

Full Length Research Paper

Evaluation of anti-athletic fatigue activity of *Schizandra chinensis* aqueous extracts in mice

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To study the anti-athletic fatigue effects of *Schizandra chinensis* aqueous extracts (SCAE), Forty Kunming male mice were randomized into 5 groups (CG, LDG, MDG, IHDG and HDG) equally based on body mass after one week adoption. The control group (CG) was given distilled water and LDG, MDG, IHDG and HDG were given various doses of SCAE (15, 30, 50 and 80 mg/kg) for 28 consecutive days, respectively. Swimming time, lactate, blood urea nitrogen (BUN) and hemoglobin (Hb) concentration were measured in the forced swimming treated mice. Results showed that SCAE had significant anti-athletic fatigue effects on mice. It extended the swimming time, increased concentration of the Hb, prevent the increase in lactate and BUN concentrations. In addition, acute toxicity studies revealed that SCAE did not exhibit any toxic symptoms in the limited toxicity evaluation in mice.

Key words: Anti-athletic fatigue, *Schizandra chinensis*, mice.

INTRODUCTION

Schizandra chinensis is a creeping vine with small red berries that is native to Northern China. In ancient China, *S. chinensis* was used as a staple food for hunting and gathering tribes (Zheng et al., 2005). As a traditional medicinal herb, *S. chinensis*, called Wu-wei-tzu in China, has been used as an astringent for a treatment for dry cough, asthma, night sweats, nocturnal seminal emissions and chronic diarrhea (Saunders, 1998; Lin et al., 2005). It is also used as a tonic for the treatment of chronic fatigue (Zhang and He, 2005). *S. chinensis* contains a number of compounds, including essential oils, numerous acids and lignans (You et al., 2006). Lignans are found in the seeds of the fruit and have a number of medicinal actions (You et al., 2006; Avula et al., 2005).

Athletic fatigue refers to that due to over-exercise. When the body cannot maintain physiologically its specific level or its predetermined exercise intensity, it is manifested as mental and physical fatigue (Li and Wei,

2005; Wang et al., 2006; Li et al., 2000). Over-intensity of exercise will lead to athletic fatigue and affect sport skills brought into play. Therefore, to prevent and release athletic fatigue is the hot topic in the researches on improving exercise quality. The exercise capacity can be improved by supplementing energetic substance, releasing metabolic production and administrating tonics, but these bring harms to the body even though retarding the fatigue (Li and Wei, 2005). During seeking for safe and effective anti-athletic fatigue methods, the specialty of Chinese herbal medicine has drawn the attentions of scholars in the world.

In the last decade, extensive research has been conducted at home and abroad on *S. chinensis* extract, regarding its chemical composition, pharmacological action and clinical medicine, and many results has been obtained (Ohsugi et al., 1999; Zhu et al., 1999; Wang et al., 2002; Xiang et al., 2003). However, related research on athletic health protection as well as its function in anti-athletic fatigue and the improvement of athletic ability of animal or human body is less available. The aim of present study is to investigate the anti-athletic fatigue activity of *S. chinensis* aqueous extracts (SCAE).

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MATERIALS AND METHODS

Plant material

The *S. chinensis* fruit used in the current study were purchased locally in Nanchang. Identification was authenticated by Professor Kaifeng Li, a biologist of East China Jiaotong University. The authentication tests included morphological examination and identification of schisandrin B content using thin-layer chromatography according to the Chinese Pharmacopoeia.

Preparation of *Schisandra chinensis* aqueous extracts (SCAE)

The hot -water extract was prepared by boiling the dried fruit with distilled water for six hours. The extract was filtered, freeze-dried and kept at 4°C (You et al., 2006). The yield of extraction was approximately 11.32% (w/w). The direct extract was dissolved in distilled water before use.

Experimental animals

Kunming male mice weighing approximately 18 to 22 g were obtained from medical scientific academy in Jiangxi (Nanchang, China) and housed individually in plastic cages at 20 to 30°C, relative air humidity of 45 to 55%, with lighting on from 6:00 AM to 6:00 PM (Fu and Cui, 2007; Hu et al., 2008; An, 2008). Mice were provided a basal diet (the Disease Control Center, Nanchang, China) and water *ad libitum*. The approval of this experiment was obtained from the Institutional Animal Ethics Committee of East China Jiaotong University (Nanchang, China) and was carried out according to the "Principles of Laboratory Animal Care" (World Health Organization (WHO) Chronicle, 1985) Forty male mice were randomized into 5 groups, equally based on body mass after one week adoption, with eight mice per group, as follows: control group (mice treated with distilled water, CG), low-dose group (mice treated with 15 mg/kg SCAE, LDG), medium-group (mice treated with 30 mg/kg SCAE, MDG), intermediate-high group (mice treated with 50 mg/kg SCAE, IHDG), high-group (mice treated with 80 mg/kg SCAE, HDG). The volume of administration was 0.5 mL and the treatments lasted for 28 days by gavage.

