

Original Paper:

The Effect of Adiantum Capillus-veneris L. Hydroalcoholic Extract on the Oxidative Stress Rate of Mice's Blood and Brain in the Depression Model Caused by Acute Immobilization Stress



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ABSTRACT

Background: The oxidant-antioxidants balance in the living organism is constantly challenged by internal and external pressures. Maidenhair or Adiantum capillus-veneris (Acv) is rich in bioactive compounds with antioxidant effects.

Objectives: The present study aimed at investigating the effect of Acv hydroalcoholic extract on the oxidative stress rate of blood and brain of mice in the depression model caused by acute immobilization stress.

Methods: In this study, 40 male Balb/C mice were randomly divided into five groups, including 1 (control, 2, 3, and 4) intervention (receiving doses of 100, 200, and 400 Acv extracts) and diazepam group. Acute stress was induced by motion limitation (2 hours) and electrochemical shock (0.5 mA, 2 min), and then the mice were treated intraperitoneally with the extract or drug for 21 days. First, the rate of depression was assessed by forced swimming. Then, the Total Antioxidant Capacity (TAC), serum Malondialdehyde (MDA), and the MDA level of the brain were determined.

Results: The prescription of different doses of Acv extract and diazepam significantly reduced the duration of immobilization in the forced swimming test compared with the control group ($P < 0.05$). Besides, Acv extract at different doses of 200 and 400 significantly increased serum FRAP (TAC) and significantly increased TAC of the brain compared with the control group. Administration of Acv extract at different doses of 200 and 400 and diazepam significantly decreased serum MDA but significantly decreased MDA of the brain of mice compared with the control group ($P < 0.05$).

Conclusion: Acv extract can reduce the symptoms of depression and protect against acute stress-related oxidative stressors.

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Introduction

Stress is a condition caused by physical and psychological pressures. In other words, stress is the response of the individual to situations that threaten the environment [1]. Also, stress is defined as a set of general reactions to external factors which are inconsistent or unexpected, or a disruption to the system and adaptation of the body to the external environment. Based on this definition, if the balance and adaptability of organisms disappear due to external factors, they will become stressed [2]. Anxiety and depression are two psychosocial illnesses with significant comorbidity and their prevalence is increasing in the international community [3]. Monoamines play an important role in the pathophysiology of depression and anxiety, in such a way that the selection of medicine that changes the activity of the neuromuscular system of monoamines determines the treatment of depression and anxiety [4].

Homeostasis is constantly challenged by internal and external stressors [5]. The excessive production of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) is stimulated under stress conditions causing a disturbance in the balance between oxidants and the antioxidant defense system and ultimately oxidative stress. In such a condition, the destructive effects of oxidants are revealed. Some destructive effects of oxidants can be the killing healthy cells, increased production of pro-inflammatory cytokines, oxidative degradation of DNA, activation of certain genes, inactivation of proteins and enzymes, oxidation of sugars and fats, and especially the production of unsaturated fatty acids and cell membrane lipoproteins [6]. Oxidative stress is involved in many degenerative diseases, including neurodegenerative diseases (Alzheimer, Parkinson, Huntington, amyotrophic lateral sclerosis, multiple sclerosis, and other aging processes), diabetes, atherosclerosis, arthritis, inflammation, and various cancers [7].

Several studies proved that exposure to stressors weakens the antioxidant defense system and increases the production of free radicals [5, 6, 8]. Plants are a rich source of terpene, phenol, flavonoids, tannins, and anthocyanin, which are the most important natural antioxidants. It has been observed that the use of a diet containing herbal compounds can reduce the oxidative damage caused by acute stress [8].

Maidenhair or *Adiantum capillus-veneris* (Acv) is a species of ferns belonging to the Pteridaceae family and the genus *Adiantum*. *Persiaoshan* has narrow stems and

tiny leave and is located in wet areas rich in organic compounds and the margins of rivers and streams [9]. This plant is grown in southern Europe, the Alps, the Atlantic coast, and the northern and southern parts of Iran. Acv is used in Iranian traditional medicine as an anesthetic, anti-febrile dysplastic, and diuretic medicine and is widely used in the treatment of respiratory diseases and digestive disorders [10].

