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Anti-Fatigue Activity of Crude Saponins from *Panax quinquefolium*

[1]Xing Jin-Ming, [1]Yuan Wen-Xue, [2]Zhang Zhi-Cheng and [3]Chen Shuai
[1]Department of Physical Education, Dalian University of Technology, 116024 Dalian, China
[2]Department of Physical Education, Jilin Agricultural University, 130118 Changchun, China
[3]School of Physical Education, Northeast Normal University, 130024 Changchun, China

Abstract: The present study was carried out to investigate anti-fatigue activity of Crude saponins from *Panax quinquefolium* (CPQS) in mice. The animals were randomly divided into four groups: control (distilled water); 50 mg kg^{-1} CPQS; 100 mg kg^{-1} CPQS and 200 mg kg^{-1} CPQS. Distilled water or CPQS were orally administered for consecutive 28 days. The 28 days later, forced swimming test was performed and then fatigue-related biochemical parameters including blood lactic acid, serum urea nitrogen, liver glycogen and muscle glycogen were detected. The results showed that CPQS could prolong the swimming to exhaustion time of mice as well as increasing the liver and muscle glycogen contents but decrease the blood lactic acid and serum urea nitrogen levels. Therefore, the present study demonstrated that CPQS possessed anti-fatigue activity.

Key words: Anti-fatigue activity, crude saponins from *Panax quinquefolium*, forced swimming test, mice, liver glycogen

INTRODUCTION

The use of herbal medicine as an unconventional health treatment is gaining considerable recognition and popularity worldwide. One of the most widely used herbs is ginseng which has been used as one of the most valuable natural medicines in China for >2000 years. Within the context of traditional Chinese medicine, ginseng is generally viewed as an “adaptogen”, a substance which can help reduce the impact of environmental stress (Barton *et al*., 2010). There are different species of ginseng, the two most common being Asian (*Panax ginseng*) and American (*Panax quinquefolius*) both from the genus *Panax* of the Araliaceae family of plants (Wen and Nowicke, 1999). *Panax quinquefolius* is native to North America and is commonly found in rich woods from Maine to Georgia and from Oklahoma to Minnesota. Saponins including ginsenosides Rg1, Re, Rb1, Rg2, Rb2, Rc and Rd have been regarded as the principal components responsible for the pharmacological activities of American ginseng (Peng *et al*., 2012). Over the past few decades, pharmacological effects of Crude saponins from *Panax quinquefolium* (CPQS) such as antimicrobial (Kochan *et al*., 2013), anxiolytic (Wei *et al*., 2007), anti-obesity (Liu *et al*., 2008), anti-cancer (Qiu *et al*., 2009), antihyperlipidemic, hypoglycemic (Zhang *et al*., 2007), antioxidative (Li *et al*., 1998), anti-hypoxia (Xu and Zhang, 2013) and cardioprotective effects (Xu *et al*., 2013) have been shown. To date, the anti-fatigue activity of CPQS has not been investigated. In this study, researchers evaluated the anti-fatigue activity of CPQS by forced swimming test and then fatigue-related biochemical parameters including blood lactic acid, Serum Urea Nitrogen (SUN), liver glycogen and muscle glycogen were explored.

MATERIALS AND METHODS

Plant material: The dried roots of *Panax quinquefolium* (native to herbal medicines planting base, China) were purchased from Jilin Medicinal Materials Co. (Changchun, China). The plant was authenticated by Professor Wan Fujian, a botanist of Dalian University of Technology (Dalian, China) and a voucher specimen (No. 11498) was deposited in the Herbarium of the Department of Biology, Dalian University of Technology. The dried roots of *Panax quinquefolium* were ground with an electric mixer prior to obtain a coarse powder (60-80 mesh).

Chemical: All chemicals were purchased from Shengmin Chemical Reagents Co., Ltd. (Dalian, China) unless otherwise indicated. Commercial diagnostic kits for blood lactic acid and Serum Urea Nitrogen (SUN) were purchased from Nanjing Jiancheng Biocompany (Nanjing, China), Commercial diagnostic kits for liver glycogen and

Corresponding Author: Xing Jin-Ming, Dalian University of Technology, No. 2 Linggong Road, Ganjingzi District, 116204 Dalian, China

muscle glycogen were purchased from Beijing Zhongsheng Biological Engineer Company (Beijing, China) and other reagents used in this study were of analytical grade.