Acute toxicity studies

Healthy male mice of either sex, starved overnight were divided into three groups (n = 6) and were gavaged with SCAE in increasing dose levels of 160, 400, 800 mg/kg. The animals were observed continuously for 2 h under the following profiles (Shirwaikar et al., 2006):

- i) Behavioral profile: Alertness, restlessness, irritability, and fearfulness.
- ii) Neurological profile: spontaneous activities, reactivity, touch response, pain response and gait.
- iii) Autonomic profile: Defecation and urination.

After a period of 24 and 72 h, they were observed for any lethality or death.

Anti-athletic fatigue activity

Anti-athletic fatigue activity was assessed 30 min after the final SCAE was administered. The apparatus used in this test was an acrylic plastic pool (50 × 40 × 50 cm) filled with water maintained at 30 ± 2°C. The water in the acrylic plastic pool was 40 cm deep. The mice were loaded with a lead block weighing approximately 5

% of their body mass attached to the tails. The end point of the swimming endurance was taken as when the mouse remained at the bottom for more than 10 s, then swimming endurance time was measured (Ma et al., 2007).

Blood biochemical analysis

In order to clarify anti-athletic fatigue mechanism, lactate, blood urea nitrogen (BUN) and hemoglobin (Hb) concentration were measured in the forced swimming treated mice (Jung et al., 2004; Wang et al., 2006). The measurements were conducted before and after swimming. Blood samples were collected from the veins on the tails of individual mice and the lactate, BUN and Hb concentrations were determined using a commercial diagnostic kit provided by Jiancheng Diagnostic Systems (Nanjing, China).

Statistical analysis

The data were analyzed with SPSS 10.0 software. ANOVA was used to determine the effects of SCAE on anti-athletic fatigue. The values were expressed as mean ± SD. The test differences were considered statistically significant when a P value was less than 0.05.

RESULTS AND DISCUSSION

Acute toxicity studies

Acute toxicity studies revealed that no obvious symptom of toxicity or significant changes in general behaviour in mice of the SCAE was observed. There was no lethality or any toxic reactions found at any of the doses selected until the end of the study period.

Effect of the SCAE on body mass in mice

Table 1 shows the body mass change of the mice during the experimental period. The body mass of the mice were measured after they were gavaged by different dosages of SCAE for 28 days. Results showed that the increased body mass in the treated groups were of no significant difference compared with the control group (p > 0.05), which means SCAE had no effect on body mass.

Swimming endurance test

Swimming endurance test was employed in our study to evaluate anti-athletic fatigue of SCAE on mice. It was commonly accepted that swimming was an experimental exercise model (Jung et al., 2004). Figure 1 showed that the swimming time of each treatment group increased significantly (p < 0.05) when compared with that of the control group. The results indicated that different doses of SCAE had significant effect on the endurance of the mice in the experimental and the dosage of 30 mg/kg was more effective.

Table 1. Effect of the SCAE on body mass in mice (mean \pm SD, n = 8).

Group	Body mass	
	Before experiment (day 0)	After experiment (day 28)
CG	20.23 \pm 1.39	29.36 \pm 2.11
LDG	20.45 \pm 1.21	28.76 \pm 3.56
MDG	20.06 \pm 1.83	28.48 \pm 2.29
IHDG	20.37 \pm 1.64	29.07 \pm 3.08
HDG	20.52 \pm 1.27	28.95 \pm 2.31

