Effect of hydro-alcoholic extract of *Rosa Damascena* Mill on anxiety and oxidant/antioxidant status in rats fed with a long-term high-fat diet

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Abstract

Today, a high-fat diet (HFD) is widely used in most communities. This diet causes anxiety by oxidative stress and inflammation. On the other hand, the *Rosa damascena* mill has potent antioxidant properties and can eliminate free radicals. In this study, the effect of hydroalcoholic extract of *R. damascena* on anxiety was investigated in male rats fed with a HFD. In this experiment, forty male Wistar rats (200–250 g) were used. The extract was administrated through gavage for one month. The HFD was consumed freely by the animals for three months. The elevated plus-maze (EPM) was used to assess anxiety. At the end of the experiment, the activity of glutathione peroxidase (GPX) and superoxide dismutase (SOD) enzymes and the levels of corticosterone also were measured. In the EPM test, the number of entries into the open arms and time spent in the open arms in the HFD + extract group significantly increased compared with the HFD group. The activity of GPX and SOD enzymes was lower in the HFD group than in the control group. The level of serum corticosterone in the HFD group was higher than in the control group, whereas the HFD + extract group was found with significantly lower corticosterone levels than the HFD group. In the EPM, a HFD caused anxiety and the extract decreased it. These effects of extract probably are due to its antioxidant properties. The extract increased the GPX and SOD activities, which is representative of its antioxidant activity.

Introduction

Consumption of a high-fat diet (HFD) has increased dramatically over the past few decades (1). Today, the diet of developed countries is rich in saturated fat and refined sugar, and it is well known that people's lifestyle and diet quality play a vital role in their neuronal and brain function (2). Increased fat intake and obesity are associated with mood and mental disorders, such as anxiety and depression (3–6). Prolonged consumption of a HFD impairs brain function through inflammation (7), oxidative stress (8, 9), and induction of insulin resistance (10). Consuming a HFD for 4 months and the resulting obesity increased protein oxidation in the frontal cortex and increased oxidative stress parameters, resulting in anxiety-like behaviors (10). In another study, HFD for 7 weeks reduced the number of new cells produced in the hippocampal gyrus, as well as increased malondialdehyde (MDA) levels and decreased levels of brain-derived neurotrophic factor (BDNF). MDA has a toxic effect on neurons producing stem cells, disrupting neurogenesis in the hippocampus, and increasing lipid peroxidation (LPO) in the brain (8).

Oxidative phosphorylation is the process, by which energy is produced as adenosine triphosphate (ATP), and this process, which is essential for energy metabolism, produces the reactive oxygen species (ROS), such as superoxide and hydrogen peroxide (11). Oxidative stress is caused by an imbalance between the production of free radicals, such as ROS, and the ability of cells to remove them and protect the cell against them (12). The brain is highly prone to oxidative stress damage due to its high oxygen consumption, relatively low antioxidant defenses, and high-fat content (13, 14). Antioxidants are compounds that, in very small amounts relative to the oxidizing substrate, significantly delay or inhibit the oxidation of the substrate. Antioxidants are biologically active compounds that protect the body against the damages caused by ROS and active nitrogen species (RNS) (15). ROS can damage cell lipids,

proteins, and DNA, and typically, antioxidants, such as superoxide dismutase (SOD), vitamins E and C, and glutathione (GPX) can reduce ROS-induced cell damage by reducing ROS levels (16). In the presence of oxidative stress, due to the high-fat content of the brain, LPO occurs, which leads to a decrease in membrane fluidity and membrane proteins and inactivation of enzymes, receptors, and ion channels (14, 17). Oxidative stress can affect neurotransmitter transmission, neuronal function, membrane integrity, whole-brain activity, and even neuronal death (18). Recently, there has been evidence that consuming a HFD can increase the production of free radicals and causes oxidative stress (19), and consuming supplements rich in antioxidants can reduce the harmful effects of free radicals on neuronal cells and cognitive function (20–22).

Recent studies have shown an association between oxidative stress and anxiety. The overexpression of glutathione reductase 1 and glyoxalase 1 in the cortex of the cingulate leads to increased anxiety-like behaviors, while inhibition of glyoxalase 1 expression reduces anxiety behavior. Thus, the association between the antioxidant status of the brain and anxiety-related behavior became apparent (23). It has also been shown that there is a close relationship between the level of intracellular ROS in peripheral blood cells (lymphocytes, monocytes, and granulocytes) and anxiety-related behaviors (24). Also, the bamboo extract showed antianxiety effects in laboratory mice fed with a diet containing saturated fatty acids for 2 months by decreasing pro-inflammatory cytokines and increasing glutathione levels (25).

In traditional medicine, the use of various herbs has been suggested to reduce anxiety. For example, *Rosa damascene* mill L. is a shrub of the family Rosaceae, which has strong antioxidant properties (26). Kalim et al. in 2010 showed that *R. damascene* due to its high phenolic, flavonoid, and ascorbic acid content has a strong antioxidant power that can scavenge harmful free radicals (27). In a study by Achuthan in 2003, it has been shown that *R. damascene* hydroalcoholic extract prevented liver damage caused by carbon tetrachloride and oxidative stress in rats and had a protective effect on the liver. These observations are probably due to its antioxidant power and neutralization of free radicals (28). The parts used in this plant include flowers, flower buds, fruits, petals, and flags. Its essential oil is prepared from its petals and was first made in Europe by Rossi in 1574. Chemical compounds in this plant are geraniol, citronellol, essential oil, fat, resin, malic acids, tartaric, christrin, gallic acid, red pigments, such as cyanine, quercetin, kaempferol, phenethyl alcohol, vitamins. C, and carotene. Several studies have also been performed on the analgesic, anti-inflammatory, antibacterial, and anti-viral properties of this plant (29). It also has sedative, anti-flatulence, fever-relieving, thirst-quenching, and invigorating properties. The essential oil of this plant is used in aromatic ointments or disinfectants, cosmetic products, and the perfume industry (30).

