by the open field device [26]. The results were then compared.

Elevated plus maze test as a standard test for anxiety evaluation

The elevated plus maze is plus shape maze that is based on four bases [26]. Two of the arms lack the lateral and end walls (open arms 50 × 10 cm). The other two arms have two side walls and one end wall, but the roof is open (enclosed arms 50 × 10 × 40 cm). A square-shaped plate is 10 × 10 cm in size at the cross section of these four arms. The maze's elevation was 50 cm. Before entering the maze, the animal was placed in a wooden box

with the size of 35 × 100 × 50 cm for 5 min. One hour after drug injection each animal was placed alone in the center of the elevated plus maze in a way that the animal's face was towards the open arm and moved freely in the maze for 5 min. All movements were recorded for 5 min and the number of times the animal entered the open arms, enclosed arms, total time spent in the open arms, and the total amount of time spent in the enclosed arm were measured. Passing four hands and feet of the animal was considered as the entry of the arms. The percentage of entering the open arm and enclosed arm that is anxiety criterion was calculated as follows:

A. The ratio of entrance to the open arm to the total entrance to arm and enclosed arm × 100 = Open Arm Entrance (OAE%)
B. The ratio of time spent in open arms to total time spent in open and enclosed arm × 100 = Open Arm Time (OAT%)

The total entrance to open arm and enclosed arm were also called locomotor activity (LA) [27].

Rotarod motor coordination test

Rotarod device includes a wheel whose speed is 0-40 rpm. The rats receiving *Artemisia persica* extract or control were placed on a rotary device one hour after receiving the extract. The wheel spinned for 300 s (max) and the time that the rat could maintain its balance, and resist the movement of the wheel, was recorded as the time of resistance of the rat, which was conducted in triplicate for each rat and the average of the three measurements was included in data analysis [24].

Open field test

In this test, a fiberglass box (transparent) with dimensions of 90 × 90 × 30 cm is used; the floor is divided into 16 squares. Rats' activity (frequency of crosses and standing on two legs) was recorded over a 5-min period [28].

Determining total antioxidant capacity of blood and brain serum using FRAP assay

The basis of this method is the ability of the serum to regenerate ferric ions Fe^{3+} into ferrous Fe^{2+} in the presence of tripyridyltriazine (TPTZ). In this method, the reaction of Fe^{2+} with the TPTZ reagent produces Fe^{+2}-TPTZ blue complex with a maximum absorption at 593 nm. The degree of regenerative capacity of the serum in different concentrations of the extract was measured accordingly [29].

Measurement of serum and brain tissue malondialdehyde

One mL of the homogenized brain tissue or the serum was poured into a 20 mL glass and incubated at 37 ± 1 °C in a metabolic shaker for 60 min. Then, one mL of 5% trichloroacetic acid (TCA) was added to one mL of 67% of thiobarbituric acid and mixed well after each stage. The combination of each vial was centrifuged at 2000 rpm for 15 min. Afterwards, the supernatant was transferred to another tube and placed in a water bath. After 10 min, the test tubes were cooled and the absorbance was measured at 535 nm [29].

Statistical analysis

Statistical analyses were performed using one-way ANOVA and Tukey's test and $p<0.05$ was considered significance level.

Results and Discussion

Entering into open arm of the plus maze device in groups that received *A. persica* extract at the doses of 100 and 200 mg/kg significantly increased compared to the control group ($p<0.05$). The group receiving diazepam also showed a significant increase ($p<0.001$) in comparison with the control group. The 400 mg/kg group showed a significant decrease ($p<0.05$) compared to the normal saline and those receiving *A. persica* extract with lower doses ($p<0.001$). The 100 and 200 mg/kg groups did not show significant difference. The group receiving 200 mg/kg extract showed a significant increase ($p<0.001$) compared to the control group receiving normal saline in the percentage of time spent in the open arm. The diazepam group also showed significant increase ($p<0.001$) compared to the control while there was no significant difference at the dose of 100 mg/kg compared with the normal saline group. The group receiving 400 mg/kg^{-} extract showed a significant decrease ($p<0.001$) compared to the groups receiving lower doses of the extract, diazepam and normal saline (figure 1).

The groups receiving doses of 100 and 200 mg/kg showed a significant increase ($p<0.05$ and $p<0.005$, respectively) in the number of movements between squares compared to normal saline alone.