Laboratory studies indicated antimicrobial [9], analgesic [11], anti-inflammatory [11], and anti-oxidant [12] effects of Acv. Phytochemical analyses indicated the presence of flavonoids, alkaloids, tannins, saponins, terpenoids, glycosides, steroids, and reducing sugars in the plant extract [9]. Active compounds in this plant include rutin, quercetin, quercetin-3-O-glucoside, nicotine fluorine, niacin, astragaloside, procyanidin, camphorol-3 sulfate, prodelphinidin, and saponin [13]. Some of these compounds including rutin, quercetin, and geranium showed antioxidant effects in vitro and animal models [14-16]. The in vivo effects of Acv on the central nervous system, the characteristics of oxidative stress, and antioxidant activity were studied by some researchers [17-21]. Also, the activity of antibacterial Acv was investigated by some authors in the in vitro condition [22-24].

Although another study investigated the protective effect of Acv hydroalcoholic extract on depression and anxiety due to chronic stress in adult male mice [25], since the antioxidant effect of Acv extract in the living creature has not been studied, this study was designed to evaluate the effect of Acv extract on the oxidative stress rate of blood and brain of mice in the depression model caused by acute immobilization stress.

Materials and Methods

Preparation of drugs

Acetic acid, Thiobarbituric Acid (TBA), Sodium Dodecyl Sulfate (SDS), FeCl₃.6H₂O, 2,4,6-Tri (2-pyridyl)-s-triazine (TPTZ) and other reagents were purchased from Sigma-Aldrich Chemical Co. (USA) and Merck Co. (Germany).

Determination of radical scavenging activity of Acv extract

Acv extracts (100, 200, 400 µg/mL) were first prepared and an equal amount of the DPPH (2,2-diphenyl-1-picrylhydrazyl) solution (1 mg/mL) was added to Acv in all concentrations. The resulting solution was kept in the dark at room temperature for 15 minutes. Finally, the op-

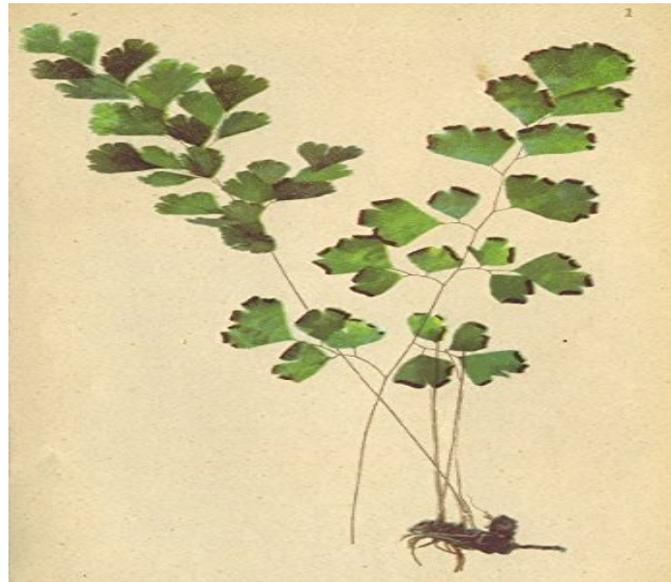


Figure 1. Forced swimming test

tical absorbance was measured at 517 nm using a spectrophotometer and then the activity of the DPPH radical inhibition was calculated (Formula 1).

$$1. IC_{50} (\%) = (A_{control} - A_{sample}) / A_{control} \times 100$$

where IC₅₀ is the concentration of the solution in which 50% of the DPPH radical is scavenged [26].

