

Effects of Radix puerariae (Ge Gen) Water Extract on Exercise-Induced Fatigue in Mice

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Abstract: To investigate the effects of Radix Puerariae water Extract (RPE) on exercise-induced fatigue, 96 mice were randomly divided into four groups: Normal Control (NC), Low dose RPE Treated (LRT), Middle dose RPE Treated (MRT) and High dose RPE Treated (HRT) groups. The mice in the treated groups received RPE (2, 4 and 8 g kg⁻¹, ig) and the mice in the normal control group received drinking water ig for 4 weeks. The exhaustive swimming time, Blood Lactic Acid (BLA), Blood Urea Nitrogen (BUN), Hemoglobin (Hb) and liver glycogen were determined after swimming test. The results indicated that RPE had significant anti-fatigue effects on mice. It extended the exhaustive swimming time of mice, increased the Hb and liver glycogen contents and decreased BLA and BUN contents.

Key words: Radix puerariae, water extract, exercise, fatigue, mice, China

INTRODUCTION

Fatigue is an unpleasant symptom that affects everyone which can be induced by burn-out, forced exercise, sickness or sleep disturbance and result in symptoms including impaired concentration, attention and memory (Gandevia *et al.*, 1996; Hunter *et al.*, 2004; Powers and Jackson, 2008). Recent researches show that physical fatigue after exercise can be caused by various biochemical mechanisms such as the depletion of energy stores, dysfunctions in cellular components that are responsible for producing energy and the production of free radicals inside muscle cells (Vollaard *et al.*, 2005; Ikeuchi *et al.*, 2006; Kim *et al.*, 2008; Liu *et al.*, 2011). Since, the available therapies for fatigue in modern medicine are very limited, potential alternatives from bioactive compounds of traditional herbal medicine and their respective mechanisms of action are worth investigating. The benefits of these bioactive compounds of traditional herbal medicine are their low toxicity, low cost and availability.

Radix puerariae is the dried root of *Pueraria lobata* (Willd), Ohwi and *Pueraria thomsonii* berth (Fabaceae) which is especially rich in the isoflavones (a class of flavonoids) such as daidzein, daidzin, puerarin, genistein and daidzein-4', 7-diglucoside (Lee *et al.*, 2002). In China, Radix puerariae is known as Ge Gen and has been used as a traditional medicine for the treatment of various diseases including cardiovascular disorders or as an antimicrobial, pain-releasing and appetite-inducing agent (Jiang *et al.*, 2005; Wang *et al.*, 2010; Bebrevska *et al.*, 2010). It has

also been proven to be useful in the treatment of alcohol abuse, diabetic and hypertension (Lee, 2004; Lukas *et al.*, 2005). However, no detailed study has been reported on the anti-fatigue effects of Radix puerariae. Therefore, the present study was designed to investigate the effects of Radix Puerariae water Extract (RPE) on exercise-induced fatigue in mice.

MATERIALS AND METHODS

Plant materials: Radix puerariae were purchased from a local medicine shop (Hangzhou, China) and was authenticated by Dr. Wang Xin in the Institute of Zhejiang and Institute of Botany (Hangzhou, China). A voucher specimen has been deposited in the Herbarium of the Zhejiang Institute of Botany.

Chemicals: The reagent kits for the determination of Blood Lactic Acid (BLA), Blood Urea Nitrogen (BUN) and Hemoglobin (Hb) were purchased from Jiancheng Biotechnology Co. (Nanjing, China). The reagent kits for the determination of liver glycogen were obtained from Leadman Biochemistry Technology Co., Ltd. (Beijing, China). All other reagents used were of analytical grade. Preparation of Radix puerariae water extract: Radix Puerariae water Extract (RPE) were prepared as described previously (Lee *et al.*, 2002; Lee, 2004). In brief, dried Radix puerariae was ground to fine powder and 100 g of this powder was extracted in 500 mL of boiling water for 4 h. After filtration, the extract was concentrated with a vacuum rotary evaporator and freeze-dried.

Tested animals and grouping: Male Kunming mice, weighing 18-22 g were purchased from Zhejiang Animal Husbandry Center (Hangzhou, China). The animals were housed in polypropylene cages and maintained under controlled conditions of 12 h light/12 dark cycle and 55±10% relative humidity at 21-23°C. The animals were fed pellet diet and water *ad libitum*. The study was approved by Institutional Animal Ethics Committee of Zhejiang Police College and the animals were maintained in accordance with the guide for the care and use of laboratory. After an adaptation period of a week, 96 mice were randomly divided into four groups: Normal Control (NC), Low dose RPE Treated (LRT), Middle dose RPE Treated (MRT) and High dose RPE Treated (HRT) groups. The mice in the treated groups received RPE (2, 4 and 8 g kg⁻¹, ig) and the mice in the normal control group received drinking water ig for 4 weeks.

