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Full Length Research Paper

Study of safflower on blood lactate concentration and exercise function of mice after exercise

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Effects of safflower extracts on anti-fatigue and exercise function of mice were studied in this paper. The blood lactate concentration (BLA) experiment results indicated that the BLA of mice in the experimental groups were significantly lower than that in the control groups ($p < 0.05$) after swimming and rolling stick exercise. The exercise function of mice was evaluated by a calculation of the rolling-stick time, swimming endurance time and hypoxia tolerance time. The results showed that both rolling-stick and swimming endurance time of mice in the experimental groups were significantly prolonged ($p < 0.05$) with regards to the control groups. Following the treatment, the hypoxia tolerance time of mice in the low-dose group was significantly longer ($p < 0.05$) when compared with the control group. It was therefore concluded that safflower can relieve the exercise-induced fatigue of mice evidently and enhance the physical strength, endurance and exercise coordinating ability of mice, while the cardiac fitness and hypoxia tolerance of mice were improved.

Key words: Safflower extracts, animal, anti-fatigue, hypoxia tolerance, blood lactate concentration.

INTRODUCTION

Safflower (Honghua in China) is the flower of *Carthamus tinctorius* L., a member of the family Compositae or Asteraceae and an annual herb. It is soft with mild odor and slightly bitter in taste (Zheng, 1999). Safflower is both edible and medicinal and is issued by the Ministry of Health in China and consumed safely (Guan et al., 1999). The main ingredients of safflower are safflower glucoside, safflower yellow and safflower quinone. In addition, it also contains a small amount of oleic acid, linoleic acid, linolenic acid, flavonoids, amino acids and polysaccharides (Zheng, 1999). Safflower grows widely in many areas of China and it is one of the traditional Chinese medicinal herb in common use with its flowers to treat coronary heart disease and thrombosis, remove blood stasis, cure pain and swelling (Zheng, 1999; Zhang et al., 2005). Moreover, it was reported that safflower had the functions of anti-thrombosis and hypoxia tolerance, and can increase coronary flow and improve microcirculation (Ling, 2002). In recent years, the use of safflower as a coloring and flavoring agent has been increased as a food additive in some Asian countries (Nobakht et al., 2000). Modern pharmacological studies have shown that the safflower polysaccharide has the activity of anti-tumor and antioxidative effect (Jin et al., 2004; Shi et al., 2010). Safflower yellow A has the functions to relieve myocardial ischemia, protect neuron against hypoxia injury and attenuate acute lung injury induced by lipopolysaccharide administration in mice (Jin et al., 2005; Ye and Guo, 2008).

Exercise-induced fatigue, due to over-exercise, refers to the body that cannot maintain its specific level physiologically or cannot maintain the predetermined exercise intensity, manifested as mental and physical fatigue (Wu et al., 2003; Chen et al., 2004; Gao and Chen, 2003). Exercise-induced fatigue can be recovered by supplemented energetic substance, releasing metabolic production and administrated tonics, but these bring harms to the body even though retarding the fatigue (Li and Wei, 2005). In addition, some of the drugs are forbidden by the International Olympic Committee. During the process of seeking for safe and effective anti-athletic fatigue methods, the specialty of Chinese herbal medicine has drawn the attentions of scholars in the world (Shenhua et al., 2009) and some research results have been reported in recent years. However, no detailed study has been reported about safflower on the antifatigue activity and

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exercise function of mice. This study evaluated the antifatigue and exercise function activity of safflower by swimming exercise, rolling stick exercise and hypoxia tolerance experiment of mice. Furthermore, fatigue-related changes in BLA of mice were also determined. The results obtained from this study may offer further information to provide scientific evidence for application of safflower in other fields such as sports food.

MATERIALS AND METHODS

Experimental animals, materials and apparatus

Kunming mice, weighing 25 ± 2 g, were obtained at the Public Health College of Hebei Medical University, Shijiazhuang, China. Animals were allowed unlimited access to laboratory standard diet (purchased from Hebei Medical University) and water *ad libitum*. They were housed in standard cages (21.5 x 32 x 14 cm, 5 mice/cage) under controlled conditions of temperature (24 ± 2°C) and humidity (50 ± 2%), and with a 12-h light–dark cycle. The experiments were carried out according to the "principles of laboratory animal care" (World Health Organization (WHO) Chronicle, 1985).