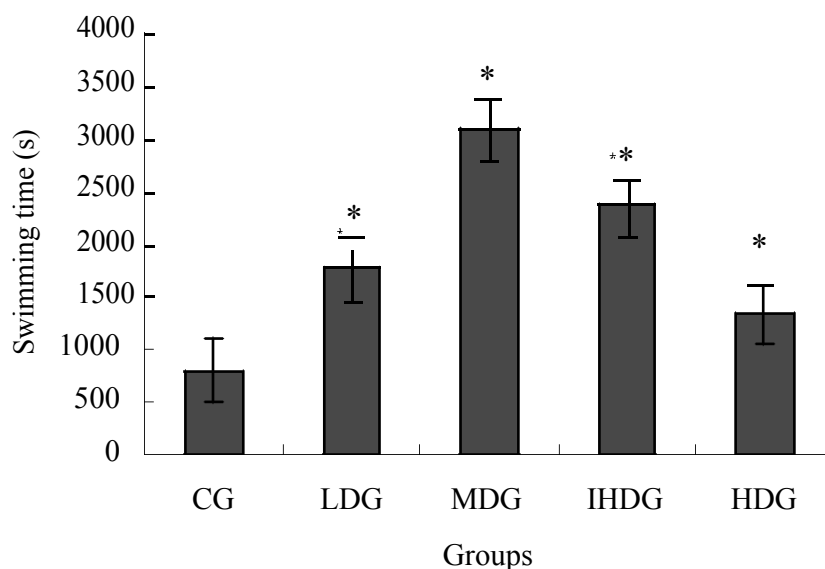


Figure 1. Effect of the Schizandra chinensis aqueous extracts (SCAE) on swimming time in mice (mean \pm SD, n = 8). CG -Control group (mice treated with distilled water); LDG- Low-dose group (mice treated with 15 mg/kg SCAE); MDG -Medium-group (mice treated with 30 mg/kg SCAE); IHDG-Intermediate-high group (mice treated with 50 mg/kg SCAE); HDG -High-group (mice treated with 80 mg/kg SCAE); *p < 0.05 as compared with the control group.

Effect of the SCAE on blood lactate in mice

Blood lactate is the glycolysis product of carbohydrate under an anaerobic condition and glycolysis is the main energy source for fierce exercise in a short time. Therefore, blood lactate 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, blood lactate represents the degree of fatigue after exercise and the condition of recovery (Wang et al., 2006; Yu et al., 2008). As shown in Table 2, there was no significant difference in the concentration of blood lactate treatment groups and the control group before swimming (p > 0.05). After swimming, the concentration of blood lactate for the MDG, IHDG and HDG were significantly lower than that of control group (p 164% achieved by the control group. Judging from the increase ratio of blood lactate concentration, it could be

Table 2. Effect of the Schizandra chinensis aqueous extracts (SCAE) on blood lactate in mice (mean \pm SD, n = 8).

Group	Lactate (mmol/L)		Increase ratios (%)
	Before swimming	After swimming	
CG	4.37 \pm 0.53	11.53 \pm 1.38	164
LDG	4.48 \pm 0.39	10.82 \pm 1.26	142
MDG	4.15 \pm 0.77	8.21 \pm 1.41*	98
IHDG	4.26 \pm 0.65	9.13 \pm 1.64*	114
HDG	4.39 \pm 0.42	9.67 \pm 1.75*	120

Increase ratio = (a - b)/b. a, the blood lactate concentration of mice after swimming; b, the blood lactate concentration of mice before swimming; CG, Control group (mice treated with distilled water); LDG, Low-dose group (mice treated with 15 mg/kg SCAE); MDG, Medium-group (mice treated with 30 mg/kg SCAE); IHDG-Intermediate-high group (mice treated with 50 mg/kg SCAE); HDG, High-group (mice treated with 80 mg/kg SCAE); *p < 0.05 as compared with the control group.

< 0.05). The increase ratios of the blood lactate of the LDG, MDG, IHDG and HDG were 142, 98, 114 and 120%, respectively, which were lower than the increase ratio of seen that the treatment groups did possess the ability to retard and lower the blood lactate produced after exercise.

Effect of the SCAE on BUN in mice

BUN is the metabolism outcome of protein and amino acid. Urea is formed in the liver and is carried by the blood to the kidneys for excretion, because urea is separated from the bloodstream by the kidneys, urea nitrogen concentration in the blood can be used as an indication of renal function. However, there are many factors other than renal disease that can cause BUN alteration. This includes protein breakdown, dehydration, stress, fatigue, etc. The BUN value was found to increase significantly after exercise (Wang et al., 2006; Xu and Luo, 2001; Wang et al., 2003). Therefore, it is considered that BUN are important blood biochemical parameters related to fatigue. The BUN changes before and after swimming for all the groups were shown in Figure 2. It was found that BUN concentration of each group had no significant difference ($p > 0.05$) before swimming. However, after swimming, BUN of MDG, IHDG and HDG were significantly lower than that of the control group ($p < 0.05$). It indicated that SCAE possessed the ability to lower or retard the formation of BUN after exercise.