Weight-loaded swimming test: After 4 weeks, 8 mice were taken out from each group for weight-loaded swimming test. The procedure used in this experiment was similar to that described by Nozawa *et al.* (2009). Briefly, 30 min after the final treatment with RPE, the mice were allowed to rest for 30 min. Then, the mice were placed in the swimming tank (50×40×50 cm) with 30 cm deep, 25±0.5°C water. A tin wire (7% of body weight) was loaded on the tail root of each mouse. The swimming period was regarded as the time spent by the mouse floating in the water with struggling and making necessary movements until exhausting its strength. The mice were assessed to be exhausted when they failed to rise to the surface of water to breathe within a 10 sec period and the time was immediately recorded.

Measurement of the contents of blood lactic acid: After 4 weeks, 8 mice were taken out from each group for Blood Lactic Acid (BLA) analyses. The procedure used in this experiment was similar to that described by Tang *et al.* (2008). Briefly, 30 min after the final treatment with RPE, 20 mL of blood were collected from the mice with a capillary tube by using the Retroorbital Bleeding Method. Then, the mice were forced to swim for 20 min after weight loading (2% body weight) another 20 mL of blood was collected 30 min after forced swimming. Then, BLA was tested according to the recommended procedures provided by the kit.

Measurement of the contents of blood urea nitrogen, hemoglobin and liver glycogen: After 4 weeks, 8 mice were taken out from each group for Blood Urea Nitrogen (BUN), Hemoglobin (Hb) and liver glycogen analyses. The procedure used in this experiment was similar to that

described by Tang *et al.* (2009). Briefly, 30 min after the final treatment with RPE, the mice were forced to swim for 90 min without weight loading, after 60 min of recess, the mice were anaesthetized with ether and blood were collected for assaying of BUN and Hb. In addition, immediately after the blood had been collected, the liver was dissected out quickly from the mice, washed with physiological saline and dried with absorbent paper. Then, the content of liver glycogen was analyzed. Then BUN, Hb and liver glycogen was tested according to the recommended procedures provided by the kit.

Statistical analysis: Statistical analysis was performed by using one-way Analysis of Variance (ANOVA) followed by Duncan's multiple test. Results are expressed as mean±SD by group. Significant differences were determined at p<0.05 by applying ANOVA followed by SAS, Version 6.12, Cary, NC.

RESULTS AND DISCUSSION

Effects of RPE on the body weights of mice: Change of body weights during the experimental period was shown in Fig. 1. Body weights of mice were recorded before experiment (initial) and after 4 weeks (final). There was no significant difference in the body weights of mice in the three RPE treated (LRT, MRT and HRT) groups compared with that in the NC group during initial and final stages in the experiment (p>0.05). The results indicated that RPE had no effect on body weights of mice.

Effect of RPE on the exhaustive swimming time of mice: The direct appearance of anti-fatigue ability is the elevation of exercise tolerance. Reduced susceptibility to

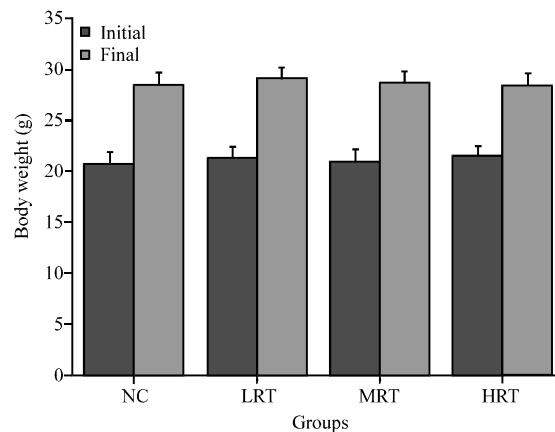


Fig. 1: Effects of RPE on the body weights of mice. Data are mean±SD; *p<0.05 compared with Normal Control (NC) group

fatigue was interpreted from a longer swim time. A weight-loaded swimming test was used to evaluate the extent of fatigue which increased the exercise intensity of mice in order to shorten the investigational time. With 6-8% body weight load, the mice could swim freely and safely (Wang *et al.*, 2008). In this experiment, the mice had a weight attached 5% body weight in the duration of the swimming to exhaustion. As shown in Fig. 2, the exhaustive swimming time of mice in LRT, MRT and HRT groups significantly increased compared to that of the NC group ($p < 0.05$) and the exhaustive swimming time increased by 53.80, 72.22 and 97.08%, respectively. The results indicated that RPE could elevate the exercise tolerance of mice and possessed anti-fatigue effects.