Ethics approval and consent to participate

This experimental study was conducted in the Experimental Animal Unit of Islamic Azad University of Izeh, Khuzestan, Iran. All animal procedures were based on the guideline for the Care and Use of Laboratory Animals. The study was reviewed by the Research Committee of Islamic Azad University of Izeh and approved by Code: 15330557962002. A standard powdered purified diet (Dampars Co, Iran) was used, which consisted of 7% protein, 20%-30% carbohydrates, 2% vegetable fat, and 10%-12% fiber. In this experimental study, 32 male Balb/C mice (25-30 g, 6-8 week) were obtained from the Animal Breeding Facility Centre of Pasteur Institute, Karaj, Iran. The mice were kept in standard conditions (21±2°C and 12:12 h light/dark cycle) with free access to water and food of the same type. The mice were randomly divided into five groups (n=8 per group): One control group under the stress of normal saline recipient, three intervention groups under the acute stress of receiving Acv extract at doses of 100, 200, and 400 mg/kg [27] and one diazepam group. To create acute stress, a standard immobilization technique and an electric shock were used. The mice were placed in a restrictive device for water,

food, and movement (Restrainer) (BorjSanat Company, Iran) for two hours. Afterward, the mice were given 0.5 mA electric shock for two minutes (each 1 second with 10 seconds of rest between each one). This process was performed for acute stress [28]. Then, the mice were treated with normal saline or extract by intraperitoneal injection for 21 days. Next, depression was assessed by forced swimming test [29]. After performing behavioral testing, the animals were subsequently put under deep anesthesia (under ether: Hakim Pharmacy, Iran). Then, their cardiac blood samples were collected, and their brains were removed. After the removal of the brain, the hippocampus, cortex, and sub-cortex were separated on ice and used for biochemical analyses. Their collected blood was centrifuged and the plasma separated was used for biochemical analyses [30].

Preparation of herbal extracts

Acv was bought from a valid herbal shop in Izeh, Khuzestan in summer. The plant was collected from Khuzestan Province (Figure 1).after the systematic verification by a botanist, the herbarium sample of this plant was registered in Herbarium (No. 7543) of Islamic Azad University, Izeh Branch, Khuzestan. To prepare the extract, we poured 1 kg of dried plant powder into 70% ethanol and placed on a shaker for two days at room temperature. Then, the obtained mixture was filtered and the solvent was removed by rotary evaporator (Heidolph Co. Germany), and Acv extract was obtained (yield 20%-25%). The extract was completely dried at 40°C and used to prepare the required concentrations. The ex-

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tract was stored at -20°C [31]. The extract was dissolved in distilled water and given to the animals.

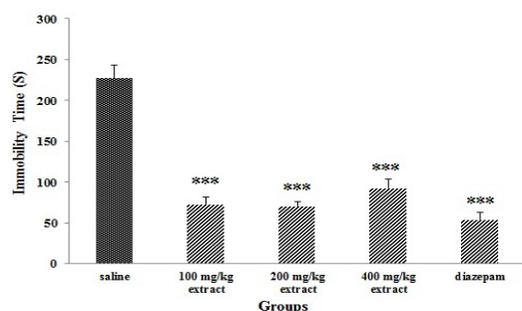
This test is one of the most reliable and commonly used examinations to test depression. In this method, a glass container with a length of 25 cm, a width of 12 cm, and a height of 15 cm were used. The dish was filled with water with a temperature of 25°C and the mouse was gently immersed in water. The discontinuation of the movement by the mouse's limbs was considered as immobilization. The experiment time was 10 minutes and the first 2 minutes was considered the adaptation of the animal to existing conditions and the immobilization time was measured for the following 8 minutes [32].

The measurement of malondialdehyde of the brain and serum

A total of 100 μL of serum samples or homogeneous tissue was added with 1.5 mL of 20% acidity, 1.5 mL of 0.8% TBA, 0.8% of Sodium Dodecyl Sulfate (SDS) 1.8%, and 700 μL of distilled water to the test tubes. The tubes were placed in boiling water for 60 minutes and then 1 mL of distilled water and 5 mL of butanol or pyridine was added to the samples. Then, they were centrifuged and the light absorbance of the supernatant was measured at 532 nm [33].