Although little is known about the effects of HFD on the nervous system, studies have shown that increased fat intake and obesity play a role in developing mood and psychological disorders, such as anxiety and depression. *R. damascene* is one of the oldest medicinal plants in traditional medicine with great antioxidant power. Therefore, in this study, we investigated the effect of hydroalcoholic extract of *R. damascene* on anxiety in rats following the consumption of HFD.

Materials And Methods

Animals

In this study, 40 male Wistar rats weighing 200–250 g were purchased from the Pasteur Institute and kept in an animal lab for two weeks before the start of the experiment. Animals were randomly placed in cages (19 × 27 × 42.5 cm) (5 rats per cage) at 22 ± 2° C under the dark and light cycles (12 hours of light and 12 hours of darkness). All rats had free access to water and food. Animal care processes were approved by the Veterinary Ethics Committee of the Hamadan University of Medical Science (Ethics Code: IR.UMSHA.REC.1394.9404232209) and were accomplished consistent with the Guidelines of the National Institutes of Health (NIH Publication 80 − 23, 1996).

Hydroalcoholic extract of *R. damascene*

The extract of *R. damascene* was obtained considering the reported previous approach with some modifications (31–33). To prepare the extract, 6 kg of *R. damascene* petals was purchased from the market in May and dried in the shade for 2–3 days, and then, 500 g of dried flowers were ground by an electric shredder. The dried plant powder was then soaked 3 times in 98% ethanol alcohol 3 times and each time for 3 days to extract the required active ingredients. It was then filtered and rotated each time with filter paper to separate the solvent from the extract, and then the resulting extract was placed in a crystallizer vessel in a bain-marie apparatus at a temperature of 80° C. After collection, the extract was weighed sufficiently for each rat, dissolved in distilled water, prepared weekly, and given to the animals daily by gavage (1 cc of the desired dose), depending on the weight of the animals.

Method of administration of hydroalcoholic extract of *R. damascene*

In the groups receiving the extract, the desired extract was gavaged orally. After weighing the rats, according to the calculations, the amount of the desired extract was gavaged based on the weight of the animals. All groups receiving the extract received 1000 mg/kg body weight. The desired dose was selected based on previous studies (34–41).

HFD

the HFD was prepared from a standard food (Behparvar Livestock and Poultry Food Company) and a combination of other compounds (42). The compositions of the high-fat and standard diets are given in Table 1.

Table 1
The composition of the high-fat and standard diets.

Standard diet (% gr)	High-fat diet (% gr)
Protein 21%	Standard food 67.7%
Fat 3.69%	Animal oil 8.3%
Carbohydrates 32.5%	Hydrogenated oil 4.05%
Raw fiber 5.5%	Soybean oil 0.85%
	Sodium cholate 0.8%
	Cholesterol 1%
	Sugar 17.3%

To prepare the HFD, the standard food of the milled rats was carefully weighed according to the percentage of the Table 1 and then mixed with the weighed sugar. Then, all oils were carefully weighed with a scale and the cholesterol and cholic acid were dissolved in the oils and added to the powder containing standard food and sugar. All ingredients in the HFD were weighed carefully according to the Table 1. After preparing the HFD, enough water was added to increase the adhesion of the ingredients, and finally, they were pelleted by a meat grinder machine. The HFD groups had free access to high-cholesterol food and water.

Experiment design

Animals were divided into the following groups:

A) Control group: This group received a normal diet and did not receive the extract.

B) Extract group: This group received a normal diet + extract at a dose of 1000 mg/kg through gavage for one month.

C) HFD group: This group received a HFD for 3 months.

D) HFD + extract group: This group received a HFD for 3 months and in the last month (from the second month onwards), the extract was administrated for one month at a dose of 1000 mg/kg through gavage. The experiment timeline is shown in Fig. 1.

Evaluation of the anti-anxiety effect: Elevated plus-maze (EPM) test

In order to assess anxiety, a device called EPM was used, which is a standard model for assessing anxiety levels in rodents and is designed based on the incompatibility of rodents with the open

environment. This test is rapid and is used to evaluate amygdala-mediated anxiety and the effects of and anti-anxiety agents. It is also used to determine the anxiolytic and anti-anxiety effects of drugs (43, 44).

The device is a wooden maze that is 50 cm away from the floor of the room and consists of two closed arms and two open arms facing each other. The two open arms with the dimensions of 30 × 10 cm, have no walls and are white and the two black closed arms are 30 × 15 × 10 cm with walls.

The test was performed between 9 am and 3 pm. Ten minutes before the experiment, the animals were placed on an elevated plate in an unfamiliar environment to reduce accidental errors in entering the arms. At the beginning of the experiment, the animals were placed in the center of the maze, with the direction of the animal's head toward the close arms. Entry to each arm is when the animals' four paws are in the arm (43). The test lasts 10 minutes and each animal is tested only once. After each experiment, the maze was cleaned with 70% ethanol alcohol. A CCTV camera attached to a computer recorded animals' behaviors inside the maze and the files were saved to analyze the data.

Animals tend to be curious about a new environment and maze arms when are located in the maze. In the new environment, the animal explores the new environment or escapes from it. Due to the height of the maze from the ground, the open condition of open arms, and the fear and anxiety of falling from these arms, animals are less inclined to enter and remain in the open arms. In addition, in the EPM, the animal actively prevents entering the open arm. Thus, the open arm entry is a measure of anxiety, while entering the closed arm is an indicator of the animal's general activity. Known anti-anxiety agents are able to increase the tendency of animals to enter and remain in open arms (45).

During the test time (10 minutes), the number of open arms (OE) entries to investigate motor activity behaviors and the length of stay in open arms (OT) to investigate anxiety behavior, and the total number of open and closed arms (TA) entries were recorded and compared between groups. The number of entrances to the open arms and the time spent in open arms were compared with the control group level (46–50); the lower number of entries and shorter time spent indicate more anxiety-like behavior (43).

