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Original Article

Effects of *Ruta graveolens* L. on Locomotor Disorders and Anxiety: Modulation of Hippocampal Antioxidant Capacity

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Abstract

Background and aims: *Ruta graveolens* L. has shown promise in alleviating anxiety and enhancing motor balance, attributed to its antioxidant properties. This plant is rich in flavonoids, such as rutin, which aid in mitigating oxidative stress—a condition associated with anxiety and locomotor disorders. Research indicates that its neuroactive compounds may contribute to the maintenance of neural function and the restoration of balance.

Methods: This study involved 56 eight-week-old male Balb/c mice divided into seven groups. The control group received normal saline (NS) intraperitoneally for five days without cold stress. Three extract groups were subjected to cold stress and administered *Ruta graveolens* L. extract (RGE) at doses of 30, 100, and 300 mg/kg. Another NS group also faced cold stress but received NS. The diazepam group received 1 mg/kg diazepam intraperitoneally under cold stress. The seventh group received 300 mg/kg RGE after flumazenil (3 mg/kg) administration. Anxiety levels were assessed using the elevated plus maze (EPM), and psychomotor coordination was evaluated with the rotarod test. Following deep anesthesia with ketamine and xylazine, blood samples were collected, and the hippocampi were extracted for biochemical analyses of antioxidant capacity and malondialdehyde (MDA) levels.

Results: Cold stress significantly decreased both the number of entries into and the duration of time spent in the open arm of the EPM ($P < 0.001$). Locomotor coordination in the NaCl group exposed to cold stress was diminished compared to the control group. Administration of RGE at all three doses improved locomotor coordination and balance movements. Furthermore, the extract at all doses enhanced the antioxidant capacity of the hippocampus and increased the duration of time spent in the open arm.

Conclusion: RGE effectively reduced anxiety, improved balance, and enhanced the antioxidant capacity of the hippocampus.

Keywords: Anxiety, Stress, Rotarod, Elevated plus maze, Antioxidant, *R. graveolens* L.

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Introduction

Cold stress has been shown to impair locomotor activity and exacerbate anxiety-like behaviors, as evidenced by various animal studies. It also induces oxidative stress in the hippocampus, disrupting the balance of antioxidant defenses and leading to damage via lipid peroxidation and protein oxidation (1, 2). While stress is distinct from anxiety, it is one of the contributing factors that can precipitate anxiety disorders, which can arise from a multitude of sources. In this study, cold exposure was employed as a stress-inducing factor. Anxiety disorders represent one of the most prevalent psychiatric conditions globally (3). These disorders are influenced by an interplay of environmental, genetic, and multi-molecular factors, yet their exact etiologies remain elusive, as no singular domain—be it genetics, neurobiology, or neuroimaging—adequately elucidates their underlying pathological mechanisms (4).

The glutamatergic system, which plays a crucial role in the hippocampus, impacts anxiety through α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-Methyl-D-aspartate (NMDA) receptors, both of which also influence learning and memory. Anxiety is mediated by various neurotransmitters, including dopamine (via D1 and D2 receptors), gamma-aminobutyric acid (GABA), serotonin, catecholamines, and sex hormones. Chronic nicotine exposure has been shown to enhance NMDA receptor responses, thereby increasing the NMDA/AMPA ratio and facilitating long-term potentiation (LTP) in hippocampal CA1 neurons (5).

GABA receptors, classified into GABA_A and GABA_B subtypes, play critical roles in mitigating fear, anxiety, and depression while modulating memory. Flumazenil, which inhibits GABAergic activity by targeting benzodiazepine receptors, is widely utilized to treat delirium resulting from benzodiazepine overdoses (6). Increased GABA concentrations in the brain can attenuate the activity of various brain nuclei, producing anxiolytic effects (7).