The measurement of the total antioxidant capacity of the brain and serum

To measure the antioxidant capacity of serum and tissue, we used FRAP method with three solutions: buffer (1.55 mL of sodium acetate and 8 mL of concentrated acetic acid, distilled water with a volume of 500 mL), iron chloride solution (270 mg $6\text{H}_2\text{OFeCl}_3$, distilled



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Figure 2. Comparing the immobilization time (s) in forced swimming test in the acute stress-induced groups of saline recipients, different doses of Acv extract

* Indicates a significant difference with the control group (saline);

*** $P < 0.001$

water with a volume of 50 mL, and a solution of triazine (47 mg of triazine dissolved in 40 mL of 40 mM HCl). The final solution was prepared by adding 10 mL of solution one, 1 mL of solution two, and 1 mL of solution three. About 25 μL of the serum or homogeneous tissue samples were added to 1.5 mL of the solution and placed on the object for 10 minutes at 37°C . Then, the optical absorption at 593 wavelengths was recorded [33].

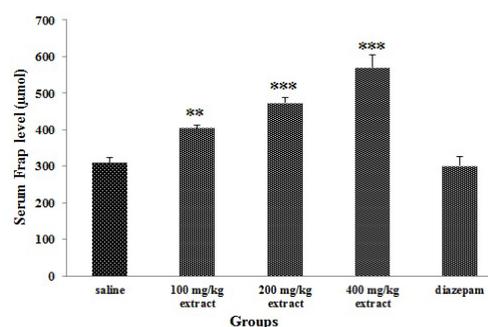
Statistical analysis

The resulting data were analyzed in SPSS version 21. Considering that the obtained results were quantitative, the assumption of normal distribution of the frequency of data was confirmed by the Kolmogorov-Smirnov non-parametric test ($P > 0.05$). The data were also analyzed by one-way ANOVA and the post hoc Least Significant Difference (LSD) tests. Also, the obtained results were reported together with the corresponding statistical calculations as Mean \pm SEM. In all cases, the difference among groups was considered significant with $P < 0.05$.

Results

DPPH radical scavenging activities of *A. capillus-veneris* extract:

The results demonstrated that the anti-radical activity of Acv extract rose with increasing its concentration. Besides, the IC_{50} of Acv extract was obtained 54.61 $\mu\text{g}/\text{mL}$. IC_{50} was directly correlated with Acv extract antioxidant activity.



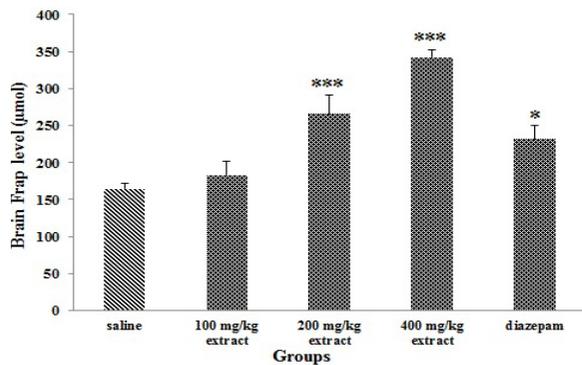
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Figure 3. Comparing the serum antioxidant capacity (FRAP) in the acute stress-induced groups of saline recipients, different doses of Acv extract

* Indicates a significant difference with the control group (saline);

** $P < 0.001$;

*** $P < 0.001$



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Figure 4. Effect of Acv extract at different doses on brain frap levels in the acute stress-induced mice

* Indicates a significant difference with the control group (saline);

