Anti-fatigue Effects of the Genistein Supplementation in Mice Subjected to Forced Swimming Test

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Abstract. The present study was designed to evaluate the anti-fatigue effects of genistein in mice subjected to the forced swimming test. Mice were divided into four groups, i.e., one control group and three genistein treated groups. The treated groups were received doses of genistein (7, 15 and 30 mg/kg) dissolved in 1.0 mL of physiological saline, and the control group received same volume of physiological saline via oral gavage once a day. After 28 days, the mice were subjected to the forced swimming test, along with the determination of exhaustive swimming times and some biochemical parameters. The results showed that genistein could prolong exhaustive swimming time of mice, which was accompanied by decreases in blood lactic acid (BLA) and serum urea nitrogen (SUN) levels, increases in liver and muscle glycogen contents, and superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) levels in serum. This indicated that genistein had anti-fatigue effects.

Introduction

Fatigue can be defined as difficulty initiating or sustaining voluntary activity [1]. It is usually accompanied by a feeling of physical or mental tiredness, resulting from severe stress and hard physical or mental work [2]. With physical fatigue, exhaustion and free radical theories have attracted the most attention [3]. The exhaustion theory suggests that several energy sources, such as liver glycogen and muscle glycogen, exhausts during long exercises and the concentration of blood glucose reduces, leading to physical fatigue [4]. The free radical suggests that intense exercise can induce the production and accumulation of excess reactive free radicals, resulting in oxidative stress injury to the body, which can cause physical fatigue [5]. Since clinical drugs have limited therapies for physical fatigue and potential alternatives from natural antioxidant components are worth investigating. Recently, it has been reported that dietary antioxidants supplementation has a major role in reducing the degree of fatigue by the oxidation of inter or intra cellular oxidizable substrate [6].

Genistein (4',5,7-trihydroxyisoflavone, $C_{15}H_{10}O_5$), a naturally occurring soy is oflavone is a flavonoid in legumes and some herbal medicines [7]. Research has shown that genistein have diverse pharmaceutical properties including, antiinflammatory, neuroprotective, anti-apoptotic, anti-diabetic and estrogenic activities, as well as protective effects against bone loss and cardiovascular diseases [8]. Furthermore, genistein has also been proven to have significant antioxidant effects in vitro and in vivo [9], which suggests that genistein might be possible to delay fatigue. Thus, the present study was designed to evaluate the anti-fatigue properties of genistein in mice subjected to the forced swimming test.

Materials and Methods

Materials and Chemicals

Genistein (98.5% purity) was purchased from Zhenzhun Biological Technology Co., Ltd (Shanghai, China). The commercial diagnostic kits for the blood lactic acid (BLA), glycogen, superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) were purchased from Jiancheng Bioengineering Institute (Nanjing, China). The commercial diagnostic kit for the serum urea nitrogen (SUN) was purchased from Biosino Biotech Science Co. (Beijing, China). All other chemicals used were of the highest purity available.

Animals and Treatment

Kunming mice (Males, SPF, 20 ± 2 g) used in this study were obtained from the Hunan Biological Supplier (Changsha, China). The mice were housed in an animal room at constant temperature (23 ± 2 ℃) and humidity (50 ± 5%) under a 12-h light–12-h dark cycle at the Central South University (Changsha, China). The animals had free access to standard rat pellet diet and water *ad libitum*. The study had been carried out in compliance with the Principles of Laboratory Animal Care published by the National Institutes of Health, as approved by the Ethical Committee of Central South University.

After an adaptation period for a week, the mice were randomly divided into four groups (n = 8 per group). i.e. control (C) group, low-dose genistein treated (GT-L) group, intermediate-dose genistein treated (GT-I) group and high-dose genistein treated (GT-H) group. The treated groups were received doses of genistein (7, 15 and 30 mg/kg) dissolved in 1.0 mL of physiological saline, and the control group received same volume of physiological saline via oral gavage once a day for 28 days.

Forced Swimming Test

After 28 days, the mice were subjected to the forced swimming test one hour after the last treatment administration. The protocol was adapted from a previous study with some modifications [10]. Briefly, the mice were dropped individually into an acrylic plastic pool (50 cm × 50 cm × 40 cm) filled with water to 30 cm deep and maintained at a temperature of 25 ± 0.5℃. A lead fish sinkers (5% of body weight) was attached to the tail root of each mouse. Exhaustion was determined by observing loss of coordinated movements and failure to return to the surface within 10 s. The exhaustive swimming times were recorded.