Individuals with anxiety often exhibit varying degrees of oxidative damage and reduced levels of antioxidant enzymes. Under normal physiological conditions, a balance exists between the production and elimination of reactive oxygen species (ROS). Disruption of this balance can result in oxidative stress and the accumulation of free radicals (8, 9). Elevated levels of ROS, such as nitric oxide and superoxide, have the potential to damage cellular macromolecules (10). Cold exposure disrupts antioxidant enzyme activity, elevates metabolic rates, and generates oxidative substances, such as hydrogen peroxide, thereby impairing the body's antioxidant defenses. Fluctuations in temperature among mice have been associated with behavioral changes and increased anxiety. Stress can destabilize homeostasis, consequently weakening the antioxidant defense system (11). Cold stress has been found to negatively impact growth performance, induce hepatocyte apoptosis, and enhance innate immunity in hybrid sturgeon as a compensatory response to additional stressors (12).

Ruta graveolens L., an herbaceous plant reaching heights of 30 to 80 cm, is commonly utilized in the Middle East due to its antispasmodic, diuretic, and sedative properties. This plant possesses numerous medicinal attributes, including anti-inflammatory, antipyretic, and antiparasitic effects. The efficacy of *Ruta graveolens* L. extract (RGE) in ameliorating inflammation and oxidative damage in hypercholesterolemia mice has been documented. Furthermore, its therapeutic potential in managing hyperlipidemia and type 2 diabetes has also been substantiated (13). Various secondary metabolites have been isolated from *Ruta graveolens* L. leaves, notably furanocoumarins, flavonoids, alkaloids, and essential oils, with quercetin being one of the significant flavonoids. Quercetin is a glycosylated form of rutin (a type of flavonoid). The furanocoumarins in *Ruta graveolens* L. have been reported to function as potassium channel blockers in nerve fibers (14). The metabolite composition of the plant's leaves includes approximately 16% terpenoids, 30.6% aliphatic acids, 11% flavonoids, 34% alkaloids, 3.7% quinones, 4% alcohols, 3% steroids, and 13% other metabolites. The antioxidant activity of RGE has been demonstrated to inhibit the oxidation of levodopa catalyzed by fungal tyrosinases, with a noted correlation to the concentration of phenolic compounds. Additionally, the anti-inflammatory and antioxidant effects of *Ruta graveolens* L. methanolic extract have been substantiated in several studies. The objective of this study was to investigate the anti-anxiety effects of *Ruta graveolens* L. (14). Specifically, the study aimed to assess the impact of cold stress on anxiety, psychomotor activity, and antioxidant indices in the hippocampus across different experimental groups.

Materials and Methods

Laboratory Animals

Fifty-six male BALB/c mice, each weighing between 25 and 30 grams, were utilized in this study. The mice were divided into the following groups: the control group, which received normal saline (NS) at a dosage of 10 ml/kg (15); the NaCl 0.9% group, which was subjected to stress and also received NS at the same dosage; the diazepam group, which underwent stress and received diazepam at a dosage of 1 mg/kg (16); and groups 4 to 6, which were subjected to stress and received RGE at dosages of 30, 100, and 300 mg/kg, respectively. The flumazenil group received flumazenil prior to the administration of the effective dose of the extract (300 mg/kg), which was given 15 minutes later (17). All injections were administered intraperitoneally over a period of five days.

Following the completion of behavioral experiments, the mice were euthanized under deep anesthesia induced by ketamine (100 mg/kg) and xylazine (10 mg/kg). Decapitation was performed using a guillotine, and the hippocampal sections were subsequently stored at -80°C until biochemical analyses were conducted (Figure 1) (18).

RGE = *Ruta graveolens* L. Extract, ip = Intraperitoneal, TAC = Total Antioxidant Capacity, MDA = malondialdehyde

Preparation of RGE

Ruta graveolens L. was collected from the highlands of northern Iran, and the herbarium specimen was registered under the code 152 following approval by a botanist at the Medicinal Plants Research Center, Shahrekord University of Medical Sciences. The collected material was ground and pulverized, and then mixed with 70% ethanol to obtain a hydroethanolic extract via maceration. Subsequently, a rotary evaporator was employed to concentrate the extract. The concentrated extract was then dried at 37°C (19).

