

Effects of *Biebersteinia multifida* DC. Root Extract on Physical Stamina in Male Mice

Mahsa Hadipour Jahromy^{1*}, Ahmad Jamshidi Mohajer¹, Leila Adibi², Shahrzad Khakpour¹

¹Herbal Pharmacology Research Centre, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran

²Biology Department, Faculty of Biological Sciences, Islamic Azad University, North Tehran Branch, Tehran, Iran

Email: [*Jahromymh@yahoo.com](mailto:Jahromymh@yahoo.com)

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Abstract

Biebersteinia multifida DC. has been used for the purpose of development of physical strength in Iranian folk medicine. In the present study, we investigated the effect of *Biebersteinia multifida* DC. root extract on performance of the Forced Swimming Test (FST), and on blood biochemical parameters related to fatigue: Blood Urea Nitrogen (BUN), Creatine Kinase (CK), Lactic Dehydrogenase (LDH), Glucose (Glc), and Total Protein (TP). *Biebersteinia multifida* DC. root extract was orally administered to mice at the doses 4, 7 and 10 mg/kg continuously once per day for 1 and 7 days using a feeding atraumatic needle. After 7 days, on FST the immobility time was decreased in the treated-groups at the doses 4 and 7 mg/kg, but after 1 day, only the immobility time of the treated-group at the dose of 4 mg/kg was decreased significantly. In addition, the content of Glc and TP in the blood serum was significantly increased in the groups receiving 4 and 7 mg/kg of *Biebersteinia multifida* DC. root extract for 7 days and LDH was decreased significantly. However, the levels of BUN and CK did not show a significant change. The results predict a potential benefit of *Biebersteinia multifida* DC. root extract as an anti-fatigue treatment and for improving physical stamina.

Keywords

Biebersteinia multifida DC., Forced Swimming Test, Physical Stamina

1. Introduction

Biebersteinia multifida DC. (Geraniaceae) is a common herb known in Iran as Adamak, with stem 20 - 70 cm

*Corresponding author.

long, leaf lacinate, flowers borne in a lax panicle, calyx hardened in fruit, petals usually yellowish and a little shorter than the sepals [1] [2] (**Figure 1**). The roots are thick and dense with a light to dark brown color. The root of this plant has been used locally in folk medicine of western region of Iran for the treatment of musculoskeletal disorders and restoring bone fractures. A few phytochemical and biological investigations have so far been carried out on this species. The anti-inflammatory and analgesic activity of *Biebersteinia multifida* DC. root extract have been reported, too [3]. Isolation of an alkaloid (vasicinone) and some polysaccharides and peptic substances from this species have been performed [4]-[6]. Zhang and colleagues (1995) [7] reported isolation of seven compounds from other species (*Biebersteinia heterostemon* Maxim.) of this genus with hypotensive, analgesic and immunity stimulating effects.

Forced swimming test (FST) is a behavioral test for rodents, which predicts the efficacy of antidepressant treatments [8]. This test induces the development of immobility as a reflection of helplessness when subjected to an inescapable situation (tank of deep water). In this paradigm; mice are placed in the tank for an extended period. After an initial swimming period, the animal exhibits immobility behavior considered a depression-like response. FST is used to examine whether certain agents have an anti-fatigue effect [9] and is also used as an endurance test [10] [11].

Blood Urea Nitrogen (BUN), Creatine Kinase (CK), Lactic Dehydrogenase (LDH), Glucose (Glc), and Total Protein (TP) are biochemical parameters related to fatigue. The BUN test is a routine test used primarily to evaluate renal function. Urea is formed in the liver as the end product of protein-metabolism. During digestion, protein is broken down into amino acids. Amino acids contain nitrogen which is removed as ammonium ion, and the rest of the molecule is used to produce energy for the cell. Serum CK and LDH are known to be accurate indicators of muscle damage [12] [13].

The normal function of CK in cells is to add a phosphate group to creatine, turning it into the high-energy molecule phosphocreatine. During the process of muscle degeneration, muscle cells break open and their contents find their way into the bloodstream. Because most of the CK in the body normally exists in muscle, an increase in the amount of CK in the blood indicates that muscle damage has occurred or is occurring. LDH catalyses the interconversion of pyruvate and lactate. Exercising muscles convert Glc to lactate. Lactate is released into the blood and is eventually taken up by the liver. The liver converts lactate back to Glc and releases Glc into



Figure 1. *Biebersteinia multifida* DC. (Geraniaceae) is a common herb known in Iran as Adamak.

the blood. Energy for exercise is derived from circulating Glc released by the liver. TP is a rough measure of serum protein. Protein measurements can reflect nutritional state, kidney disease and liver disease [14].

The aim of the present study is to examine the effect of *Biebersteinia multifida* DC. root extract on FST. In addition, the contents of BUN, CK, LDH, Glc and TP in the blood serum were measured.