Dry safflower was obtained from the local retailer in Shijiazhuang, China; revolving evaporator was purchased from Yarong Biochemical Instruments Co. Ltd (Shanghai, China); vacuum filter was provided by Taikang Biological Technology Co., Ltd (Shanxi, China); multi-function extractor and lactic acid meter were both purchased from ShunYi Tech. Co. Ltd (Shanghai, China) and Zhongxitaian Tech. Co. Ltd (Beijing, China).

Preparation of safflower extracts

Safflower reflux was extracted twice by multi-function extractor with 80% ethanol for 1 h at each time. The ratio (w/v) for safflower to ethanol was 1:8 at the first time and 1:6 at the second time. Following this, the vacuum filtered the mixture twice with extracted liquids and concentrated the mixture with revolving evaporator until the safflower content reached 0.5 g/ml extracts. As such, the extracts were used for the following experiments.

Determining the BLA experiment of mice

Mice (male/female) were assigned randomly into six groups (A to F) on the basis of basic diets:

Group A (control): Mice were administered 0.3 ml distilled water daily.
Group B (control): Mice were administered 1 ml distilled water daily.
Group C and Group E: Mice were administered 0.3 ml safflower extracts daily.
Group D and Group F: Mice were administered 1 ml safflower extracts daily.
Water and safflower extracts were both administered by oral gavages to the mice for 7 days.

Rolling-stick experiment

A 2.5 cm diameter wooden bat, operated by an electrical motor, was moved along the horizontal direction continuously, and the rotational speed was 16 r/min. Ten mice were taken out from each group (A, B, C and D) to make the rolling-stick experiment, and as such, the mice were rotated on the stick for 20 min.

Forced swimming experiment

The swimming exercise was employed in the study to evaluate the anti-fatigue activity of safflower extracts on mice. Ten mice were taken out from each group (A, B, E and F) to make the forced swimming experiment. The mice were forced to swim in an acrylic plastic pool (90 x 45 x 145 cm) filled with water to a depth of 35 cm (Matssumoto et al., 1996; Kamakura et al., 2001) and the temperature of the water was maintained at 34 ± 1°C. To avoid the influence of circadian variations in physical activity, swimming exercise was done from 11:00 to 17:00, a period during which minimal variation of endurance capacity has been confirmed in mice (Matsumoto et al., 1996). The mice must swim or float without their hind limbs or tail touching the bottom, and from which they cannot escape. Each of the mice had a weight attached (6% body weight) to the tail and swam for 10 min.

Determination of BLA

After swimming and rolling stick exercise, blood samples were collected from the veins on the tails of the individual mice, 20 min after swimming and rolling stick exercise. To avoid blood dilution with residual water at the tail of the animal, the mice were quickly dried with a towel immediately before blood collection and the BLA were determined by using a commercial diagnostic kit provided by Jiancheng Diagnostic System (Nanjing, China).

Determination of mice experiments on exercise function

Rolling-stick experiment

Female mice, weighing 25 ± 2 g, were randomly assigned into four groups on the basis of basic diets:

Group CA (control A): Mice were administered 0.3 ml distilled water daily.
Group CB (control B): Mice were administered 1 ml distilled water daily.
Group LD (low dose): Mice were administered 0.3 ml safflower extracts daily.
Group HD (high dose): Mice were administered 1 ml safflower extracts daily.

After water and safflower extracts were both administered by oral gavages to the mice for 7 days, ten mice were taken out from each group to prepare the rolling-stick experiment. As such, the continuous crawling time of mice in each group on the rolling stick was determined.

Swimming endurance experiment

Animals and treatments were the same as those previously discussed. Each of the mice had a weight attached (6% body weight) to the tail for the duration of the swimming exercise. The mice were assessed to be fatigued when they failed to rise to the surface of the water to breathe within 5 s (Ikeuchi et al., 2006, 2009) and as such, the swimming endurance time was determined.

Hypoxia tolerance experiment

Animals and treatments were the same as those previously discussed. Each of the mice was placed in the 250 ml airtight glass

Table 1. Effects of safflower extracts on BLA of mice after swimming.

Group	Dose (ml/10 g body weight)	BLA (mmol/l) (Mean ± SD)
A	0.3 (water)	9.1±1.56
C	0.3 (extracts)	7.0±1.06*
B	1 (water)	8.3±1.22
D	1 (extracts)	8.0±1.39

*p < 0.05 is significantly different from the control. BLA, blood lactate concentration

Table 2. Effects of safflower extracts on BLA of mice after rolling stick exercise.