Determination of the Antioxidant Activity of RGE Using Diphenylpicrylhydrazine (DPPH)

Each extract and stock solution of butylated hydroxytoluene (BHT) were prepared at a concentration of 1 mg/ml, while DPPH was prepared at a concentration of 0.1 mg/ml. Using these stock solutions, six concentrations of the extract were prepared, ranging from 5 to 100 µg, specifically at 10, 20, 40, 60, 80, and 100 µg in 2 ml volumes. Corresponding concentrations of BHT were also prepared in a similar manner.

To each of the six concentrations of extract or BHT, 2 ml of DPPH solution was added, and the mixtures were maintained in the dark for 15 minutes. Additionally, a control tube containing 2 ml of ethanol and 2 ml of DPPH was prepared alongside the samples. After the 15-minute

incubation period, the spectrophotometer was calibrated to zero using ethanol at a wavelength of 517 nm, and the absorbance of the samples was subsequently measured. The concentration at which 50% of DPPH was neutralized was reported as the IC50, expressed in micrograms per milliliter of extract (20).

Measurement of Total Phenolic and Flavonoid Content in RGE

The total phenolic content of the RGE was quantified using the Folin-Ciocalteu method. The extract was prepared at a concentration of 10 mg/mL. A volume of 5 mL of the extract was combined with 2.5 mL of 2 N Folin-Ciocalteu reagent and stirred for 5 minutes. Subsequently, 2 mL of a 20% sodium carbonate solution (75 g/L) was added to the mixture. The absorbance of the samples was measured at 760 nm using an ultraviolet spectrophotometer against a methanol blank after a 2-hour incubation at room temperature. The total phenolic content was calculated using a standard curve based on milligrams of Gallic acid equivalent per gram of dry extract.

The total flavonoid content of the extract was assessed using a colorimetric method. The extract was similarly prepared at a concentration of 10 mg/mL. To this, 0.5 mL of the extract was dissolved in 1.5 mL of methanol, followed by the addition of 0.1 mL of a 10% aluminum chloride solution. Then, 0.1 mL of 1 M potassium acetate solution and 2.8 mL of distilled water were incorporated into the mixture, which was allowed to stand at room temperature for 30 minutes. The absorbance of the resulting solution was measured at a wavelength of 415 nm. The total flavonoid content was reported based on a standard curve expressed as mg/g of dry extract (21).

Determination of TAC of Mouse Hippocampus and Serum Using the Ferric Reducing Antioxidant Power (FRAP) Method

The antioxidant capacity of the hippocampus and serum was assessed by measuring their ability to reduce Fe^{3+} to Fe^{2+} using the FRAP method. The FRAP working solution was prepared by combining 25 mL of acetate buffer, 2.5 mL of tripyridyltriazine (TPTZ) solution, and 2.5 mL of $FeCl_3$. The hippocampus was excised, homogenized, and then centrifuged at 10 000 rpm; the resulting supernatant was utilized to evaluate the antioxidant capacity of the hippocampus. A volume of 50 µL of this supernatant or mouse serum was added to 1.5 mL of the freshly prepared working solution and incubated at 37°C for 10 minutes. During this incubation, a complex between Fe^{2+} and TPTZ was formed, resulting in the development of a blue color. The absorbance of this solution was measured at a wavelength of 593 nm, with $FeSO_4$ serving as the standard solution in this method (22).

Cold Stress Induction

Cold stress was induced in mice by placing them in a refrigerator set at 4°C for 30 minutes each day over a period of 5 days. Behavioral assessments were conducted on the fifth day following exposure to the cold stress (23).

Behavioral Assessments

Behavioral tests, including the Elevated Plus Maze (EPM) and the Rotarod, were conducted in the following order.

EPM

The EPM consists of a cross-shaped design featuring two open arms (0.25 × 5 × 30 cm) and two closed arms (0.25 × 5 × 15 cm) that face each other, connected by a central platform measuring 5 × 5 cm. The maze is elevated 50 cm above the ground and the experiment was conducted in a semi-dark, quiet room. During the procedure, the mouse was gently and carefully placed in the central area of the apparatus, oriented such that its head faced an open arm. The number of entries into each arm and the time spent in the arms were recorded over a period of 5 minutes. The device was thoroughly cleaned with a cloth soaked in alcohol after each use (24).