2. Materials and Methods

2.1. Animals

Male BALB/c mice, weighing 20 - 25 g were used in these experiments. They were group housed under the following laboratory conditions: temperature $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$ humidity 40% - 60%, 12:12-L/D cycle, lights on at 07:00. Food and water were available *ad libitum*. All the experiments were carried out between 10:00 and 15:00 in testing rooms adjacent to the animal rooms. Each experimental group consisted of seven mice. Mice were treated in accordance with the current law and the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Plant Material

The dried root of the plant was obtained from Zandjan province, western region in Iran. The plant was authenticated in the Department of Pharmacognosy, Tehran University of Medical Sciences. A voucher specimen (No. B-37) was preserved at the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences. The roots were cleaned, air-dried and chopped into small pieces, powdered and stored in air-tightened, light protected containers.

2.3. Extraction Procedures

Powdered plant material (800 g) was mixed with 2 L ethanol at room temperature and refluxed for 2 days followed by filtration through filter paper. The filtrate was evaporated to dryness under reduced pressure and weighed (28.8 g, 3.6%).

2.4. Drug Administration

Male BALB/c mice weighing 20 - 25 g were used in this study. The animals were randomized into control and experimental groups and divided into eight groups of seven animals each. Animals in Groups 1 and 2 were administered with normal saline for one and seven days, respectively (0.9% NaCl). Animals in Groups 3, 4, 5, 6, 7 and 8 were administered with the extracts of *Biebersteinia multifida* DC. at the doses of 4, 7 and 10 mg/kg. All drugs were orally administered for 1 or 7 days, respectively. The behavioral tests were conducted 1 h after the last treatment. The results of acute toxicity studies showed LD50 to be greater than 100 mg/kg in mice.

2.5. Forced Swimming Test

Swimming sessions were conducted by placing mice into individual glass cylinders (46 cm height \times 20 cm diameter) containing 23°C - 25°C water 30 cm deep, so that mice could not support themselves by touching the bottom with their tail or paws. During the 6 min of the forced swimming test, the duration of immobility was measured as previously described by Porsolt, Bertin, and Jalfer (1977) [15]. The total duration of immobility, after a delay of 2 min, was measured during a period of 4 min. Each mouse was considered to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. We measured the immobility time at the 1 and 7 days after the administration of saline or *Biebersteinia multifida* DC. root extract.

2.6. Preparation and Ingredient Analysis of Blood Serum

Changes in blood biochemical parameters were measured in 7-day treated groups after the FST. Due to the observation of no further changes in immobility time in ground receiving 10 mg/kg of the extract, the blood analysis was not performed. Mice were anesthetized with an intra-peritoneal injection of Ketamine (80 mg/kg) and Xylazine (4 mg/kg). After anesthetization, blood was withdrawn from the heart of forced swimming-treated mice into syringes. Then serum was prepared by centrifugation at 3000 rpm at 4°C for 10 min. Contents of BUN,

CK, LDH, Glc and TP were determined by autoanalyzer (Hitachi 747, Hitachi, Japan).

2.7. Statistical Analysis

Results were expressed as the mean \pm S.E. The significance of the mean difference was determined by Student's *t*-test and Anova-Oneway for independent data. All statistical analyses were performed using Origin VI statistical analysis software. A value of $p < 0.05$, $p < 0.01$ was considered to indicate statistical significance.

3. Results

3.1. Effect of *Biebersteinia multifida* DC. Root Extract on Immobility

Male BALB/c mice weighing 20 - 25 g were used in this study. First, we investigated the effect of oral administration of *Biebersteinia multifida* DC. root extract at the doses 4, 7 and 10 mg/kg after 1 and 7 days on FST. Mice, when placed into the cylinders for the first time, swam around vigorously, apparently searching for an exit. After 2 min their activity subsided, replaced by periods of immobility of increasing duration where the mice passively remain floating in the water in a semi-horizontal position with their heads just above the water. The mice were divided into a control group and treated groups to match the swimming time in each group. As a result of test, the immobility time was decreased in the *Biebersteinia multifida* DC. root extract-treated groups at the doses 4 and 7 mg/kg after 7-day treatment, and after 1-day treatment, only the immobility of the treated group at the dose of 4 mg/kg was decreased significantly compared with controls, shown in **Figure 2**.

FST is generally used for assessing anti-depressive effects. Recently, the FST has been used as an anti-fatigue test and endurance test. As shown in **Figure 1**, the duration of immobility on the 1 and 7 days was shortened by feeding of *Biebersteinia multifida* DC. root extract. These phenomena suggest that the decreased duration of immobility in mice may be caused by a change of certain metabolites in the system.

3.2. Effect of *Biebersteinia multifida* DC. Root Extract on Blood Biochemical Parameters

In order to clarify its mechanisms, we assessed the levels of several blood biochemical parameters in mice after FST. Contents of BUN, CK, LDH, Glc and TP were determined by autoanalyzer. As shown in **Table 1**, when *Biebersteinia multifida* DC. root extract was administered at the doses 4 and 7 mg/kg for 7 days orally to mice, the Glc and TP levels significantly increased ($p < 0.05$) in the treated-groups receiving 4 and 7 mg/kg of root extract. Amount of CK increased in group receiving 4 mg/kg of the extract chronically. LDH was decreased significantly at both concentration administered, however, the levels of BUN did not show significant changes.