Biochemical parameters

After three months, blood samples were taken from the inferior vena cava, and the biochemical parameters and levels of the oxidative stress biomarkers were determined in the plasma.

Measurement of serum corticosterone

For the measurement of serum corticosterone, 2 ml of blood sample was collected and was allowed to clot for 30 minutes at room temperature. The serum was separated by centrifugation at 2500 rpm for 5 minutes and subsequently, it was stored at -20° C. The serum samples were then subjected to estimation of serum corticosterone. Corticosterone levels were estimated by an automated chemiluminescence immunoassay system using a corticosterone ELISA kit (DRG, USA). This immunoassay kit allows for the in vitro quantitative determination of endogenic corticosterone concentration in serum.

Measurement of GPX activity

The levels of GPX were determined with spectrophotometry. Phosphate buffer solution (1 mL; 0.4 M; pH 7.0) containing 0.4 mM EDTA, 1 mL of 5 mM NaN3, 1 mL of 4 mM GSH, and 200 µL of supernatant was preincubated at 37°C for 5 min. Then, 1 mL of 4 mM H2O2 was added and incubated at 37°C for a further 5 min. The surplus amount of GSH was quantified by the DTNB procedure as previously demonstrated by Sharma and Gupta (51). One unit of GPX is described as the amount of enzyme needed to oxidize 1 nmol GSH/min. The enzyme activity was represented as units/mg protein (52).

Determination of SOD activity

SOD activity was determined using a spectrophotometric method according to the procedure described by Dhindsa et al. (53). Accordingly, 1.0 mg of SOD (3000 units) was dissolved in 10 ml of dH2O to prepare a stock solution. For the preparation of samples for the assay of SOD activity, 5 ml of enzyme sample was added to the solution, including 0,1 ml of 1.5 M Na2CO3, 0.2 ml of 200 mM methionine, 0.1 ml of 2.25 mM NBT, 0.1 ml of 3 mM EDTA, 1,5 ml of 100 mM PBS (pH 7.5), and 1 ml of dH2O. Then, 0.1 ml of 60 mM riboflavin solution was added. The prepared enzyme samples were kept under the fluorescence light for 15 minutes and then, the reactions were stopped by keeping the samples in a dark place. The absorbance of the samples was measured at the wavelength of 560 nm against the blank. Enzyme samples were kept on ice until the treatment (54).

Statistical Analysis

The obtained results will be analyzed using SPSS 16 software and ANOVA test and if there is any significance, Tukey post hoc test will be used. p Value less than 0.05 ($P < 0.05$) considered as the level of significance of the difference.

Results

Comparison of the average time spent in open arms of the EPM

The results of the one-way ANOVA and Tukey's test showed that the time spent in open arms in the HFD group was significantly shorter than the time spent in open arms of the EPM in the control ($p < 0.01$) and HFD + extract ($p < 0.05$) groups. Also, this time was much longer in the extract group than in the control group ($p < 0.05$). These results show that the HFD increased anxiety in rats and the extract reduced anxiety in both the extract and HFD + extract groups (Fig. 2).

Comparison of the average number of entries into the open arms of the EPM

The results of the one-way ANOVA and Tukey's test showed that the number of entries into the open arms of the EPM in the HFD group was significantly lower than the control group ($p < 0.05$). Also, the number of open arms entries in the extract group was significantly higher than in the control group ($p < 0.05$).

Meanwhile, the number of open arms entries in the HFD + extract group increased significantly compared with the HFD group ($p < 0.05$; Fig. 3).

Compare the total number of entrances to the open and closed arms of the EPM

According to the results of the one-way ANOVA and Tukey's test, regarding the total number of entrances into open and closed arms of the EPM, the mean number of entrances in the HFD ($p < 0.01$) and HFD + extract ($P < 0.05$) groups decreased significantly compared with the extract group (Fig. 4).

Comparison of the GPX activity between the HFD and extract groups

The results of the one-way ANOVA and Tukey's test showed that the activity of this enzyme in the HFD group was significantly lower than in the control group ($p < 0.05$). Also, GPX activity in the extract group was significantly higher than in the HFD group ($p < 0.01$; Fig. 5).

Comparison of SOD activity between the HFD and extract groups

According to the results of the one-way ANOVA and Tukey test, the SOD activity in the HFD group was significantly lower than in the control group ($p < 0.05$). Also, SOD activity in the extract group was significantly higher than in the HFD group ($p < 0.05$) (Fig. 6).

Comparison of the level of corticosterone between the HFD and extract groups

The results of one-way ANOVA and Tukey's test showed that the corticosterone level in the HFD group was significantly higher than the control group ($p < 0.01$). Also, the corticosterone level in the extract group was significantly lower than in the HFD group ($p < 0.01$) (Fig. 7).

Discussion

In the present study, consumption of a HFD in the HFD group reduced the number of entrances into the open arms and reduced the duration of stay in the open arms of the EPM, which means an increase in anxiety. Also, following the consumption of the extract in the HFD + extract group, the number of entrances into the open arms and the length of stay in the open arms of the EPM system increased. Also, in the extract group, these two indicators increased, which indicates the anti-anxiety effect of this extract. The results of this study also showed that the activity of GPX and SOD enzymes (as an indicator of antioxidant activity) was lower in group HFD than in the control group. In addition, the level of serum corticosterone in the HFD group was higher than the control group and in the HFD + extract group was significantly lower than the HFD group.