Group	Dose (ml/10 g body weight)	BLA (mmol/l) (Mean ± SD)
A	0.3 (water)	18.3±2.39
E	0.3 (extracts)	16.9±2.17
B	1 (water)	17.5±2.06
F	1 (extracts)	13.7±2.28*

*p < 0.05 is significantly different from the controls

bottle containing 15 g lime each, respectively, and as such, the survival time (tolerance to hypoxia) of the mice was determined.

Statistical analysis

The data were analyzed using mean ± SD analysis and Student's t-test, while the probability (P) values below 0.05 were considered to be significantly different.

RESULTS AND DISCUSSION

Effects of safflower extracts on the BLA of mice after forced swimming and rolling stick exercise

The experimental results were shown in Tables 1 and 2. Blood lactate is one of the important indicators for judging the degree of fatigue (Yu et al., 2008; Lin et al., 2005) and it has been reported that blood lactate is accumulated during exercise (Dawson et al., 1971; Banister et al., 1983). Results in Table 1 showed that, the BLA of mice in 0.3 mL safflower extracts group were significantly lower than that in the control group ($p < 0.05$) after swimming. It could been seen from Table 2 that the BLA of mice in 1 ml safflower extracts group were significantly lower than that in the control group ($p<0.05$) after rolling stick exercise. The results suggested that safflower extracts could inhibit the production of blood lactate during exercise and relieve the exercise-induced fatigue greatly.

Effects of safflower extracts on exercise function of mice

The experimental results were shown in Figures 1 to 3. Figures 1 and 2 showed that both the rolling-stick time and swimming endurance time of mice in experimental groups (LD and HD) were significantly prolonged ($p<0.05$) than that in the control groups (CA and CB). It reflected that the extracts may enhance the physical strength, endurance and exercise coordinating the ability of mice evidently.

Results in Figure 3 indicated that the survival time of mice in the low-dose group (LD) was significantly longer ($p<0.05$) compared to the control group (CA) at hypoxia condition, which indicated that the extracts can enhance myocardium and oxygen utilization coefficient of mice and reduce the myocardial oxygen consumption to a certain extent.

There was no significant direct association that was observed between the dosage and effect from the experimental data. It seemed that the effect of the low-dose was more obvious than that of the high-dose. This had some correspondences with the previous reports that safflower extracts could enhance myocardium of mice at low dose, while it had inhibitory effects at high dose (Zheng, 1999). It was believed that safflower yellow contains many kinds of water-soluble mixtures such as safflower yellow A, B and C, which were the main functional factors of safflower currently (Zheng, 1999). In addition, it was reported that safflower had the effects of anti-inflammation and acesodyne (Ling, 2002) which was also helpful in reducing the pain and inflammation induced by exercises. However, the action mechanisms of safflower at present are unclear and it needed to be further studied.

Conclusion

Results from this study suggest that the safflower had

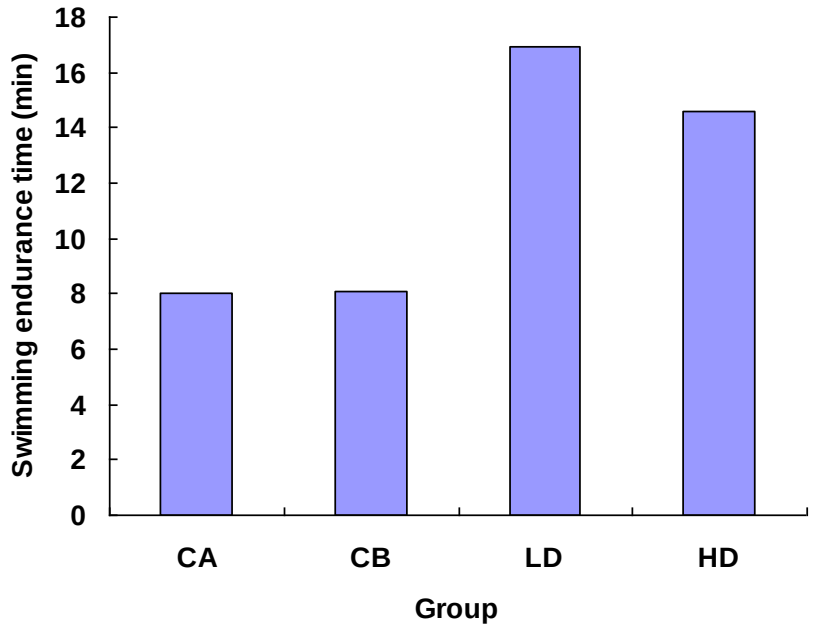

Figure 1. Effects of safflower extracts on rolling time of mice. n = 10; (Mean ± SD) p< 0.05 as compared with group CA or CB.