Psychomotor Coordination Test Using the Rotarod Device

This test is utilized to assess motor balance. The presence of muscle relaxant effects or a decrease in motor coordination can interfere with the outcomes of anxiety assessments. The rotarod device features a horizontal rod with a diameter of 3 cm that initially rotates at a speed of 10 revolutions per minute (rpm). After 20 seconds, the rotation speed increases to 20 rpm. Prior to the test, the mice underwent a training session 24 hours in advance; those that were unable to remain on the rotating rod for at least 30 seconds were excluded from the study. On the day of testing, assessments were conducted 30 and 60 minutes after the administration of the drug. The maximum duration for each animal in this test was set at 300 seconds (25).

Measurement of MDA in Mouse Hippocampus and Serum

To measure MDA levels, 1 mL of homogenized hippocampus tissue or serum was placed in a 20 mL glass tube and incubated at (37 ± 1°C) in a metabolic shaker for 60 minutes. Following the incubation period, 1 mL of 5% trichloroacetic acid (TCA) was added, along with 1 mL of 67% thiobarbituric acid (TBA), and the mixture was well-mixed after each addition. The resulting mixture from each vial was then transferred to a centrifuge tube and centrifuged at 2000 rpm for 15 minutes. The supernatant was collected and placed in a boiling water bath for 10 minutes. After boiling, the test tubes were allowed to cool, and the optical absorbance was measured at 535 nm to assess MDA levels (26).

Data Analysis

Data were analyzed using Prism 8 software and presented

as Mean ± SEM (Standard Error of the Mean). Statistical analysis was performed using One-Way Analysis of Variance (ANOVA) followed by Tukey's post hoc test to assess differences among groups. Statistical significance was set at an alpha level of 0.05.

Results

Comparison of the Number of Entries and Duration of Time Spent in the Open Arms in the EPM Test Among the Studied Groups

In this study, the primary objective of using flumazenil was to elucidate the mechanism of action of *Ruta graveolens* L. Consequently, all graphs presented compare the flumazenil + RGE 300 group with three distinct doses of *Ruta graveolens* L., specifically 30 mg/kg, 100 mg/kg, and 300 mg/kg. The results indicated that both the number of entries and the duration of time spent by mice in the open arms of the EPM were significantly reduced in the group receiving 0.9% saline (NS) under stress conditions, when compared to the control group ($P < 0.001$). In contrast, administration of diazepam resulted in a significant increase in both the number of entries and the duration of time spent in the open arms of the EPM, relative to the group receiving 0.9% NS under stress ($P < 0.001$). Furthermore, RGE at doses of 30 mg/kg, 100 mg/kg, and 300 mg/kg significantly enhanced both the number of entries and the duration of time spent in the open arms when compared to the group receiving 0.9% NS under stress ($P < 0.001$). Notably, flumazenil effectively inhibited the impact of RGE at a dose of 300 mg/kg on the time spent in the open arms of the EPM ($P < 0.001$) (Figures 2A and 2B).

RGE = *Ruta graveolens* L. Extract, Flum = Flumazenil, s = second; Data were analyzed using ANOVA followed by Tukey's post hoc test. Values are expressed as mean ± standard error. Statistical significance is indicated as follows: ###$P < 0.001$ compared to the control group; **$P < 0.01$ and ***$P < 0.001$ compared to 0.9% NaCl; $$P < 0.001$ compared to the Flumazenil + RGE 300 group.

Comparison of Duration of Balance Maintenance in the Rotarod Test Among the Studied Groups

The duration of balance maintenance on the rotating rod of the rotarod test was significantly reduced in the group receiving NS combined with stress compared to the control group ($P < 0.001$). Diazepam significantly increased the duration of balance maintenance on the rotarod compared to the NS + stress group ($P < 0.001$). Administration of RGE at doses of 30 mg/kg, 100 mg/kg, and 300 mg/kg also significantly enhanced the duration of balance maintenance compared to the NS + stress group ($P < 0.001$). Furthermore, flumazenil effectively prevented the increase in time spent on the rotarod induced by RGE at a dose of 300 mg/kg ($P < 0.001$) (Figure 3).