Effects of RPE on the blood lactic acid of mice: Generally, lactic acid is a product of glycolysis under anaerobic condition. Glycolysis is the primary form of metabolism used during intense exercises in a short time which can increase lactic acid production to a point that exceeds the rate of lactic acid removal (Wang *et al.*, 2008). The increased level of lactic acid will bring about a reduction of pH in muscle tissue and blood and also induce many side effects of various biochemical and physiological processes which are harmful to the body performance and produce fatigue (Zhang *et al.*, 2006). Therefore, Blood Lactic Acid (BLA) is closely related to workload intensity and is one of the important indicators for judging the intensity of the exercise or the degree of fatigue. In other words, BLA represents the degree of fatigue after exercise and the condition of recovery. As shown in Fig. 3, there was no significant difference in the contents of BLA between three RPE treated (LRT, MRT and HRT) groups and the control group before swimming ($p > 0.05$). After swimming, the BLA contents of mice in LRT, MRT and

HRT groups significantly decreased compared to that of the NC group ($p < 0.05$) and the BLA contents decreased by 36.18, 47.12 and 68.31%, respectively. The results indicated that RPE effectively could delay the increase of BLA contents and postpone the appearance of fatigue.

Effects of RPE on the blood urea nitrogen of mice: Blood Urea Nitrogen (BUN) is a sensitive index to evaluate the bearing capability when human bodies suffer from a physical load caused by catabolism of proteins and amino acids. Protein and amino acids have a stronger catabolic metabolism when body cannot obtain enough energy by sugar and fat catabolic metabolism. Therefore, there is a positive correlation between the urea nitrogen *in vivo* and the exercise tolerance (Wei *et al.*, 2010). As shown in Fig. 4, after swimming the BUN contents of mice in LRT,

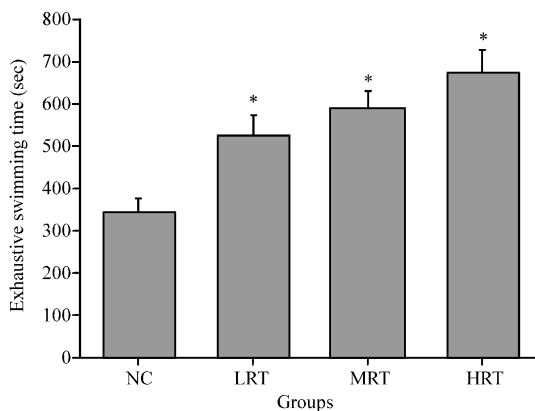


Fig. 2: Effects of RPE on the exhaustive swimming time of mice. Data are mean±SD; * $p < 0.05$ compared with Normal Control (NC) group

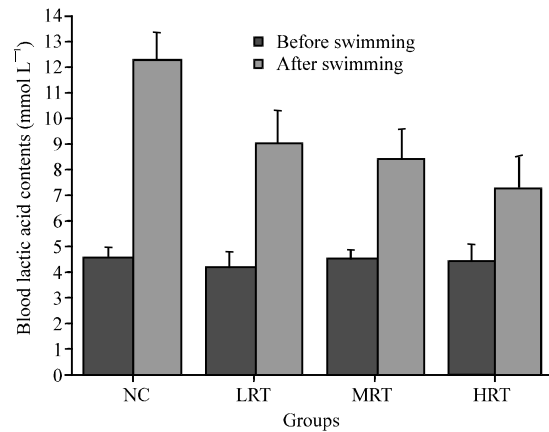


Fig. 3: Effects of RPE on the blood lactic acid of mice. Data are mean±SD. * $p < 0.05$ compared with Normal Control (NC) group

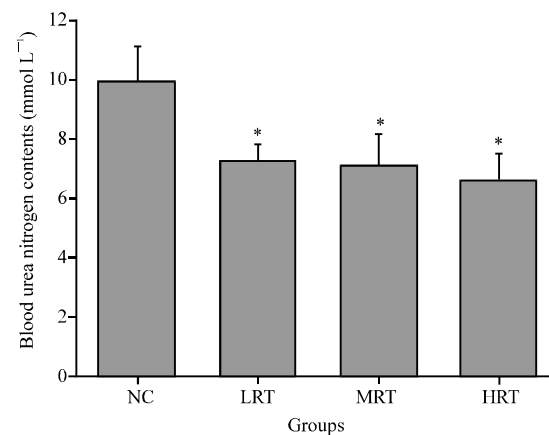


Fig. 4: Effects of RPE on the blood urea nitrogen of mice, Data are mean±SD; * $p < 0.05$ compared with Normal Control (NC) group