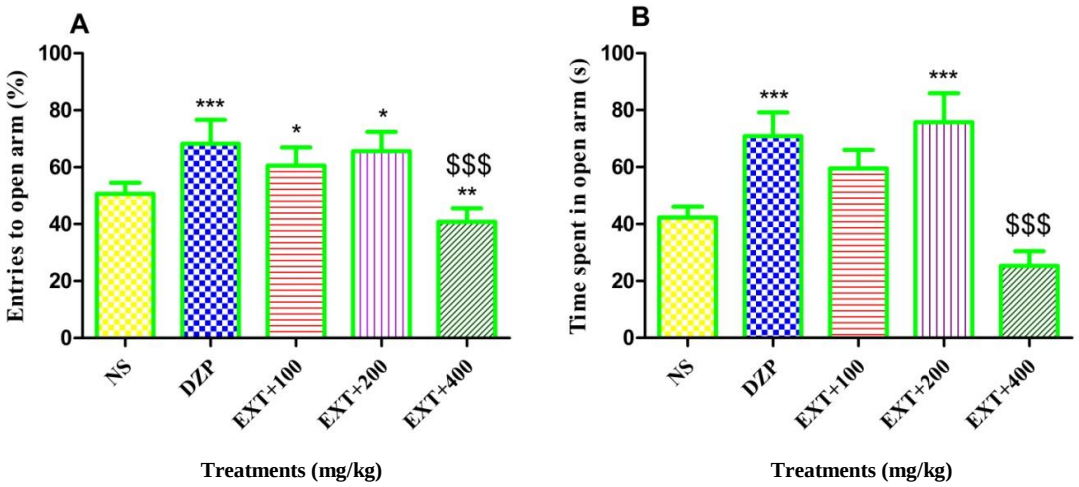

Figure 1. The effect of intra peritoneal injection of different concentrations of *Artemisa persica* extract on the percentage of entries (A) and time spent in the open arm of the plus maze device (B); NS: normal saline; DZP: diazepam; EXT: *Artemisa persica* extract; * vs control group (*p <0.05, ** p <0.005, *** p <0.001), $ vs 100 and 200 *Artemisa persica* extract ($$$ p <0.001).

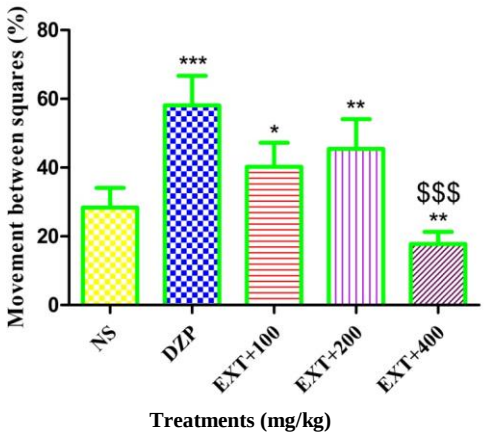

Figure 2. Effect of intra peritoneal injection of different concentrations of *Artemisa persica* extract on the number of rats movement between squares in the open field device; NS: normal saline; DZP: diazepam; EXT: *Artemisa persica* extract; * vs control group (*p <0.05, ** p <0.005, *** p <0.001); $ vs 100 and 200 of *Artemisa persica* extract ($$$ p <0.001).

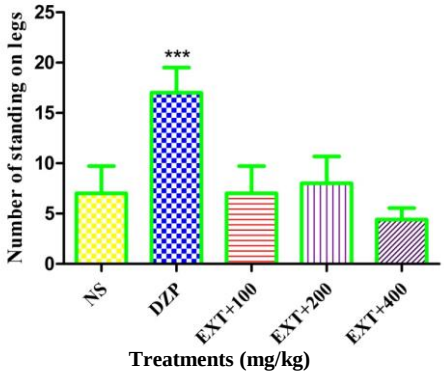

Figure 3. Effect of intraperitoneal injection of different concentrations of *Artemisa persica* extract on the number of rats standing on two legs in open field device; NS: normal saline; DZP: diazepam, EXT: *Artemisa persica* extract; * vs control group (*** p <0.001)

Also, diazepam group showed a significant increase (p<0.001) while the group receiving 400 mg/kg showed a significant decrease (p <0.005). No significant difference was found between diazepam and the dose of 200 mg/kg (figure 2).