*** P<0.001

Results of animals

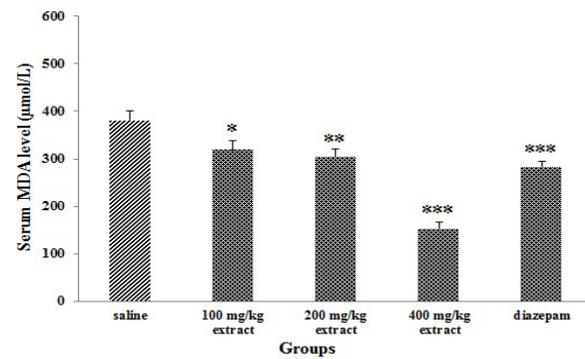
The duration of immobilization (seconds) in the forced swimming test in different groups under acute stress was investigated. The groups receiving the different doses of the extract and the diazepam showed a significant decrease in immobility period compared with the control group (saline) (P=0.000) (Figure 2).

The treatment of acute stress-induced mice by Acv at doses of 100, 200, 400 mg/kg resulted in a significant increase in serum antioxidant capacity compared with the control group (P=0.002, P=0.000) (Figure 3).

The treatment of acute stress-induced mice by Acv at doses of 200 and 400 mg/kg and diazepam significantly increased brain antioxidant capacity (P<0.05) but treatment by a dose of 100 mg/kg extract showed no significant effect on the duration of animal immobilization in the forced swimming test (P=0.444) (Figure 4).

The results of the serum and brain MDA levels in the groups studied are shown in Figures 5 and 6. The treatment of acute stress-induced mice by Acv at doses of 100, 200, 400 mg/kg and diazepam resulted in a significant decrease in serum MDA compared to the normal saline group (P=0.013, P=0.002, P=0.000) (Figure 5).

The treatment of acute stress-induced mice by Acv at doses of 200 and 400 mg significantly reduced MDA in the brain (P=0.044, P=0.026) but did not have a significant effect at a dose of 100 mg/kg (P=0.642) (Figure 6).



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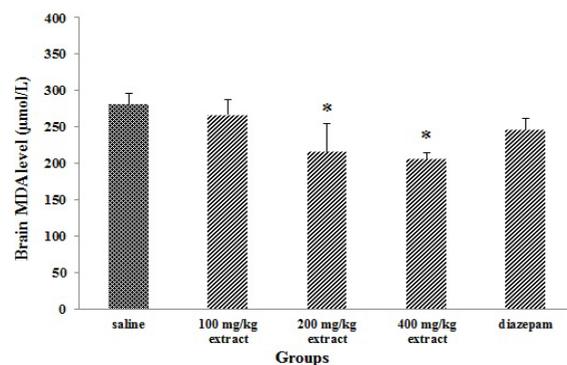
Figure 5. Comparing the serum Malondialdehyde (MDA) in the acute stress-induced groups of saline recipients, different doses of Acv extract

* Indicates a significant difference with the control group (saline);

* P<0.05; ** P<0.001; *** P<0.001

Discussion

The present study aimed to investigate the effect of Acv extract on depression and oxidative stress indices after acute stress exposure. The results showed that the immobilization time as an indicator of depression significantly decreased in mice receiving Acv extract. The serum and brain antioxidant capacity in mice exposed to acute stress was significantly higher than those of the control group receiving saline. Besides, the levels of serum and brain malondialdehyde in the acute stress-induced groups were significantly lower than that of saline recipients. These results indicated the beneficial effects of Acv extract against oxidative stress caused by acute stress.



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Figure 6. Comparing the brain Malondialdehyde (MDA) in the acute stress-induced groups of saline recipients, different doses of Acv extract

* Indicates a significant difference with the control group (saline);

* P<0.05

In general, the precise mechanism of increasing oxidative stress indices after exposure to acute stress has not been properly explained. The researchers believe that the sharp increase in the activity of the HPA axis and the levels of glucocorticoids in acute stress play some role. It has been observed that cellular contact with high levels of glucocorticoids, due to acute stress, increases the production of ROS and RNS and inhibits the dehydrogenation capacity of the enzyme endogenous antioxidants (SOD, GPX, and CAT) and non-enzymatic (glutathione) in the brain, and therefore the neural cells become susceptible to the adverse effects of ROS and RNS [6]. The increase in oxidative stress indices following acute stress has been shown in several animal studies. The exposure to acute stress increases the index of oxidative stress in the gastrointestinal tract in mice [34]. Besides, in the rats exposed to acute stress for 6 hours, an increase in oxidative stress indices was observed with increasing Nitric Oxide (NO) and induced Nitric Oxide (iNOS) [6]. In another study, the exposure of rats to acute stress has reduced the antioxidant potential of the liver, kidney, heart, and serum [5].