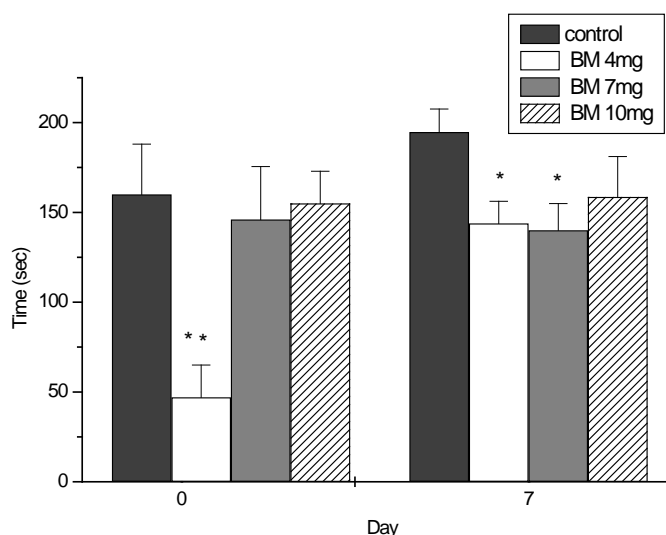


Figure 2. Effects of *Biebersteinia multifida* DC. root extract on forced swimming-induced immobility in mice after one and seven days; Values are the means \pm S.E. * $p < 0.05$, ** $p < 0.01$ versus saline-treated group.

Table 1. The levels of BUN, Glc, CK, TP and LDH in the serum after administration of *Biebersteinia multifida* DC. root extract (4 mg/kg and 7 mg/kg for 7 days) orally in mice. Each data value indicates the mean \pm S.E. * $p < 0.05$ versus control group.

	BUN (mg/dl)	Glc (mg/dl)	CK (U/l)	TP (g/l)	LDH (IU/l)
Control	54.42 \pm 4.87	285 \pm 17.89	1820 \pm 219	10.75 \pm 0.91	4247.14 \pm 276.42
Bieb.extract 4 mg/kg	59.14 \pm 5.9	364.57 \pm 29.1*	1980 \pm 229*	15.15 \pm 2.3*	3632.85 \pm 90.73*
Bieb.extract 7 mg/kg	58 \pm 3.17	374.42 \pm 9.75*	1879 \pm 185	14.04 \pm 1.63*	3081.42 \pm 181.69*

4. Discussion

The results of the present study suggest that *Biebersteinia multifida* DC. root extract may act as an energy source because the immobility time decreased in chronic administration at all concentration.

Anti-stress and anti-fatigue effect of fermented rice bran [9] and effects of L-Malate on physical stamina and activities of related enzymes [14] and many herbal medicines in folk remedies have been reported so far.

Herbkines consist of several medicinal herbs has been used for the purpose of development of physical strength. Performance of the Forced Swimming Test (FST) and on blood biochemical parameters related to fatigue: Blood Urea Nitrogen (BUN), Creatine Kinase (CK), Lactic Dehydrogenase (LDH), Glucose (Glc), and Total Protein (TP) were measured. On FST, the immobility time was decreased in the Herbkinines-fed group. In addition, the content of BUN in the blood serum was significantly decreased. However, the levels of CK, LDH, Glc, and TP did not show a significant change. Their results predicted positive effects of Herbkinines as an anti-fatigue treatment and for improving physical stamina [16].

In this research, in the treated-groups receiving 4 and 7 mg/kg of root extract the LDH level was decreased ($p < 0.05$), CK level was increased ($p < 0.05$) in the treated-group receiving 4 mg/kg of root extract and but the BUN level showed no change in the treated-groups compared with the control. CK and LDH are known to be accurate indicators of muscle damage [12]-[14].

In general, the swimming exercise is known to indicate blood biochemical changes. The BUN, CK, LDH, Glc, and TP are blood biochemical parameters related to fatigue. Therefore, our results indicate that the fatigue metabolism of mice was influenced by *Biebersteinia multifida* DC. root extract treatment. However, further studies are needed to clarify the detailed mechanisms involved in the anti-fatigue-like properties of *Biebersteinia multifida* DC. root extract in order to support present findings.

In conclusion, the *Biebersteinia multifida* DC. root extract-fed groups decreased their immobility time during FST, and the metabolites related to fatigue were changed.

5. Conclusion

Therefore, the present results suggest that *Biebersteinia multifida* DC. root extract may be useful for the development of physical strength. In addition, it might be suggested that *Biebersteinia multifida* DC. root extract has an anti-fatigue-like effect. However, this suggestion, based on our preliminary results, should be confirmed by further experimental studies.

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