Regarding the number of times standing on two legs in the open field device, none of the groups receiving the 100, 200, and 400 mg/kg *A. persica* extract showed significant difference compared to the control group (p> 0.05). The rats receiving diazepam significantly increased their frequency of standing on legs (p<0.001) (figure 3).

The groups receiving normal saline, diazepam and doses of 100 and 200 mg/kg of *A. persica* extract did not show any significant difference in balance maintenance on Rotarod device, but the group receiving 400 mg/kg showed a significant decrease compared to other groups (p<0.001) (figure 4).

There was a significant decrease in serum malondialdehyde levels in diazepam and doses of 100 and 200 mg/kg compared to normal saline (p<0.001). There was no significant difference between the dose of 400 mg/kg of and normal saline (p>0.05). There was a significant decrease

in the level of malondialdehyde of brain in diazepam groups ($p<0.001$) and doses of 100 ($p<0.05$) and 200 mg/kg ($p<0.001$), respectively, compared to control group. There was no significant difference between the dose of 400 mg/kg extract and normal saline ($p> 0.05$) (figure 5).

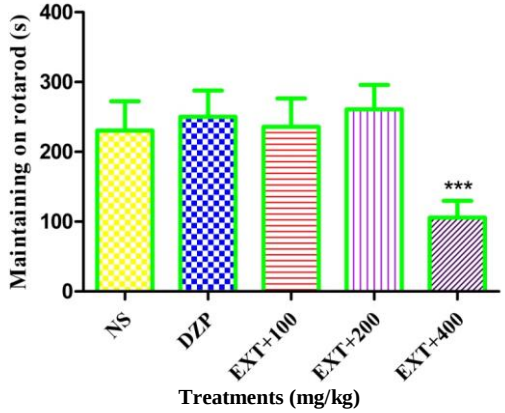

Figure 4. Effect of intraperitoneal injection of different concentrations of *Artemisa persica* extract on the duration of balance of rats on the rotarod system; NS: normal saline; DZP: diazepam; EXT: *Artemisa persica* extract; * vs other groups (*** $p<0.001$)

Regarding the level of total antioxidant capacity, as shown in figure 6, the groups receiving the doses of 100 and 200 mg/kg presented a significant increase ($p<0.05$ and $p<0.001$, respectively), compared to the control group. The diazepam group also showed a significant increase ($p<0.001$).

Assessing the total antioxidant level of the brain of the rats, the results showed that the 100 and 200 mg/kg groups showed significant increase ($p<0.05$ and $p<0.001$, respectively) in comparison to the control group. The diazepam group also showed a significant increase ($p<0.001$) while the dose of 400 mg/kg of the extract did not show a significant difference. There was no significant difference between the dose of 100 mg/kg extract and diazepam.

The role of secondary metabolites in medicinal plants, especially flavonoids, has been demonstrated in several studies related to oxidative stress such as anxiety [30]. *Artemisia persica* has a strong antioxidant property that is related to its phenolic compounds [31] including chlorogenic acid and gallic acid [32]. Researches have shown that chlorogenic acid has anti-anxiety effects due to its antioxidant properties [33].

Mirzaei et al. evaluated the total phenol and antioxidant activity of *Artemisia martima*. They used 2,2-diphenyl-1-picrylhydrazyl (DPPH) and antioxidant power tests. Hydroalcoholic extract of *Artemisia martima* showed different levels of antioxidant activity in all studied models and the most considerable antioxidant activity was attributed to *A. martima* [34].

Diazepam is classified as a central nervous system (CNS) inhibitor [35]. Inhibition of these subcortical areas (primary limb, thalamus, and hypothalamus) from the central nervous system with diazepam or other benzodiazepines results in anti-anxiety effects [35]. Anti-anxiety drugs may interact with glutamic acid decarboxylase (GAD) or GABA-T, which ultimately affect the brain's GABA level and neurotransmitter [36]. The flavonoids christine and epigene derived from medicinal plants have shown anti-anxiety properties in mice exposed to behavioral experiments. [36].

In the present study, diazepam was expected to increase the number of entry and duration of presence in the open arm in the plus maze test. In the open field test, the motor activity of the rats increased. In the rotarod test, diazepam did not cause abnormal imbalance. Sodepalm et al. showed that diazepam acutely impaired the balance of mice in the rotarod system at doses higher than 2 mg/kg [37]. Considering that the dose of diazepam in this study was 1.2 mg/kg, the balance of the rats was not reduced.