Medicinal herbs have long been employed because of their main medicinal substances (55–59). The extract of *R. damascene* has 22 main compounds, of which anthocyanins, cyanidin 3, and 5-diglucoside account for more than 95% of anthocyanins and it also contains kaempferol and quercetin (60, 61). Several studies have shown that some flavonoids are a new family of ligands, which have a selective affinity for benzodiazepine receptors; therefore, have anti-anxiety properties (62, 63). Also, *R. damascene*, due to its flavonoids, such as quercetin could prevent oxidative stress damage by intervening with high nitric oxide. Also, by preventing the oxidation of LDL, it prevented irreversible damage to the cell membrane. Flavonoids, such as quercetin also reduce inflammatory factors by inhibiting both lipoxygenase and cyclooxygenase pathways, thereby reducing anxiety (64). Studies have shown that the small amount of *R. damascene* oil reduces anxiety differently from benzodiazepines and also has a different mechanism compared with diazepam (65). Citronellol, 2-phenethyl alcohol, and geranium are other compounds available in *R. damascene*, of which 2-phenethyl alcohol and citronellol have anti-inflammatory properties, and geranium, (66) due to its estrogenic properties, can reduce anxiety (67). Finally, *R. damascene* oil represents the properties of modern anti-anxiety drugs, such as serotonergic agents than the properties of benzodiazepines (68). The results of our study showed that the activity of two GPX and SOD enzymes decreased in the HFD group. However, in the extract group, the activity of these two enzymes increased compared with the HFD group. Our results are consistent with a study, in which an association was reported between oxidative stress and anxiety, and the overexpression of glutathione reductase-1 and glyoxalase 1 in the cortex of the cingulate resulted in increased anxiety-like behaviors, while inhibition of glyoxalase 1 expression decreased anxiety behavior. Thus, the association between the antioxidant status of the brain and anxiety-related behavior became apparent (23). In this regard, a HFD leads to anxiety-like behavior with a sharp decrease in serum glutathione levels, because a decrease in glutathione was reported to be associated with increased anxiety (69).

Our results are consistent with those reported by Moon et al., who showed that consuming a HFD for 4 months, and the resulting obesity, increased protein oxidation in the frontal cortex and increased oxidative stress parameters, resulting in anxiety-like behaviors (70). Also, the results of this study are consistent with a study by Del Rosario et al., in which a diet high in saturated fat reduced blood glutathione while increased anxiety, and consuming this diet along with bamboo extract reduced anxiety in rats through decreased inflammatory cytokines and increased glutathione, indicating the anxiolytic effect of the bamboo extract. This regimen was prescribed for 2 months (25). In this regard, it was found that there is a close relationship between intracellular ROS in peripheral blood cells (lymphocytes, monocytes, and granulocytes) and anxiety-related behaviors (24). Contrary to our study, Prasad et al. showed that consuming a HFD for a short time (one week) reduced the anxiety response in rats fed with a HFD. In this study, the possible role of secondary increase in corticosterone in the blood and its effect on the reduction of corticotropin-releasing hormone (CRH) in the hypothalamic-pituitary-adrenal (HPA) axis leading to reduced anxiety were shown (71). The results of our study showed that the level of serum corticosterone in the HFD group was higher than the control group, whereas in the HFD + extract group was significantly lower than the HFD group. Numerous studies have shown a relationship between corticosteroids levels and the levels of anxiety (72–75). In this regard, the role of corticosterone in

anxiety- and depressive-like behavior and HPA regulation following prenatal alcohol exposure has been reported (76). The effects of metyrapone as a corticosterone synthesis inhibitor on anxiety-related behaviors in Lurcher mutant mice have been studied. According to the result of this experiment, it seems that the behavioral disinhibition of the mutants is caused in part by their stress-provoked high corticosterone levels (77).

Most studies have suggested that anxiety is controlled by both the GABAergic and serotonergic systems (78, 79), and it was also found that anxiety levels in offspring of primates whose mothers consumed a HFD during pregnancy were higher than the control group and this increase in anxiety was due to disturbance in the serotonergic system of the brain and decreased serotonergic tone (80). It has also been shown that a HFD for 3 weeks induced anxiety-like behavior by reducing BDNF in the striatum and frontal cortex, as well as reducing neuropeptide Y levels. On the other hand, a diet containing omega-3 unsaturated fatty acids increased BDNF levels and showed beneficial effects on preventing anxiety and depression (81).

It has also been shown that *R. damascene* compounds, such as geraniol, citronellol, nerol, and phenylethyl alcohol, and especially phenylethyl alcohol have a positive effect on the inhibition of acetylcholinesterase and their strong antioxidant properties (82). Quercetin has been shown to be a flavonoid found in *R. damascene* with a positive effect on the formation of new dendrites and the volume and density of the hippocampus and causes neurogenesis in the hippocampus (39). Given that the 7-week HFD reduced the number of new cells produced in the dentate gyrus of the hippocampus, it disrupted neurogenesis in the hippocampus and increased LPO in the brain (8). Accordingly, the effects of the extract are more likely due to its high antioxidant properties or possibly inhibitory properties on the cholinesterase or its positive effect on the process of neurogenesis. In addition, the unexpected results of memory deficit in the extract group may be justified by the positive effect of ROS on cellular signaling involved in passive avoidance memory (83). Alzoubi et al. showed that a HFD for 6 weeks reduced SOD and catalase in the hippocampus and administration of vitamin E at a dose of 100 mg/kg at the same time prevent memory impairment by increasing the antioxidant capacity (84). Also, memory function was significantly impaired in rats receiving a HFD, and this diet caused oxidative stress and dysfunction and decreased the number of cholinergic neurons in the temporal cortex and hippocampus (61, 62). Accordingly, it can be concluded that the effects of extract may be due to its antioxidant properties, neutralization of free radicals, and inhibition of LPO due to oxidative stress.

Conclusion

In this study, the effect of *R. damascene* hydroalcoholic extract on anxiety in HFD rats was investigated. The used HFD increased anxiety. One of the suggested mechanisms for these changes is oxidative damage to the brain. Subsequently, *R. damascene* extract reduced anxiety in rats receiving a HFD as well as the rats of the extract group, which is probably due to flavonoids that reduce inflammatory factors or, more likely, through anti-inflammatory properties. These effects also are likely due to a strong antioxidant activity of the extract because the extract group was found with higher GPX and SOD activity. According

to the obtained results, although more studies are needed to confirm the findings, emphasis should be placed on its use in reducing anxiety. Also, its compounds should be further studied and used for the synthesis of new anti-anxiety drugs. However, the use of molecular techniques to study intracellular pathways to more accurately understand the effects of the extract on the process of anxiety in the conditions of HFD is suggested.