Biochemical Analysis

After forced swimming test, the mice were sacrificed immediately by decapitation under anesthesia with sodium pentobarbital (40 mg/kg bw, ip). Blood samples were collected in the test tube and the serum was prepared by centrifugation for the BLA, SUN, SOD, GPx and CAT analyses. Then the liver and muscle were also removed, washed with physiological saline, and frozen in liquid nitrogen for storage at -80° C until required for the glycogen analyses. All the biochemical parameters were determined according to the recommended procedures provided by the commercial diagnostic kits.

Statistical Analysis

All data are shown as the mean ± SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc multiple comparison test

using SPSS16.0 (SAS Institute, Cary, NC, USA). $p<0.05$ was considered as statistically significant.

Results and Discussion

Effects of Genistein on Exhaustive Swimming Times of Mice

Forced swimming test is a commonly used technique for animal model of behavioral despair, which has been used extensively for the evaluation of anti-fatigue properties of novel compounds [4]. The length of the exhaustive swimming times indicated the degree of fatigue [11]. Effects of genistein on exhaustive swimming times of mice are shown in Figure 1. As shown in Figure 1, exhaustive swimming times in the GT-L, GT-I and GT-H groups were significantly longer compared with that in the C group ($p<0.05$). The results indicated that genistein had anti-fatigue effects.

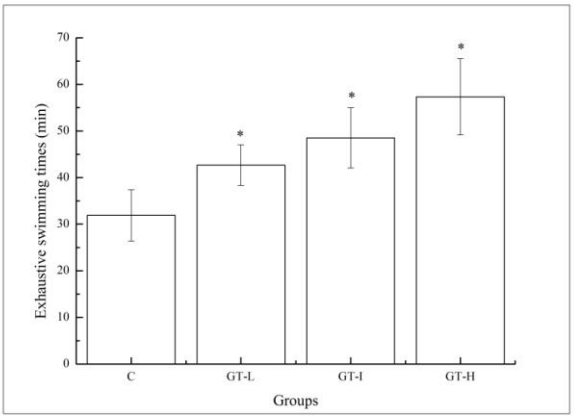


Figure 1. Effects of genistein on exhaustive swimming times of mice. Data are expressed as mean ± SD. *, $p<0.05$ compared with the C group

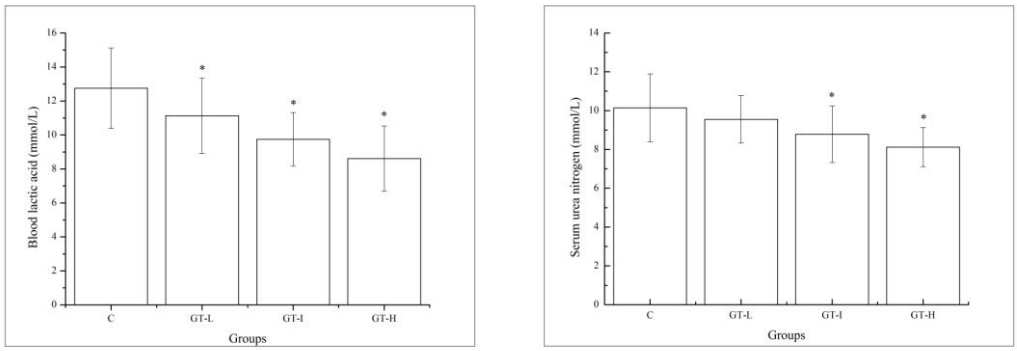
Effects of Genistein on Blood Lactic Acid of Mice

Several studies have demonstrated that glycolysis was the main energy source for intense exercise over a short time, and lactate acid was the glycolysis product of carbohydrate under anaerobic conditions [4]. The increased level of lactic acid will induce many side effects of various biochemical and physiological processes, which were harmful to the body performance [12]. Therefore, the BLA is one of the important indicators for judging the degree of fatigue, and the inhibition of BLA accumulation or reduction of BLA levels represents an anti-fatigue effect [13]. Effects of genistein on BLA of mice are shown in Figure 2(A). As shown in Figure 2(A), BLA levels in the GT-L, GT-I and GT-H groups were significantly lower compared with that in the C group ($p<0.05$). The results indicated that genistein could effectively retard and lower the BLA produced, and postpone the appearance of fatigue.