Preparation of crude saponins from *Panax quinquefolium*: Saponins from *Panax quinquefolium* (CPQS) was extracted according to the procedure reported by Wei *et al.* (2007) with some modifications. The powder was extracted with 70% EtOH three times (2 h for each time) under reflux. After filtration, excess solvent was removed under reduced pressure. The EtOH extract was suspended in water and defatted with ether followed by partitioning with n-BuOH. The combined n-BuOH layers were concentrated to dryness. The dried extract was subjected to HPD100 resin column chromatography, washed with water and eluted with EtOH to obtain CPQS.

Animals and housing: Male Kunming mice weighing approximately 18-20 g were obtained from the Experiment Animal Center of Dalian City (Dalian, China). The mice were housed under standard conditions (temperature 20±1°C, humidity 60±10%, light from 6:00 am to 6:00 pm) with free access to water. All animal use procedures were in accordance with the Regulations of Experimental Animal Administration issued by State Committee of Science and Technology of the People's Republic of China on November 14th, 1988. This study was approved by the Medical Ethics Commission of Dalian University of Technology.

Experiment design: After an acclimation period of 1 week, the mice were randomly divided into four groups (i.e., 10 mice per group): control (distilled water); 50 mg kg^{-1} CPQS (CPQS-50); 100 mg kg^{-1} CPQS (CPQS-100) and 200 mg kg^{-1} CPQS (CPQS-200). This dosage was chosen according to the results of preliminary experiments that examined the efficacy of CPQS. Distilled water or CPQS were orally administered with a volume of 10 mL kg^{-1} body weight at 8:00 am for consecutive 28 days. The 28 days later, forced swimming test was performed and fatigue-related biochemical parameters were detected.

Forced swimming test: Forced swimming test was used as described earlier with some modifications (Tang *et al.*, 2008). The test was induced by forcing animals to swim until exhaustion. Briefly, 30 min after the last oral administration of distilled water or CPQS, the mice were dropped individually into a plastic pool (90×45×45 cm). The water depth and temperature were 35 cm and 30±1°C, respectively. A lead block (5% of body weight) was loaded on the tail root of the 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 (Yan and Wang, 2010; Cai *et al.*, 2010; Yan *et al.*, 2012).

Determination of fatigue-related biochemical parameters: The mice were anesthetized with ethyl after the forced swimming test and the blood samples were collected in heparinized tubes by heart puncture at mice. Serum was prepared by centrifuga tion at 1000×g, 4°C for 15 min and the levels of blood lactic acid, serum urea nitrogen were analyzed with commercial diagnostic kits. In the following order, liver and gastrocnemius muscle were quickly dissected out, washed with 0.9% saline and blotted dry with filter papers. The samples were accurately weighed and homogenized in 8 mL of homogenization buffer for liver and muscle glycogen contents analysis using commercial diagnostic kits.

Statistical analysis: All the data were expressed as means±SD and Analysis of Variance (ANOVA) was used. Results were considered statistically significant for $p<0.05$. These analyses were carried out using SPSS for Windows, Version 13.0 (SPSS, Chicago, IL).

RESULTS AND DISCUSSION

Effects of CPQS on body weight change of mice: Change of body weight during the experimental period were shown in Fig. 1. Body weight was recorded before experiment (initial) and after 28 days (final) and weight gain was computed. There was no significant difference between the control group and all CPQS (CPQS-50, CPQS-100 and CPQS-200) groups ($p>0.05$) which meant CPQS had no effect on the body weight and weight gain.

Effects of CPQS on swimming to exhaustion time of mice: The effects of CPQS on swimming to exhaustion time were shown in Fig. 2. Swimming to exhaustion time of mice in all CPQS (CPQS-50, CPQS-100 and CPQS-200) groups were significantly prolonged compared with that in the control group ($p<0.05$) which was 1.29, 1.59 and 1.81 times longer that in the control group, respectively.