Declarations

Author contributions

Data collection: A.R.K., S.K, and I.S., Design of the study: A.K, and Z.G., Statistical analysis: A. R.K., S.K., and M.R., Analysis and interpretation of the data: M.R, and M.K.A., Drafting the manuscript: M.K.A, and Z.G., Critical revision of the manuscript: A.K., and I.S., Study supervision: A.K. All authors read and approved of the final manuscript.

Availability of data and material

All relevant data and material are within the manuscript and its Supporting Information files.

Declaration of competing interest

The authors declare that they have no known competing financial interests in this work.

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Compliance with Ethical Standards

Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical Approval

Animal care processes were approved by the Veterinary Ethics Committee of the Hamadan University of Medical Science (Ethics Code: IR.UMSHA.REC.1394.9404232209) and were accomplished consistent with the Guidelines of the National Institutes of Health (NIH Publication 80-23, 1996).

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Figures

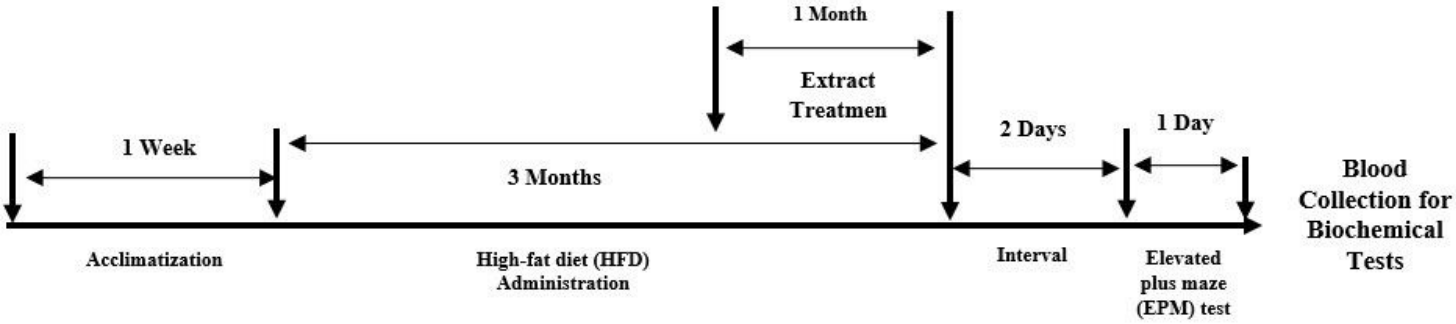

Figure 1

Timeline of the experiment

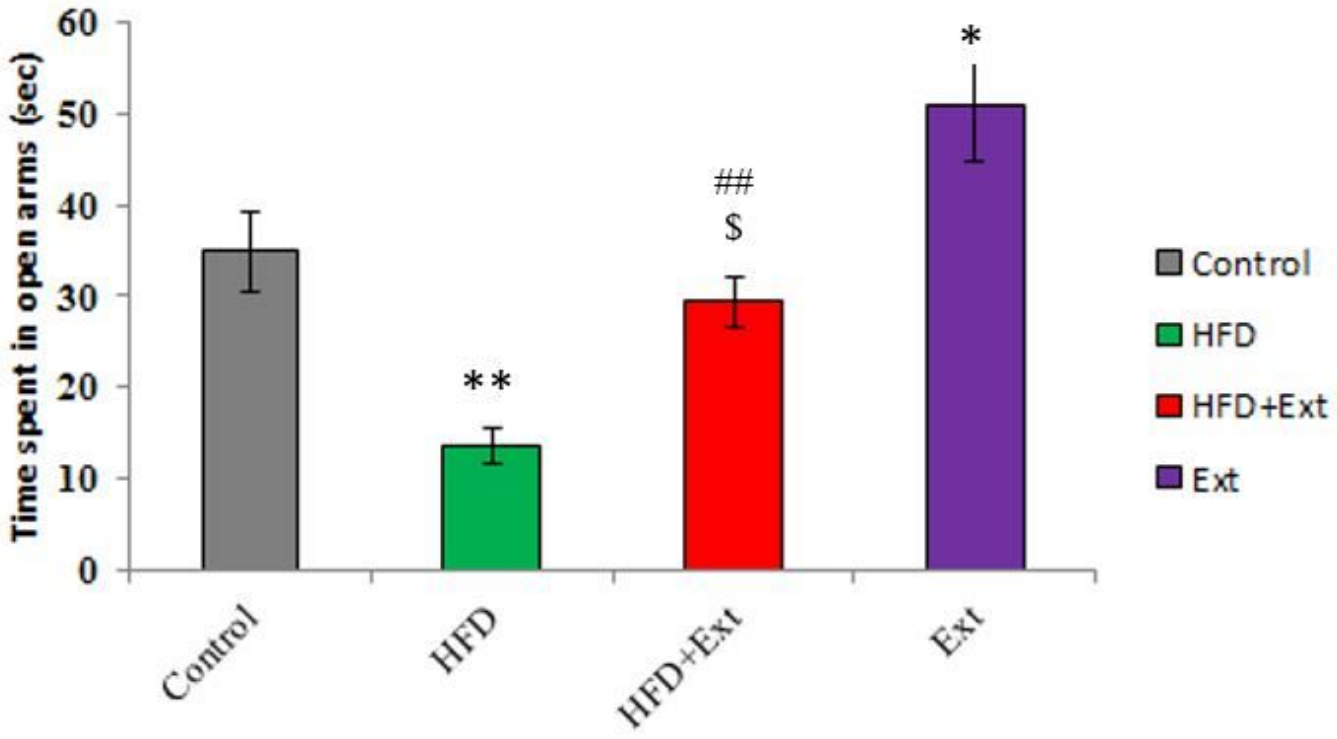

Figure 2

Average time spent in open arms of elevated plus-maze (EPM) in different groups. Control group, Extract (Ext) group, high-fat diet (HFD) group, and HFD+extract group (HFD+Ext). ** HFD group in comparison with the control group ($p < 0.01$); * Extract group in comparison with the control group ($p < 0.05$); ## HFD + extract group in comparison with the extract group ($p < 0.01$); and $ HFD + extract group in comparison with the HFD group ($p < 0.05$).