Effects of Genistein on Serum Urea Nitrogen of Mice

Urea is formed in the liver as the end product of protein metabolism. During digestion, protein is broken down to amino acids. Amino acids contain nitrogen, which is removed as NH_4^+ (ammonium ion), while the rest of the molecule is used to produce energy or other substances needed by the cell [14]. When the body is unable to obtain sufficient energy from sugar and fat catabolism, it utilizes proteins and amino acids that have a stronger catabolism [15]. Therefore, SUN is another important indicator of

fatigue status. There is a positive correlation between the SUN and the exercise tolerance [16]. Effects of genistein on serum urea nitrogen of mice are shown in Figure 2(B). As shown in Figure 2(B), SUN levels in the GT-I and GT-H groups were significantly lower compared with that in the C group ($p<0.05$). The results indicated that genistein could might reduce protein catabolism for energy and ameliorates fatigue.



(A) blood lactic acid (B) serum urea nitrogen

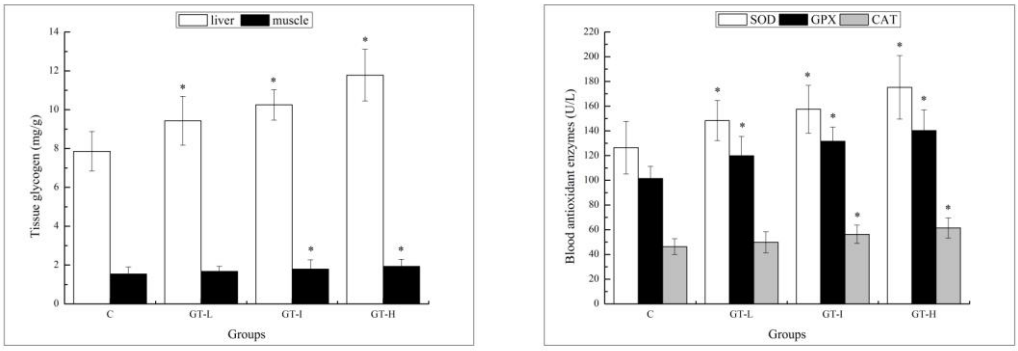
Figure 2. Effects of genistein on blood lactic acid and serum urea nitrogen of mice. Data are expressed as mean ± SD. *, $p<0.05$ compared with the C group

Effects of Genistein on Liver and Muscle Glycogen of Mice

It has been reported that energy for exercise is derived initially from the breakdown of glycogen in muscle, after strenuous exercise may be depleted and at later stages the energy will be derived from liver glycogen. With the exhaustion of liver glycogen, fatigue will set in [17]. Therefore, the glycogen contents are sensitive parameters related to fatigue [16]. Effects of genistein on liver and muscle glycogen of mice are shown in Figure 3(A). As shown in Figure 3(A), liver glycogen contents in the GT-L, GT-I and GT-H groups as well as the muscle glycogen contents in the GT-I and GT-H groups were significantly higher compared with that in the C group ($p<0.05$). The results indicated that genistein increases liver and muscle glycogen contents by improving glycogen reserve or reducing the glycogen consumed during exercise, or both. This may be one of the mechanisms of its anti-fatigue effects.

Effects of Genistein on Serum Antioxidant Enzymes of Mice

Excessive reactive oxide species (ROS) produced during exhaustive exercise has been proven to be the major component of physical fatigue [18]. Previous studies demonstrated that endogenous free radicals are removed by a set of antioxidant enzymes, including SOD, GPx and CAT [19]. SOD converts superoxide radicals to hydrogen peroxides. GPx utilizes reduced glutathione (GSH) as a hydrogen donor for the removal of peroxides. CAT catalyzes the breakdown of hydrogen peroxide to form water, sharing this function with GPx [20]. Therefore, the improvement in the antioxidant enzymes activities can help to fight against fatigue. Effects of genistein on serum antioxidant enzymes of mice are shown in Figure 3(B). As shown in Figure 3(B), SOD and GPx levels in the GT-L, GT-I and GT-H groups as well as the CAT levels in the GT-I and GT-H groups were significantly higher compared with that in the C group ($p<0.05$). The results indicated that genistein could enhance antioxidant enzymes activities to protect against physical fatigue.



(A) liver and muscle glycogen (B) serum antioxidant enzymes

Figure 3. Effects of genistein on liver and muscle glycogen and serum antioxidant enzymes of mice. Data are expressed as mean ±SD. *, $p<0.05$ compared with the C group

Summary

The present findings indicated that genistein could prolong exhaustive swimming time of mice and had anti-fatigue effects. the possible mechanisms anti-fatigue mechanisms of genistein due to decreased BLA and SUN levels as well as increased liver and muscle glycogen contents, and SOD, GPx and CAT levels in serum. Further research need to be done to clarify the anti-fatigue molecular mechanisms of genistein.

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