

*Full Length Research Paper*

# Antifatigue and sport performance enhancement effects of safflower extracts in mice

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**In this study, the antifatigue and sports performance enhancement effects of safflower extracts were evaluated in mice. Antifatigue effect of mice was judged by determining Blood Lactate Concentration (BLA) after a rolling stick and forced swimming exercise. Sports performance was evaluated by calculation of swimming endurance time and hypoxia tolerance time. BLA in test groups (weather low dose or high dose) was significantly lower than that of control groups ( $p < 0.05$ ) after forced swimming and rolling stick exercise. The swimming endurance time in the test groups was significantly prolonged ( $p < 0.05$ ) relative to the control groups. Furthermore, the hypoxia tolerance time in low-dose group was significantly longer ( $p < 0.05$ ) as compared to the control group. Safflower extracts detectably relieve exercise-induced fatigue; as well as significantly increase the exercise endurance capacity and hypoxia tolerance in mice.**

**Key words:** Anti-fatigue, safflower, sports performance, swimming exercise, hypoxia tolerance, blood lactate concentration.

## INTRODUCTION

Exercise-induced fatigue refers to the listless condition resulting from excessive exertion ("over-exercise"), leading to diminished bodily and mental functions (Wu et al., 2003; Chen et al., 2004; Gao and Chen, 2003). Exercise-induced fatigue has been attributed to the following factors. First, free radicals cause metabolic disturbance, which is the major factor in exercise-induced fatigue (Guo and Wu, 2007). Both normal and exhaustive exercises can cause an increase in free radicals in hepatic tissues and cause liver cell damage (Voces et al., 1999; Gul et al., 2006). Second, exercise causes the production and accumulation of products of metabolism, such as lactic acid. Third, exercise promotes the mobilization and consumption of energy sources such as glycogen (Grenhaff and Timmonns, 1998; Ikeuchi et al., 2006). In addition, "over exercise" diminishes the brain's supply of oxygen, which leads to fatigue (Ding and Li, 2009).

Exercise-induced fatigue can be assuaged by energy

supplements, but these can be deleterious (Li and Wei, 2005). It is a well known fact that some chemical agents or drugs forbidden by International Olympic Committee are a major problem in sport administration. Consequently, the search for approved, safe and effective antifatigue agents known to Chinese herbal medicine has drawn the attentions of scholars worldwide (Cao et al., 2009).

Safflower (named Honghua in China) is the flower of safflower (*Carthamus tinctorius* L.), a member of the family Compositae or Asteraceae, an annual herb. The herb has a faint aroma and is slightly bitter (Zheng, 1999). Safflower is both an edible and medicinal plant issued by 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 (Zheng, 1999). Safflower grows widely in many areas of China and it is one of the traditional Chinese medicinal herb in common use. The flowers are used to treat coronary heart disease and thrombosis, remove blood stasis, and cure pain and swelling (Zheng, 1999; Zhang et al., 2005). Moreover, it was reported that safflower has the functions of anti-thrombosis and hypoxia tolerance, can increase coronary flow and

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improve microcirculation (Ling, 2002). In recent years, the use of safflower as a coloring and flavoring agent in food is increasing in popularity in some Asian countries (Nobakht et al., 2000). Modern pharmacology has shown that safflower has free radical scavenging activity and neuroprotective effect. It can be used to improve neuropsychological disorders (Hiramatsu et al., 2009; Zhao et al., 2009).

However, there has been little research of safflower's antifatigue effects and sport performance enhancement in mice. This study evaluated the antifatigue and sport enhancement activity of safflower, assessed by forced swimming and rolling stick exercises, and hypoxia tolerance in mice. Furthermore, fatigue-related changes in Blood Lactate Concentration (BLA) were also determined. The results obtained from this study may offer further information for application of safflower in sports dietetics and nutrition.

## MATERIALS AND METHODS

### Animals

Kunming mice, weighting  $25 \pm 2$  g, were used for the experiments. They were obtained from 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 ( $24 \pm 2^\circ\text{C}$ ), humidity ( $50 \pm 2\%$ ), and with a 12 h light-dark cycle. The experiments were carried out according to the "Principles of Laboratory Animal Care" (World Health Organization Chronicle, 1985).

### Materials and instruments

Dry safflower was obtained from a 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), respectively.

### Preparation of safflower extracts

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

### Determining experiment of BLA of mice

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

Group A (control): mice were administered 0.3 ml distilled water daily by oral gavage for 7days.