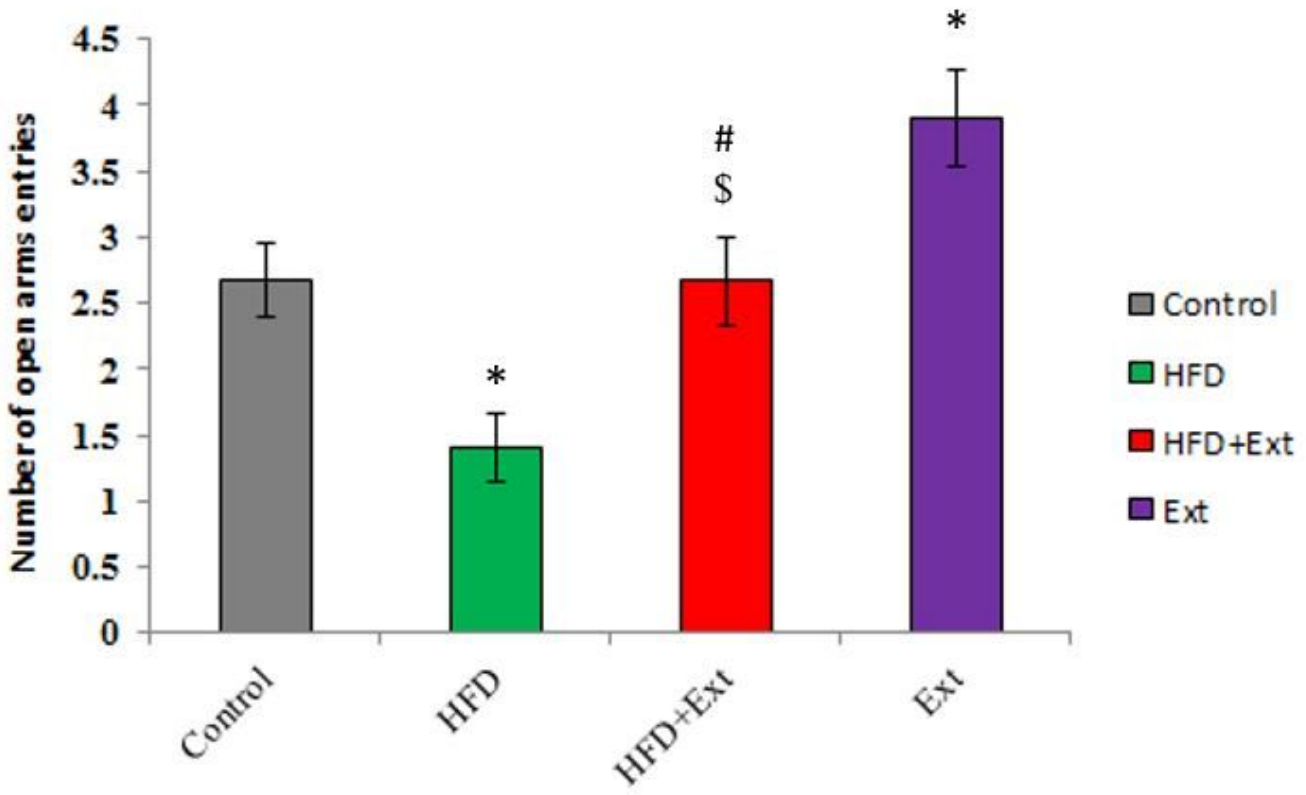

Figure 3

The average number of entrances into the open arms of the elevated plus-maze (EPM) in different groups. Control group, Extract (Ext) group, high-fat diet (HFD) group, and HFD+extract group (HFD+Ext). * In comparison with the control group ($p < 0.05$); # HFD + extract group in comparison with the extract group ($p < 0.05$); and $ HFD + extract group in comparison with the HFD group ($p < 0.05$).