In general, the best and most common method for measuring free radicals and oxidative stress is to determine the products derived from the reaction of free radicals with biological molecules as biomarkers [35]. The goals of ROSs are proteins, lipids, and nucleic acids, in which their metabolites are used as the oxidative stress biomarkers in a variety of diseases [36]. Oxidative stress biomarkers are clinically prominent and the study of their rate is used in blood, urine and other body fluids to determine pathological conditions and to diagnose diseases [37]. One of the primary targets of ROSs is the fatty acids with more than one double bond or polyunsaturated fatty acids in the cell membrane, causing the oxidation of lipid or more than a dual bond through the process of lipid peroxidation. The result is lipid peroxidation of metabolites, such as MDA, which causes an alteration in cellular receptor structure and cellular damage by binding to proteins and altering their function, creating enzymatic inhibition [14].

In the present study, MDA was used as an indicator of lipid peroxidation and oxidative stress, and it was observed that treatment by Acv reduced its levels in mice under acute stress. In the present study, the protective effects of Acv extract against chronic stress-induced depression can be due to the fighting the adverse effects of oxidative stress and boosting the antioxidant defense system. Therefore, in mice under stress, Acv extract significantly reduced the serum and brain MDA levels, as a lipid peroxidation marker, and significantly increased antioxidant capacity.

It seems that the plant's activity in reducing the symptoms of depression is due to the presence of flavonoids and phenolic compounds with biological and neuroprotective properties. We found no study that investigated the antioxidant effects of Acv extract in animal models, but the antioxidant effects of this herb were observed in several *in vitro* [12] and cell culture [34] studies. Acv extract is rich in flavonoids such as rutin, quercetin, quercetin-3-O-glucoside, nicotine fluorine, astragaloside, procyanidin, camphorol-3 sulfate, pro-delphinidin, and Paponin [13].

In rats with subarachnoid hemorrhage, quercetin treatment reduces the oxidative stress indices by reducing MDA of the brain and increasing the activity of antioxidant enzymes [38]. Furthermore, quercetin decreased oxidative stress indices in mice with ischemia-induction and strengthened the antioxidant defense of the brain. Alzheimer and Parkinson rats treated with rutin have been reported to reduce the peroxidation of brain lipids and increase the activity of antioxidant enzymes and glutathione [15, 36]. Similar effects have also been reported by catechin, procyanidin [8], and camphorol [35] in animal models. It seems that Acv can cope with oxidative stress and enhance antioxidant defense due to the presence of flavonoid compounds with antioxidant effects.

Acv can improve the symptoms of depression and decrease the oxidative stress indices caused by exposure to acute stress through decreasing lipid peroxidation and enhancing antioxidant defense in mice.

Ethical Considerations

Compliance with ethical guidelines

All animal procedures were based on the guideline for the Care and Use of Laboratory Animals. The study was reviewed by the Research Committee of Islamic Azad University of Izeh and approved by Code: 15330557962002.

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This article was extracted from the MSc. thesis of Jafar Ahmadpouri in Department of Biology, Izeh Branch, Islamic Azad University, Izeh.

Authors' contributions

Conceived the study: Saeid Valipour Chahardahcharic and Mahbubeh Setorki; Collected the data: Jafar Ahmadpouri; Carried out data analysis: Saeid Valipour Chahardahcharic; Wrote the paper: Mahbubeh Setorki; Drafted and finalized the paper, read and approved the final manuscript: All authors.

Conflict of interest

The authors declare that there is no conflict of interest.

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