Group B (control): mice were administered 1ml distilled water daily by oral gavage for 7days.

Group C and Group E: mice were administered 0.3 ml safflower extracts daily by oral gavage for 7days.

Group D and Group F: mice were administered 1 ml safflower extracts daily by oral gavage for 7days.

### Forced swimming experiment

The swimming exercise was employed in the study to evaluate anti-fatigue activity of safflower extracts on mice. Ten mice were taken out from each group (A, B, C, D) to make 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 (Matsumoto et al., 1996; Kamakura et al., 2001). The temperature of the water was maintained at  $34 \pm 1^\circ\text{C}$ . 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.

### Rolling-stick experiment

A 2.5 cm diameter wooden bat operated by electrically motor moved along the horizontal direction continuously, the rotational speed was 16 r/min. Ten mice were take out from each group (A, B, E, F) to make rolling-stick experiment. The mice rotated on the stick for 20 min.

### Determination of BLA

After swimming and rolling stick exercise, blood samples were collected from the veins on the tails of 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 sport performance in mice

#### Swimming endurance experiment

Female mice, weighting  $25 \pm 2$  g, were randomly assigned into four groups on the basis of basic diets:

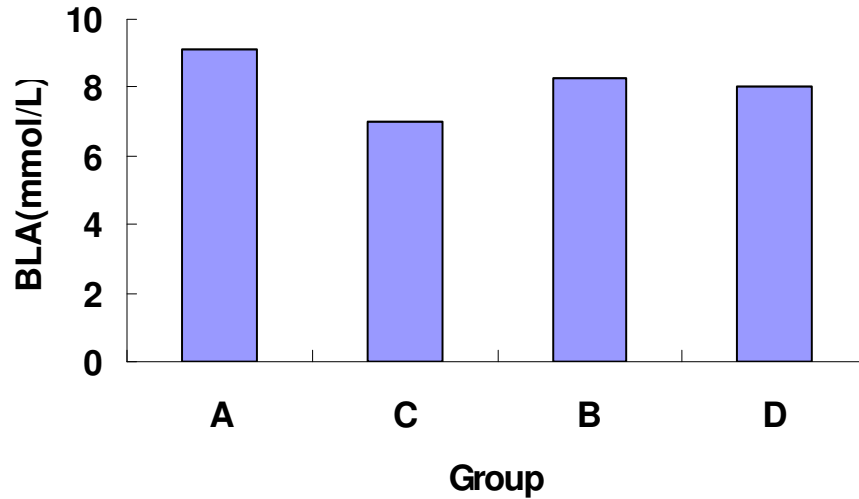
Group CL (control L): mice were administered 0.3 ml distilled water daily by oral gavage for 7days.

Group CH (control H): mice were administered 1 ml distilled water daily by oral gavage for 7days.

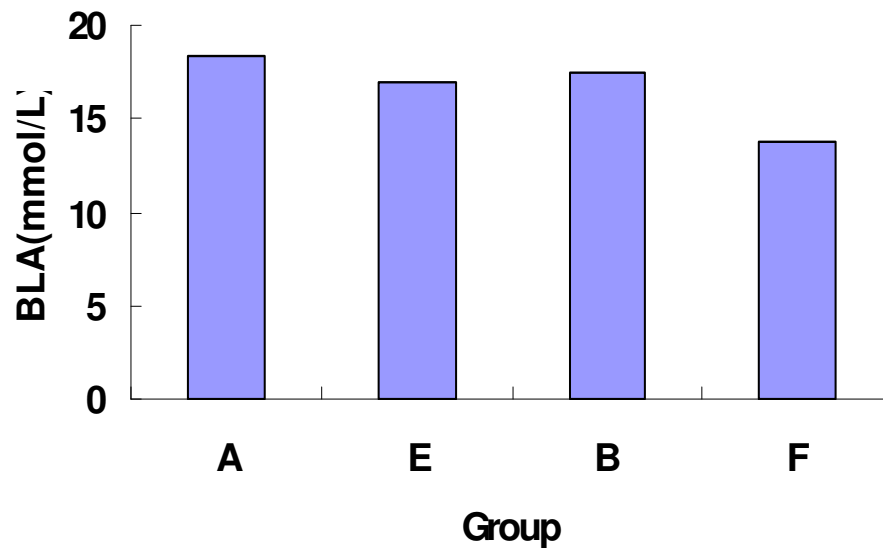
Group LD (low dose): mice were administered 0.3 ml safflower extracts daily by oral gavage for 7days.