The improvement of exercise endurance is the most powerful macro representation of anti-fatigue enhancement. Forced swimming test is perhaps one of the most commonly used animal models of behavioural despair and has been used as an exercise endurance test (Shin *et al.*, 2006; Huang *et al.*, 2011). In this study, the anti-fatigue activity of the CPQS was measured using a

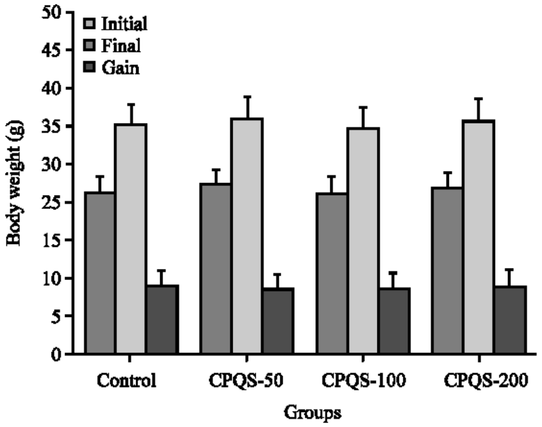

Fig. 1: Effects of CPQS on body weight change of mice. The data was expressed as means±SD (n = 10 per group)

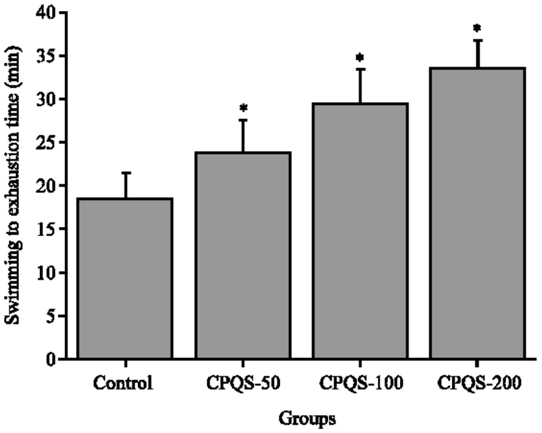

Fig. 2: Effects of CPQS on swimming to exhaustion time of mice. The data were expressed as means±SD (n = 10 per group). *p<0.05 when compared to the control group

forced swimming test in male mice. The length of the swimming to exhaustion time indicated the degree of fatigue. The present results showed that different doses of CPQS could prolong the swimming to exhaustion time which suggested that CPQS could elevate the exercise tolerance and possessed anti-fatigue activity.

Effects of CPQS on blood lactic acid of mice: The effects of CPQS on blood lactic acid were shown in Fig. 3. Blood lactic acid levels of mice in all CPQS (CPQS-50, CPQS-100 and CPQS-200) groups were significantly lower compared with that in the control group (p<0.05).

The anaerobic glycolysis product, lactic acid is an important biochemical parameter related to fatigue and the accumulation of blood lactic acid is considered a major inducer of muscle fatigue (Zhang *et al*., 2012). The muscle produces a great quantity of lactate when it obtains enough energy from anaerobic glycolysis during high-intense exercise. The increased lactate level further reduces pH value which can induce various biochemical and physiological side effects including glycolysis and phosphofructokinase and calcium ion release, through muscular contraction (Wang *et al*., 2012). Therefore, reduction in the accumulation of blood lactic acid is beneficial for alleviation of fatigue (Derave *et al*., 2007; Xu and Zhang, 2013). The present results showed that different doses of CPQS could effectively delay the increase of blood lactic acid which suggested that CPQS could postpone the appearance of fatigue.

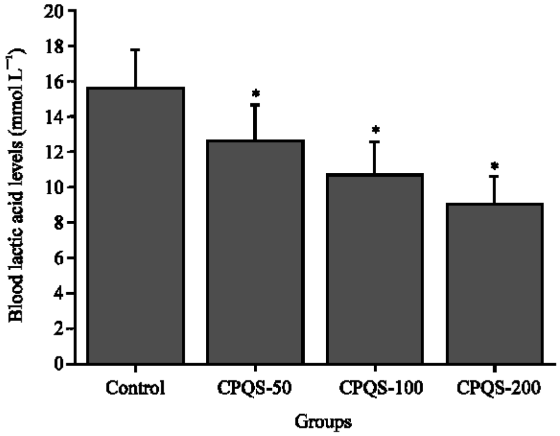

Fig. 3: Effects of CPQS on blood lactic acid of mice. The data were expressed as means±SD (n = 10 per group). *p<0.05 when compared to the control group

Effects of CPQS on serum urea nitrogen of mice: The effects of CPQS on Serum Urea Nitrogen (SUN) were shown in Fig. 4. SUN levels of mice in CPQS-100 and CPQS-200 groups were significantly lower compared with that in the control group (p<0.05). Although, the SUN levels in CPQS-50 group were also decreased, no significant difference was observed (p>0.05).