Diazepam reduced the levels of malondialdehyde in the brain and serum in rats. It also increased the antioxidant capacity of the brain and serum. Mousavi and Kakkar reported that short-term use of diazepam induced antioxidant effects and reduced peroxidation of fatty acids. They proposed that the binding of diazepam to the membrane of cells would increase the stability of the membrane [38]. Flavonoids extracted from *A. persica* are probably a relative agonist for benzodiazepine receptors [39].

Emadi et al. showed that *Artemisia annua* has a sedative effect that is probably caused by the pathway for benzodiazepine receptors [40].

Rezaei et al. observed that intra peritoneal injection of *Artemisia persica* extract in rats at different doses significantly reduced the sleep induction time and created more sleep time compared to diazepam.

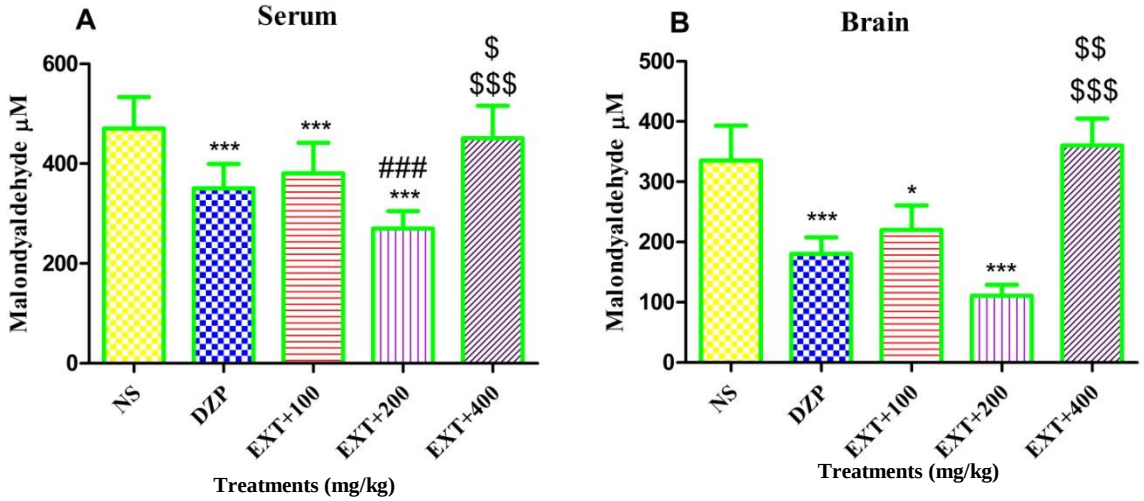

Figure 5. Effect of Intraperitoneal injection of different concentrations of *Artemisa persica* extract on serum malondialdehyde in rats (A) and brain (B); NS: normal saline; DZP: diazepam; EXT: *Artemisa persica* extract; * vs control group (*** p <0.001); ### vs NS (### p <0.001); $ vs 100 mg/kg of extract (p <0.05); $$ Vs DPZ and 100 mg/kg of extract ($$ p <0.01); $$$ Vs 200 mg/kg of extract ($$$ p <0.001)