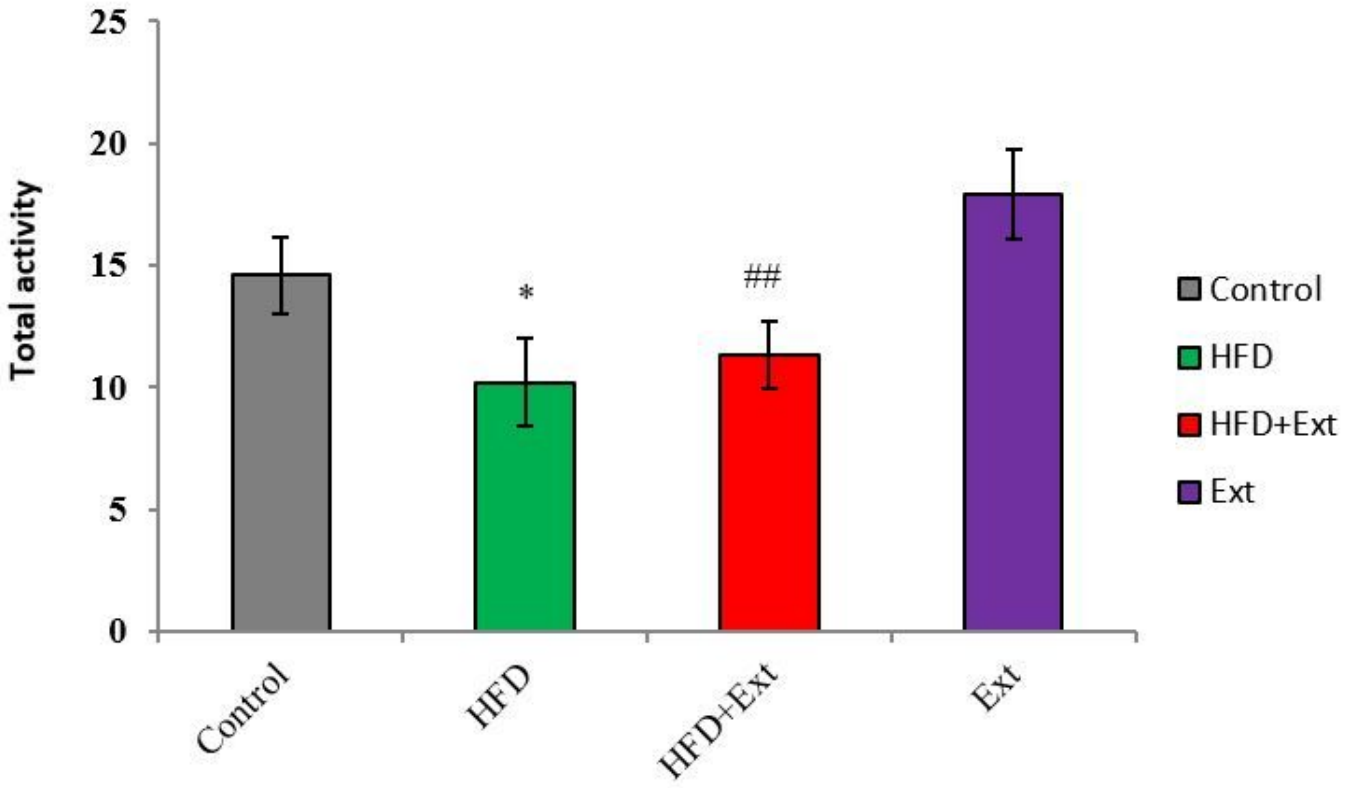

Figure 4

Comparison of the total number of entrances into the open and closed arms of the elevated plus-maze (EPM) in different groups: Control group, Extract (Ext) group, high-fat diet (HFD) group, and HFD+extract group (HFD+Ext). * HFD group in comparison with the control group ($p < 0.05$) and ## HFD + extract group in comparison with the extract group ($p < 0.01$).

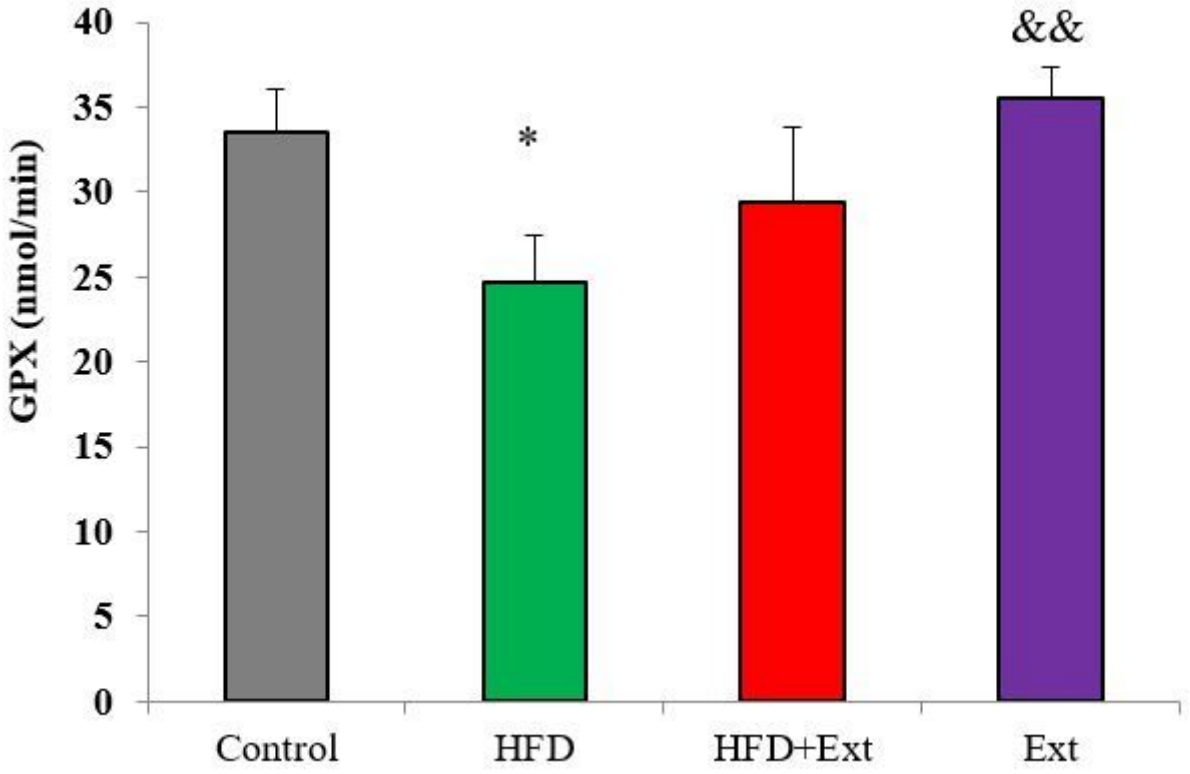

Figure 5

Glutathione peroxidase (GPX) activity in different groups. Control group, Extract (Ext) group, high-fat diet (HFD) group, and HFD+extract group (HFD+Ext). * In comparison with the control group ($p < 0.05$) and && Extract group in comparison with the HFD group ($p < 0.01$).