Group HD (high dose): mice were administered 1ml safflower extracts daily by oral gavage for 7days.

Ten mice were taken out from each group to make swimming endurance exercise. 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). The swimming endurance time was determined.



**Figure 1.** Effects of safflower drink on BLA of mice after forced swimming exercise (mean  $\pm$  SD, n=10). Compared the administered group with the control group (C Vs A),  $p < 0.05$ .



**Figure 2.** Effects of safflower extracts on BLA of mice after rolling stick exercise (mean  $\pm$  SD, n=10). Compared the administered group with the control group (F Vs B)  $p < 0.05$ .

### Hypoxia tolerance experiment

Animals and treatments were the same as above. Each of the mice was placed in the 250 ml airtight glass bottle containing 15 g lime each respectively. Determined the survival time (tolerance to hypoxia) of mice.

### Statistical analysis

The results are expressed as mean  $\pm$  SD and the Student's t-test was used as a test of the null hypothesis. The level of statistical significance was set at  $p < 0.05$  or below.

## RESULTS AND DISCUSSION

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

The experimental results were shown in Figures 1 and 2. Blood lactate is an important indicator for judging the degree of fatigue (Yu et al., 2008; Lin et al., 2005). It has been reported that blood lactate accumulates during exercise (Grenhaff and Timmonns, 1998; Ikeuchi et al., 2006). Results in Figure 1 showed that the BLA of mice in

0.3 ml safflower extract group (C) was significantly lower than that in control group (A,  $p < 0.05$ ) after forced swimming exercise. It can be observed from Figure 2 that the BLA of mice in 1 ml safflower extracts group (F) were significantly lower than that in control group (B,  $p < 0.05$ ) after rolling stick exercise. The results suggested that safflower extracts may inhibit the production of blood lactate during exercise and relieve the exercise-induced fatigue.

### Effects of safflower extracts on sports performance in mice

The experimental results were shown in Tables 1 and 2.

The data from Table 1 showed that the swimming endurance time of mice in experimental groups (LD, HD) were significantly prolonged ( $p < 0.05$ ) than that in control groups (CL, CH). It reflected that the extracts may enhance the physical strength, endurance and exercise coordinating ability of mice.

Results in Table 2 indicated that the survival time of mice in LD group was significantly longer ( $p < 0.05$ ) compared with the control group (CL) at hypoxia condition, indicating that the extracts can improve myocardial function, oxygen utilization coefficient of mice and reduce the myocardial oxygen consumption to a certain extent.

There was no significant direct association observed between the dosage and effect from the experimental data. It seems that the effect of low-dose was more obvious than that of high-dose. This has some correspondences with the previous reports that safflower extracts could enhance myocardium of mice at low dose while having inhibitory effects at high dose (Zheng, 1999). It is currently believed that safflower yellow, which contains many kinds of water-soluble ingredient such as safflower yellow A, B and C, is the main functional factor of safflower (Zheng, 1999).

### Conclusion

Results from this study suggest that safflower has significant antifatigue effects and sport activity enhancement in mice. The herb extended the swimming endurance time, rolling stick time, hypoxia tolerance time and significantly prevented increases in BLA. Further studies to clarify the detailed mechanisms involved in the antifatigue and performance enhancement properties of safflower are necessary.

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**Table 1.** Effects of safflower extracts on swimming endurance time of mice; (mean  $\pm$  SD, n=10). Compared the administered groups with their respective control groups (LD Vs CL; HD Vs CH), \* $p < 0.05$ .

Group	Swimming endurance time (min) (mean $\pm$ SD)
CL	8.0 $\pm$ 3.2
CH	8.1 $\pm$ 3.1
LD	16.9 $\pm$ 5.7*
HD	14.6 $\pm$ 2.6

**Table 2.** Effects of safflower extracts on hypoxia tolerance time of mice; (mean  $\pm$  SD, n=10). Compared the administered group with the control group (LD Vs CL), \* $p < 0.05$ .

Group	Hypoxia tolerance time (min) (mean $\pm$ SD)
CL	39.6 $\pm$ 7.5
CH	41.5 $\pm$ 8.7
LD	56.7 $\pm$ 9.3*
HD	42.7 $\pm$ 9.1

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