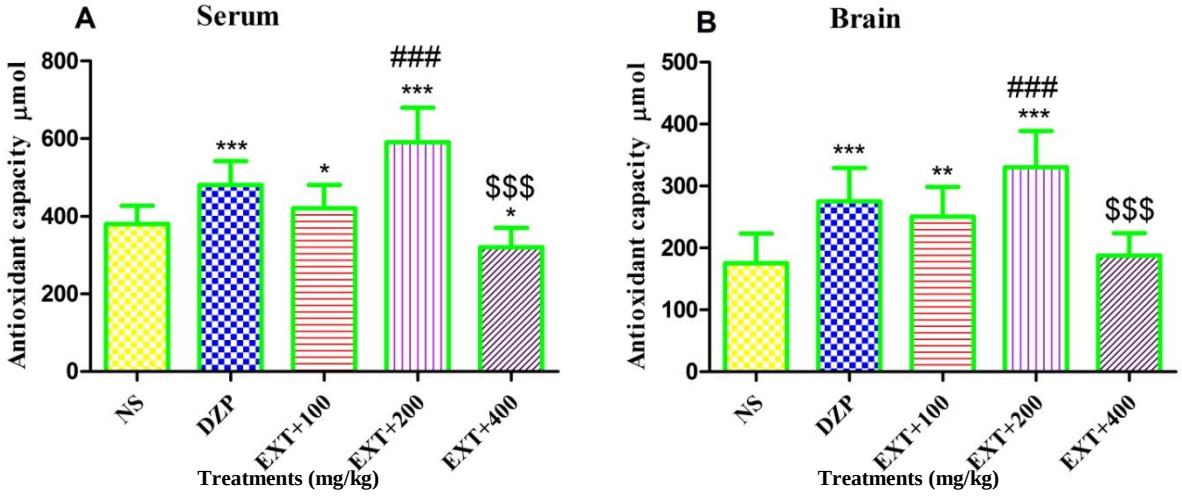

Figure 6. Effect of intraperitoneal injection of different concentrations of *Artemisa persica* extract on total antioxidant capacity of serum (A) and brain (B) of rats; NS: normal saline; DZP: diazepam, EXT: *Artemisa persica* extract; * vs control group; (*p <0.05, *** (p <0.001). # vs other groups (### p <0.001), $ vs 100 and 200 mg/kg extract and diazepam ($$$ p <0.001)

According to the results of the study, the extract showed more considerable sedative and anesthetic effects compared to diazepam with the most effective dose to be 400 mg/kg [22].

Mino et al. conducted a study to investigate the psychopharmacologic effects of *A. copa*. They used different doses of this extract to a maximum of 1.5 mg/kg. High doses caused drowsiness. Doses of 0.5 and 1.5 mg/kg, increased and decreased the spontaneous motor activity (SMA), respectively. The number of times that the rats hang their heads from the hole is directly related to their motor activity. According to the results, the dose of 1.5 g/kg caused a significant increase in drug latency and a decrease in seizure duration and death caused by 75 g/kg pantoprazole in mice [41].

These results are consistent with the results of the present research. In both cases, the high dose of *the extract* showed less anti-anxiolytic effects

(400 mg/kg hydroalcoholic extract of *Artemisia persica* in this study and 1.5 g/kg of extract in the study by Mino et al.) and reduced the motor and exploratory activity. While lower doses of the extract produce more anti-anxiety effects. In the plus maze test, the group which received the *A. persica* extract at a dose of 400 mg/kg showed a significant decrease in both entries to the open arm and the time spent in the open arm. They also demonstrated reduced activity in the open field test.

In the present study, in behavioral and biochemical tests, the dose of 200 mg/kg showed a moreconsiderable effect on oxidative stress and anxiety in rats. This indicates the antioxidant and oxidative anti-anxiety effects depending on the dose of the extract. However, the dose of 400 mg/kg showed a reversed. The reason is probably due to the lack of dependence of the dose with the response when taking medications. Rafiean et al. found that antioxidants may act as pro oxidants in certain conditions and may exacerbate oxidative stress [41]. Therefore, in the present study, increasing the dose of the extract, converting the antioxidant compounds to the pro-oxidants may have led to oxidative effects.

Overall, the effects of *A. persica* extract were dose-depended. However, reduced anti-anxiolytic effects was observed at a higher dose which should be more studied to examine the mechanisms involved in the process.

Acknowledgments

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Author contributions

Najmeh Asgharzadeh, Mehrdad Shahrani and Hossein Amini-Khoei designed and supervised the study; Azam Ghaderian performed the experiments; Marzieh Mardani and Mohamad Taghi Moradi analyzed the data; Zahra Lorigooini was the pharmaceutical consultant

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

GABA: gamma aminobutyric acid; ROS: reactive oxygen species; RNS: reactive nitrogen species; DNA: deoxyribonucleic acid; SRIs: serotonin reuptake inhibitors; SSRIs: selective serotonin reuptake inhibitors; BZDs: benzodiazepines; OAE: open arm entrance; OAT: open arm time; LA: locomotor activity; FRAP: ferric reducing antioxidant power; TPTZ: 2,4,6-tripyridyl-S-triazine; CNS: central nervous system; GAD: glutamic acid decarboxylase; GABA-T: GABA transporter