SUN is the metabolism outcome of protein and amino acid and a sensitive index to evaluate the bearing capability when bodies suffer from a physical load (Chen and Zhang, 2011; Cao *et al*., 2012). Urea is formed in the liver as the end product of protein-metabolism and is carried by the blood to the kidneys for excretion. Many studies have shown that SUN levels of human bodies rises with increase in exercise load (Liu *et al*., 2011; Zhang *et al*., 2012; Xu and Li, 2012). In other words, the worse the body is adapted for exercise tolerance, the more

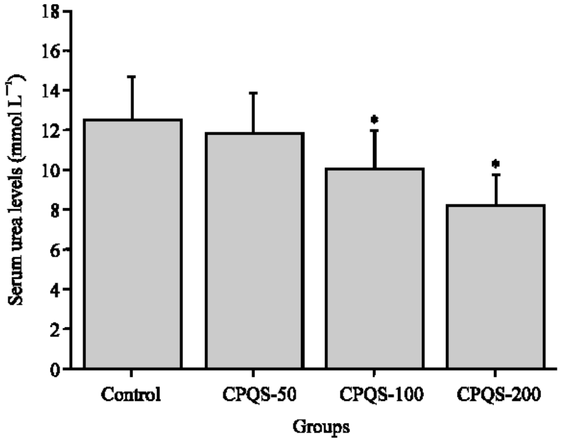

Fig. 4: Effects of CPQS on serum urea nitrogen of mice. The data were expressed as means±SD (n = 10 per group). *p<0.05 when compared to the control group

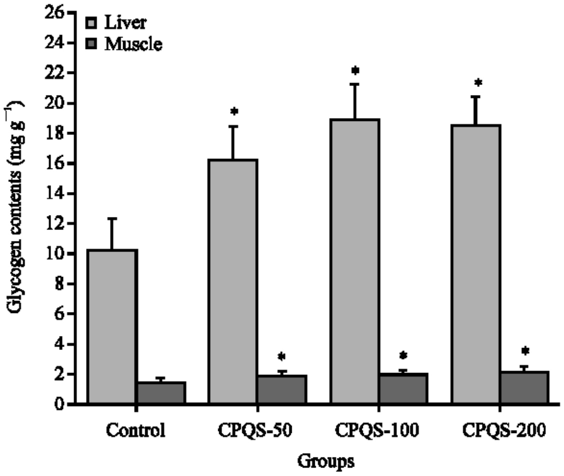

Fig. 5: Effects of CPQS on liver and muscle glycogen of mice. The data were expressed as means±SD (n = 10 per group). *p<0.05 when compared to the control group

significantly the SUN level increases. The present results showed that moderate (100 mg kg^{-1}) and high (200 mg kg^{-1}) doses of CPQS reduced the catabolism of protein for energy, increased adaptive capacity to exercise load and ultimately improved exercise tolerance and anti-fatigue activity.

Effects of CPQS on liver and muscle glycogen of mice: The effects of CPQS on liver and muscle glycogen were shown in Fig. 5. Liver and muscle glycogen contents of mice in all CPQS (CPQS-50, CPQS-100 and CPQS-200) groups were significantly higher compared with that in the control group ($p<0.05$). Energy for exercise is derived initially from the breakdown of glycogen in muscle and later from circulating glucose released by the liver as a result of glycogenolysis (Saraf *et al*., 2011; Li *et al*., 2012). The depletion of liver and muscle glycogen might be an important factor in the development of fatigue because as glycogen is depleted during exercise there is an inability to maintain blood glucose level and the simultaneous hypoglycemia could result in impaired nervous function (Liang *et al*., 2012). The present results showed that different doses of CPQS could increase the liver and muscle glycogen storage which suggested that the anti-fatigue activity of CPQS might be related to the activation of energy metabolism.

CONCLUSION

The present results showed that CPQS could prolong the swimming to exhaustion time of mice as well as increasing the liver and muscle glycogen contents but decrease the blood lactic acid and serum urea nitrogen levels. This study provided strong evidence that CPQS possessed anti-fatigue activity. However, further investigational studies need to be done to clarify the mechanisms involved in the anti-fatigue activity of CPQS.

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