RGE = *Ruta graveolens* L. extract; Flum = flumazenil; s = seconds; Data were analyzed using ANOVA followed by Tukey's post hoc test. Values are presented as mean ± standard error. Statistical significance is indicated as follows: ###$P < 0.001$ compared to the control group; **$P < 0.01$ and ***$P < 0.001$ compared to 0.9% NaCl; $$P < 0.01$, $$$P < 0.001$ compared to the Flumazenil + RGE 300 group.

Comparison of TAC and MDA Levels in the Hippocampus of Mice Among the Studied Groups

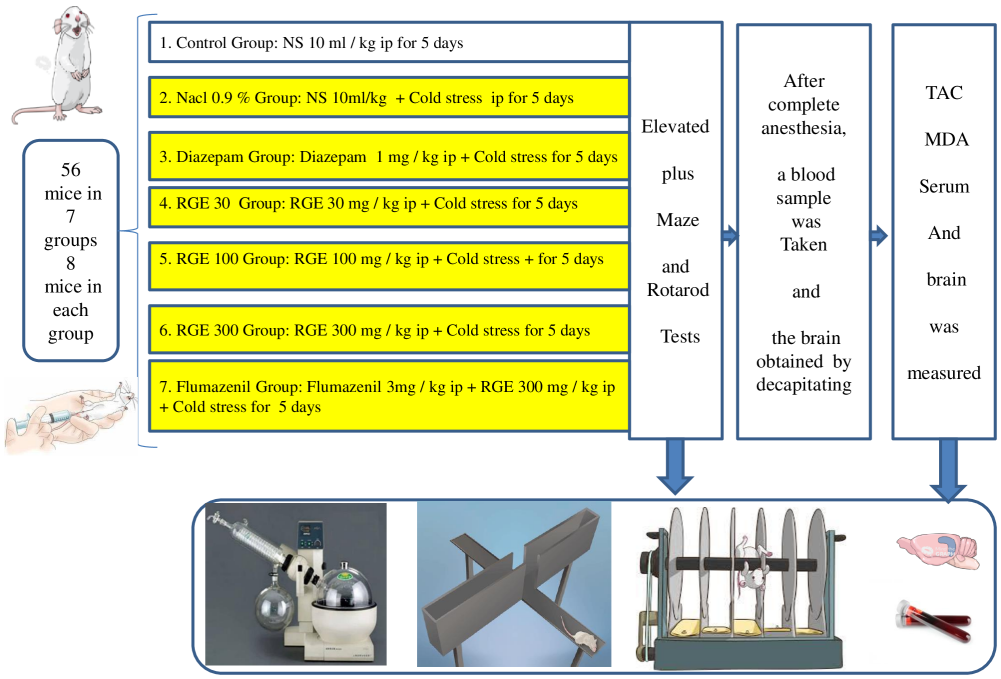

Figure 1. Schematic diagram of the study design

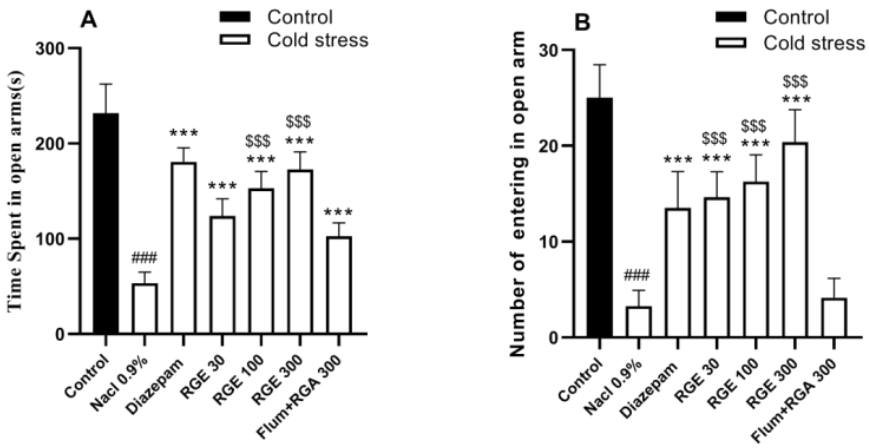

Figure 2. Effects of RGE, diazepam, and flumazenil on the duration (A) and number (B) of entries made by mice into the open arms of the EPM