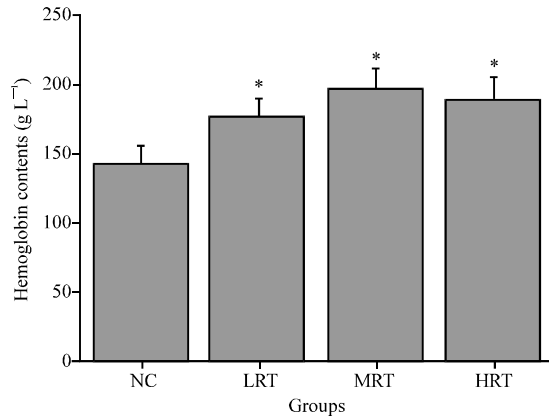


Fig. 5: Effects of RPE on the hemoglobin of mice. Data are mean±SD. *p<0.05 compared with Normal Control (NC) group

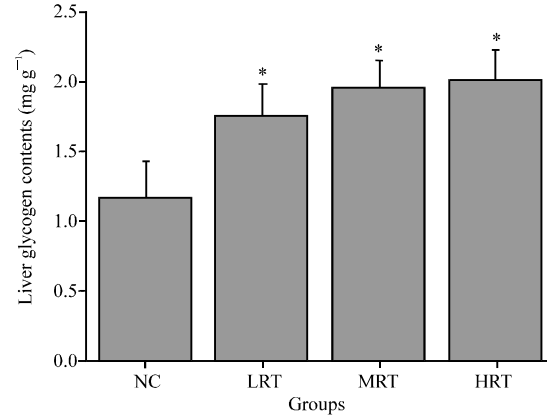


Fig. 6: Effects of RPE on the liver glycogen of mice. Data are mean±SD; *p<0.05 compared with Normal Control (NC) group

MRT and HRT groups significantly decreased compared to that of the NC group (p<0.05) and the BUN contents decreased by 36.51, 39.41 and 53.14%, respectively. The results indicated that RPE could reduce catabolic decomposition of protein for energy.

(p<0.05) and the liver glycogen contents increased by 50.86, 68.97 and 73.26%, respectively. The results indicated that RPE might have promoted glycogenolysis restraint and/or gluconeogenesis.

Effects of RPE on the hemoglobin of mice: Hemoglobin (Hb) is the main component of erythrocyte. Its main function is to serve as the carrier for the erythrocyte to transport oxygen and partial carbon dioxide. Hb also has the effect on maintaining the body fluid's acidalkali balance. Therefore, it can directly affect the substance metabolism and the energy metabolism in the body and in turn, affect body function and exercise ability of the human body, the exercise's loading capacity and fatigue. Hb normally is one of the indicators to reflect the degree of recovery from fatigue after exercise and in a certain range, higher level of Hb is helpful to improve the exercise ability (Cao *et al.*, 2009). As shown in Fig. 5, after swimming, the Hb contents of mice in LRT, MRT and HRT groups significantly increased compared to that of the NC group (p<0.05) and the Hb contents increased by 22.91, 38.31 and 34.50%, respectively. The results indicated that increase the Hb contents might be another pathway of RPE's anti-fatigue effects.

CONCLUSION

Results from this study suggested that RPE had significant anti-fatigue effects on mice. It extended the exhaustive swimming time of mice, increased the hemoglobin and liver glycogen contents and decrease blood lactic acid and blood urea nitrogen contents. The anti-fatigue effects of RPE seemed to be a comprehensive effect of its various constituents. To clarify the mechanism underlying the anti-fatigue effects and active component of RPE, further studies are warranted by using new adequate animal models.

Effects of RPE on the liver glycogen of mice: Energy for exercise is derived initially from the breakdown of glycogen and later from circulating glucose released by the liver. As one of the sources of blood glucose, liver glycogen plays an important role in controlling the availability of cellular energy (Li *et al.*, 2008; Koo *et al.*, 2008). So, liver glycogen are sensitive parameters related to fatigue. As shown in Fig. 6, after swimming, the liver glycogen contents of mice in LRT, MRT and HRT groups significantly increased compared to that of the NC group

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