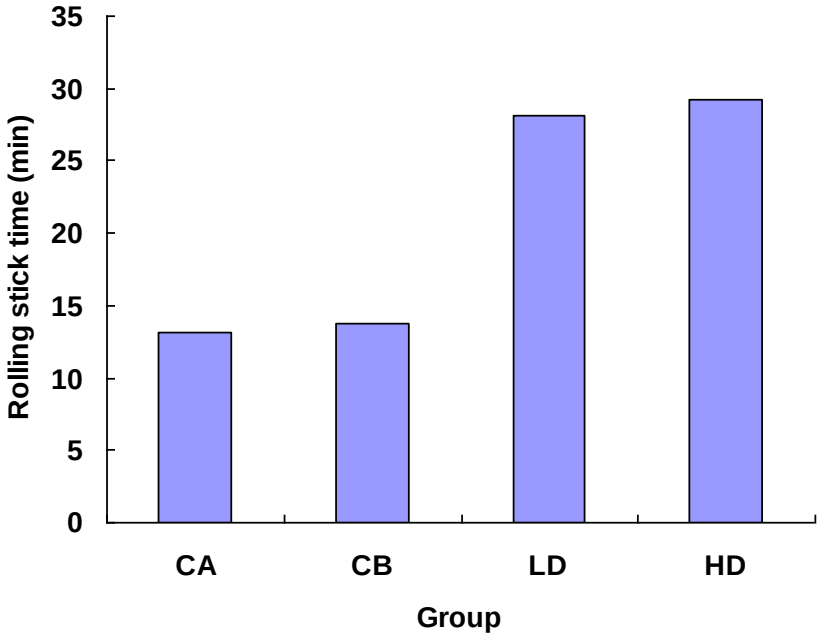

Figure 2. Effects of safflower extracts on swimming endurance time of mice. n = 10; (Mean ± SD) p<0.05 as compared with group CA or CB.

significant anti-fatigue effects and exercise on mice. It extended the swimming endurance time, rolling stick time and tolerance hypoxia time of mice evidently, and as such, it prevented the increase in BLA of mice. However, further studies to clarify the detailed mechanisms involved in the anti-fatigue and exercise properties of safflower are necessary.

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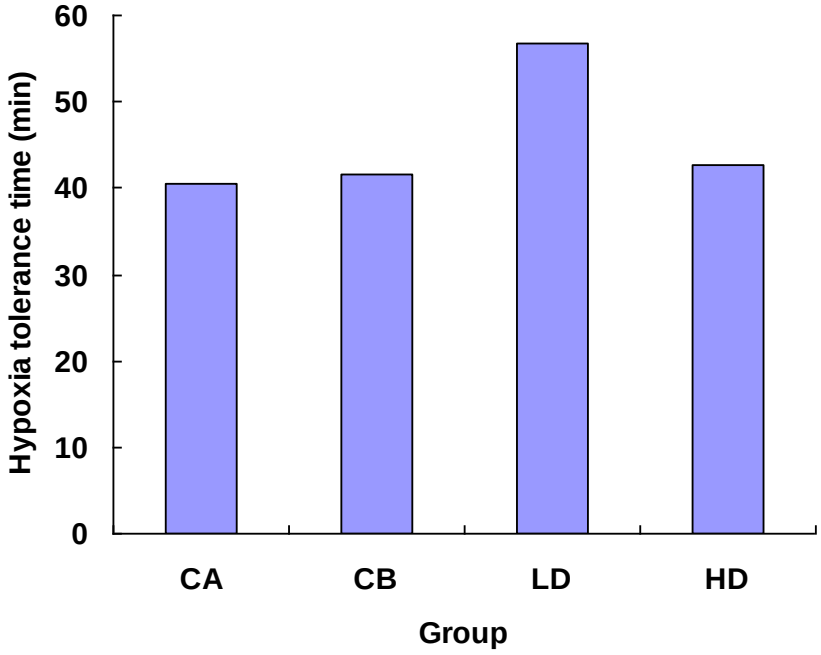

Figure 3. Effects of safflower extracts on hypoxia tolerance activity of mice. n = 10; (Mean ± SD) $p < 0.05$ as compared with group CA.

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