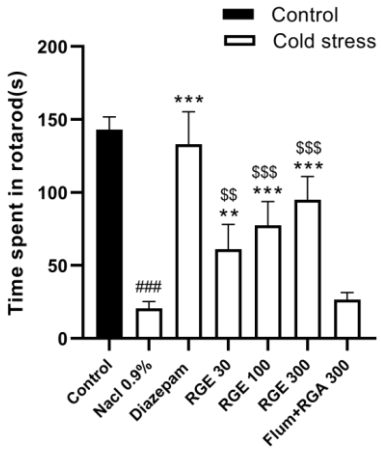

Figure 3. Effects of RGE, diazepam, and flumazenil on the duration of balance maintenance on the rotarod apparatus

The TAC level in the hippocampus of the stress group receiving NS was significantly reduced compared to the control group ($P < 0.001$). Treatment with diazepam and RGE at doses of 30 mg/kg, 100 mg/kg, and 300 mg/kg resulted in a significant increase in hippocampal TAC ($P < 0.001$). Additionally, administration of RGE at a dose of 300 mg/kg in conjunction with flumazenil also led to a significant increase in hippocampal TAC ($P < 0.001$) (Figure 4A).

Conversely, the MDA level in the hippocampus of the stress group receiving NS was significantly elevated compared to the control group ($P < 0.001$). Treatment with diazepam resulted in a significant decrease in hippocampal MDA levels ($P < 0.001$), as did RGE at doses of 100 mg/kg ($P < 0.05$) and 300 mg/kg ($P < 0.01$) (Figure 4B).

RGE = *Ruta graveolens* L. Extract, Flum = Flumazenil, nmol/ml = Nanomole/milliliter; Data were analyzed using ANOVA and Tukey's post hoc test. Values are shown as mean ± standard error, ###$P<0.001$ compared to the control group, *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared to NaCl 0.9%, \$$P<0.05$, \$\$$P<0.01$, \$\$\$$P<0.001$ compared to Flum + RGE 300.

Comparison of TAC and MDA Levels in the Serum of Mice in the Studied Groups

The TAC level in the serum of mice in the stress group receiving NS showed a significant decrease compared to the control group ($P<0.001$). Diazepam, RGE at doses of 30 mg/kg, 100 mg/kg, and 300 mg/kg, as well as Flumazenil and RGE 300 mg/kg, caused a significant increase in serum TAC levels ($P<0.001$) (Figure 5A). The serum MDA level in the stress group receiving NS significantly increased compared to the control group ($P<0.001$). Diazepam and RGE at a dose of 300 mg/kg caused a significant decrease in serum MDA levels ($P<0.01$). Injection of a dose of RGE 300 mg/kg and Flumazenil also caused a reduction in serum MDA ($P<0.01$) (Figure 5B).

RGE = *Ruta graveolens* L. Extract, Flum = Flumazenil, nmol/ml = Nanomole/millilitre; Data were analyzed using ANOVA and Tukey's post hoc test. Values are shown as mean ± standard error, ###$P<0.001$ compared to the control group, **$P<0.01$, ***$P<0.001$ compared to NaCl 0.9%, \$\$$P<0.01$, \$\$\$$P<0.001$ compared to Flum + RGE 300.