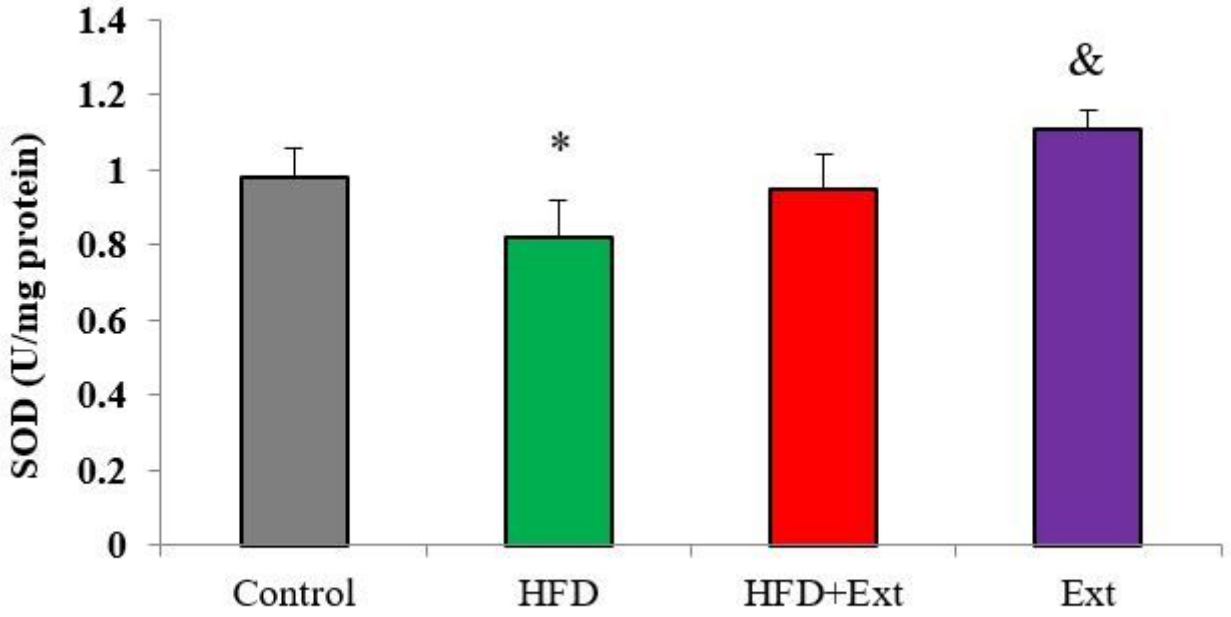

Figure 6

Superoxide dismutase (SOD) activity in different groups. Control group, Extract (Ext) group, high-fat diet (HFD) group, and HFD+extract group (HFD+Ext). * In comparison with the control group ($p < 0.05$) and & Extract group in comparison with the HFD group ($p < 0.05$).

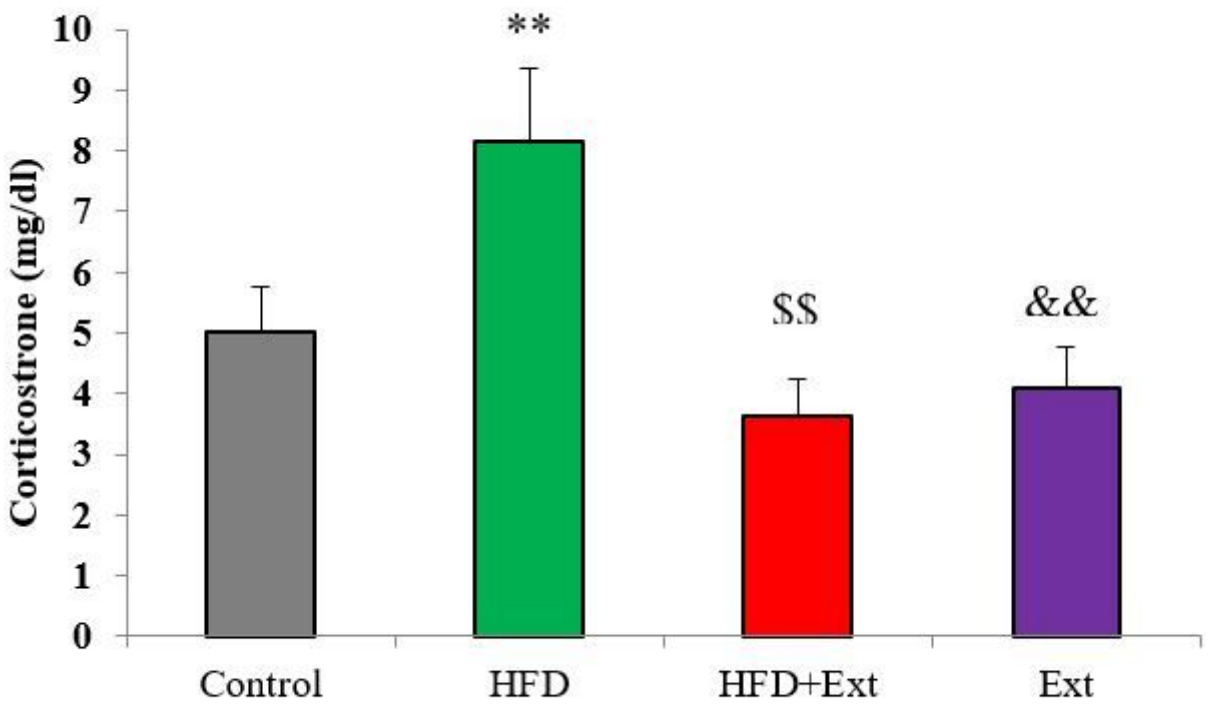

Figure 7

Serum corticosterone levels in different groups. Control group, Extract (Ext) group, high-fat diet (HFD) group, and HFD+extract group (HFD+Ext). * In comparison with the control group ($p < 0.05$); && Extract group in comparison with the HFD group ($p < 0.01$); and $ HFD + extract group in comparison with the HFD group ($p < 0.05$).