Effect of the SCAE on hemoglobin in 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 acid-alkali 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 (Wang et al., 2006). As shown in Figure 3, it could be seen that the difference among the hemoglobin concentration of each group before swimming was not significant. However, the hemoglobin concentration of MDG and IHDG were significantly increased than that of the control group after swimming. The results showed that SCAE could affect the concentration of the Hb in the blood of mice after exercise.

Conclusion

Results from this study suggest that SCAE had significant anti-athletic fatigue effects on mice. It extended the

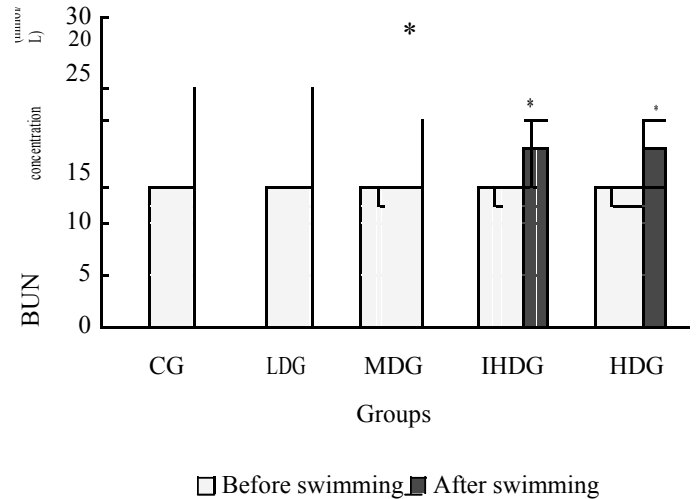


Figure 2. Effect of the *Schizandra chinensis* aqueous extracts (SCAE) on BUN in mice (mean \pm SD, $n = 8$). CG, Control group (mice treated with distilled water); LDG, Low-dose group (mice treated with 15 mg/kg SCAE); MDG, Medium-group (mice treated with 30 mg/kg SCAE); IHDG, Intermediate-high group (mice treated with 50 mg/kg SCAE); HDG, High-group (mice treated with 80 mg/kg SCAE); * $p < 0.05$ as compared with the control group.

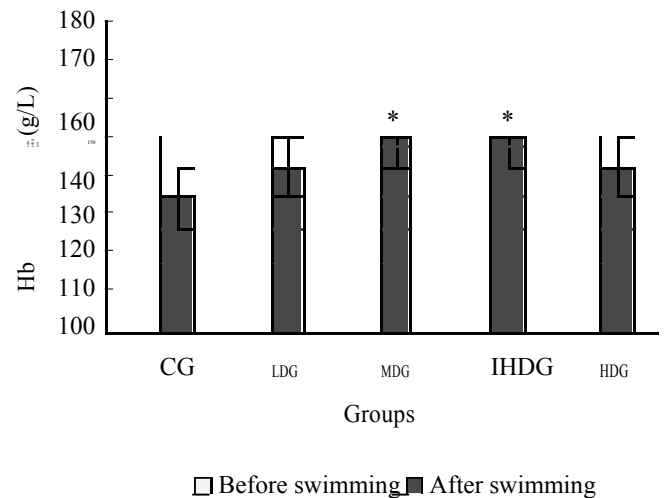


Figure 3. Effect of the *Schizandra chinensis* aqueous extracts (SCAE) on Hemoglobin in mice (mean \pm SD, $n = 8$). CG, Control group (mice treated with distilled water); LDG, Low-dose group (mice treated with 15 mg/kg SCAE); MDG, Medium-group (mice treated with 30 mg/kg SCAE); IHDG, Intermediate-high group (mice treated with 50 mg/kg SCAE); HDG, High-group (mice treated with 80 mg/kg SCAE); * $p < 0.05$ as compared with the control group.

swimming time, increased concentration of the Hb, prevent the increase in lactate and BUN concentrations. Toxicity data have already proved that the SCAE did not show any toxic reactions. Further studies to clarify the detailed mechanisms involved in the anti-athletic fatigue properties of SCAE are necessary.

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