Measurement of phenol, flavonoid and antioxidant power of RGE

The phenol content of RGE, measured by the Folin-Ciocaltio method, was determined to be 14.1 mg Gallic acid equivalent per gram dry weight of the extract. The flavonoid content of RGE was also measured by colorimetric; this amount was determined to be 15.8 mg rutin equivalent per gram dry weight of the extract. In the present study, the DPPH stable radical scavenging test was used to investigate the antioxidant effects of RGE. In this test, the ability of different concentrations of the RGE to scavenge DPPH free radicals was determined. The concentration of RGE that caused 50% inhibition of DPPH free radicals was 157.9 µg/mL.

Discussion

Anxiety disorders are one of the most common mental illnesses in the world. Given the high prevalence of these disorders in different societies, their disturbing

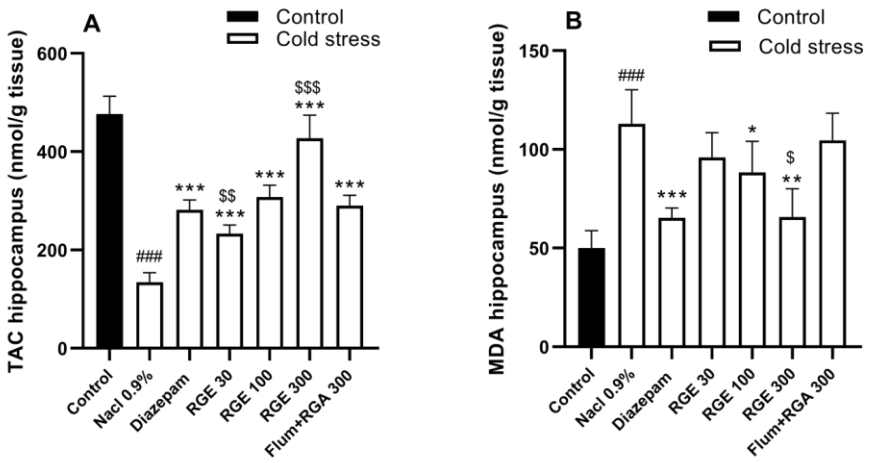

Figure 4. Effect of RGE, diazepam and Flumazenil on the hippocampus TAC (A) and MDA (B)

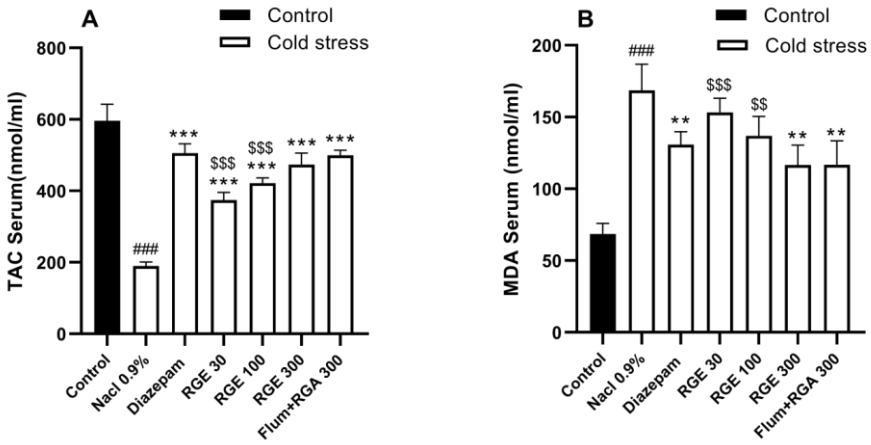

Figure 5. Effect of RGE, diazepam and Flumazenil on serum TAC (A) and MDA (B)

consequences, and some of their destructive effects, prevention and treatment of these disorders have always been a concern for humanity (27). Due to the side effects of chemical drugs, research on medicinal plants has grown, focusing on their therapeutic benefits. Flavonoids, particularly in plants like *Ruta graveolens* L., play a proven role in combating oxidative stress-related diseases, such as anxiety. This plant is rich in flavonoids, notably rutin, contributing to its antioxidant effects (28, 29).

In this study, anti-anxiety and anti-oxidative stress effects were investigated and tested. In this study, the effects of the RGE at concentrations of 30 mg/kg, 100 mg/kg and 300 mg/kg on anxiety, motor activity, serum and hippocampus MDA levels and serum and hippocampus antioxidant capacity of adult mice were investigated and compared with diazepam, which is a common anti-anxiety drug. Also, to find the anti-anxiety activity of the RGE through GABAergic receptors, Flumazenil, which is a benzodiazepine antagonist, was used (30). Anxiety was evaluated using the Elevated plus-maze test, a standard rodent model. *Ruta graveolens* L. showed an antioxidant capacity (IC50) of 157.9 µg/mL and a total phenol content of 1.14 mg Gallic acid equivalent per gram dry weight of the extract.

Due to high oxygen consumption, the hippocampus is susceptible to oxidative damage from free radicals like ROS and RNS, which harm cellular components. Antioxidant defenses, such as enzymes (SOD, catalase) and nutrients (vitamins C, E), help mitigate oxidative stress (31). Disruption in the balance between free radicals and antioxidants leads to oxidative stress, which accelerates aging and contributes to depression, anxiety, and central nervous system diseases. Oxidative stress also causes cellular damage through lipid peroxidation. It is linked to diseases like cancer, neurodegenerative disorders, and autoimmune conditions. Anxiety disorders worsen oxidative damage by impairing antioxidant defenses.

Medicinal plant extracts and essential oils help counter oxidative stress due to their antioxidant properties (32). In a study by Habtemarian et al., the effect of rutin was proven as a natural treatment for Alzheimer's disease. The mechanism of this effect was introduced as regulating the antioxidant system and reducing inflammatory substances that damage neurons (33). In a study by S.K. Raghav et al. that investigated the anti-inflammatory effects of RGE on adipose macrophage cells, it was found that *Ruta graveolens* L. plant has an active flavonoid metabolite, rutin constituting a high level of its flavonoid metabolites

(34). A study by Ratheesh et al. that investigated the anti-oxidative and anti-inflammatory effects of the *Ruta graveolens* L. plant on the heart and liver of hypercholesterolemia mice showed that the plant *Ruta graveolens* L. reduced the activities of cyclooxygenase-2 and myeloperoxidase and the concentration of the active ingredient TBA and increased the antioxidant activities of enzymes such as glutathione, which indicates the anti-oxidative stress activity of the *Ruta graveolens* L. plant (35). This study used cold stress to induce stress because freezing causes the production of oxidative substances and increases the body's metabolism (12). The flavonoid content of the *RGE* was also measured by colorimetry, which was determined to be 15.8 mg of rutin equivalent per gram of dry weight of the extract. Considering the above studies and the results of this study, it can be argued that the *RGE*, due to the presence of flavonoid compounds, reduces anxiety, increases the TAC of serum and hippocampus, and reduces the amount of MDA in serum and hippocampus of mice. It is also argued from the results of Flumazenil injection before extract injection that the positive effects of this plant may have exerted its anti-anxiety activity, possibly through GABA receptors. These values indicate the presence of antioxidant substances, including flavonoids, with rutin at the top of them, which is responsible for the high antioxidant capacity of this plant extract.

Conclusion

In summary, this study's results suggest that the plant extract has anti-anxiety and anti-oxidative stress properties in BALB/C mice. The extract of this plant also increases the TAC of the serum and hippocampus. It reduces the level of MDA in the serum and hippocampus of mice. The anti-oxidative stress effects of the plant extract are dose-dependent, and this effect increases with increasing doses. At a dose of 300 mg/kg, this plant most likely exerts its anti-anxiety activity through GABA receptors; however, further studies are needed to investigate other mechanisms involved in this process.

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Supervision: Mehrdad Shahrani Korrani.
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Conflict of Interest Disclosure

Although one of the authors in this article is the journal's editor-in-chief, the whole process of reviewing and publishing the article is like that of other articles in the journal, and there is no difference in its review from other ones.

Data Availability

The data supporting this study's findings are available on request from the corresponding author.

Ethical Approval

All procedures on mice were carried out with the approval of the Ethics Committee of Shahrekord University of Medical Sciences with the ethics code IR.SKUMS.REC.1394.231.

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