Full Length Research Paper

Effect of aqueous extract of *Lycium barbarum* on serum CK, CK-MB activities and myocardium injury in exhausted swimming rats

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Studies were undertaken to evaluate the effects of aqueous extract of *Lycium barbarum* on serum creatine kinase (CK) and creatine kinase mass (CK-MB) activities in exhausted swimming rats. Forty Wistar rats were randomly assigned to five groups: normal control group (NC), exhausted swimming model control group (MC), low dose of LBE administration group (I), middle dose of LBE administration groups (II) and high dose of LBE administration groups (III). From the 31st day, except for normal control rats, all rats received exhausted swimming exercise for 1 week. Results showed that LBE pre-treatment can reduce serum CK, CK-MB activities and myocardium injury in exhausted swimming rats.

Key words: Creatine kinase, creatine kinase mass, exhausted swimming.

INTRODUCTION

Exhausting (Khanna et al., 1999, Gul et al., 2003; Oztasan et al., 2004) or moderate (Gul et al., 2001) exercise, in rats may increase ROS production exceeding the capacity of antioxidant defences. Oxidative stress is the imbalance of pro- and anti-oxidants in favor of the former. Exercise-induced oxidative stress was also reported in thoroughbred racehorses after a 1000 m race at maximum velocity (White et al., 2001). Increased oxidative stress can be harmful to all cellular macromolecules, such as lipids, proteins and DNA (Halliwell and Gutteridge, 1984). In contrast, beneficial effects of endurance training on antioxidant defense mechanisms in various tissues have been reported by swim (Kanter et al., 1985; Venditti and Di Meo, 1996; Oluduro and Aderiye, 2009) and treadmill-trained normal (Sen et al., 1992; Oztasan et al., 2004) and diabetic (Gul et al., 2002; Sevindi et al., 2010) rats, and also in horses (Avellini et al., 1999). Lycium barbarum L. belongs to the plant family Solanaceae. Red-colored fruits of L. *barbarum* have been used as a traditional Chinese herbal medicine for thousands of years (Gao et al., 2000). The earliest known Chinese medicinal monograph documented medicinal use of L. barbarum around 2,300 years ago. L. barbarum fruits have a large variety of biological activities and pharmacological functions and play an important role in preventing and treating various chronic diseases, such as diabetes, hyperlipidemia, cancer, hepatitis, hypo-immunity function, thrombosis, and male infertility (Gao et al., 2000; Li, 2001). In the present study, we examined serum CK and CK-MB activities and myocardium injury in exhausted swimming rats.

MATERIALS AND METHODS

Aqueous extract of *L. barbarum* was prepared in our laboratory.

Experimental animals and design

Forty male Wistar rats were purchased from the Institute of Experimental Animals, (Guangzhou, China) and were randomly divided into 5 groups (n = 8 per group): normal control group (NC), exhausted swimming model control group (MC), low dose of LBE administration groups (I), middle dose of LBE administration groups (II) and high dose of LBE administration groups (III). Rats in group NC and MC were fed with basic diet for 30 days. Rats in LBE-treatment groups were fed with basic diet containing 0.5, 1 and 1.5% *L. barbarum* extract, respectively for 30 days.

The swimming exercise was carried out in a tank ($60 \times 100 \times 60$ cm), filled with water to 30 cm depth and maintained at a temperature of 30 ± 1 °C. The swimming time to exhaustion was used as the index of the forced swimming capacity. The rats were assessed to be exhausted when they failed to rise to the surface of water to breathe within a 7 s period (Jung et al., 2004; Ishola et al., 2008; Alim et al., 2009). The exercise last for 1 week. Normal control

Table 1. Effect of LBE on rats' body weight.

Group	Body weight
Normal control	263.51±22.98
Exhausted swimming	264.87±20.74
Lycium barbarum extract (I)	268.26±25.05
Lycium barbarum extract (I)	267.12±21.47
Lycium barbarum extract (I)	264.92±28.31

 Table 2. Effect of LBE on serum CK activity in rats.

Group	CK (U/L)
Normal control	163.51±11.73
Exhausted swimming	893.59±37.23 ^b
Lycium barbarum extract (I)	712.43±29.67 [°]
Lycium barbarum extract (II)	503.62±30.11 ^d
Lycium barbarum extract (III)	353.19±25.03 ^d

^b P<0.01, vs normal control rats; ^c P<0.05, ^d P<0.01, vs exhausted swimming model control rats.

Table 3. Effect of LBE on serum CK-MB activity in rats.

Group	CK-MB
Normal control	37.86±2.07
Exhausted swimming	207.43±19.68 ^b
Lycium barbarum extract (I)	168.33±14.22 ^d
Lycium barbarum extract (I)	127.84±10.31 ^d
Lycium barbarum extract (I)	82.23±3.29 ^d

^b P<0.01, vs normal control rats; ^c P<0.05, ^d P<0.01, vs exhausted swimming model control rats.

rats did not receive exhausted exercise. The experimental protocol was approved by the Animal Studies Committee of china.

After the treatment (24 h after the last administration), the animals were sacrificed by decapitation always between 8:00 and 10:00 AM and fresh blood was immediately collected into heparinised test tubes for routine biochemical analysis.

Biochemical analysis

Serum creatine kinase and creatine kinase mass activities were measured by Autoanalyser Synchron CX5, (using reagents supplied by Wipro Biomed).

Statistical analysis

The statistical significance comparing data between groups was assessed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range posthoc tests. Statistical analysis was performed using SPSS (Windows, Version Rel. 10.0.5, 1999, SPSS Inc., Chicago, IL). Statistical significance was declared when P < 0.05.

RESULTS

After experiment, there was significant (P>0.05) difference in rats' body weight between groups (Table 1). Creatine kinase (CK), also known as creatine phosphokinase (CPK) or phospho-creatine kinase (and sometimes wrongly as creatinine kinase), is an enzyme expressed by various tissues and cell types. CK catalyses the conversion of creatine and consumes adenosine triphosphate (ATP) to create phosphocreatine (PCr) and adenosine diphosphate (ADP). This CK enzyme reaction is reversible, such that also ATP can be generated from PCr and ADP (Goldblatt, 1969). The determination of serum CK and CK-MB is a well-established and widely accepted method for the laboratory diagnosis and follow-up of myocardial infarction.

Strenuous exercise can damage skeletal muscle, a condition known as exertional rhabdomyolysis (Lemos et al., 2004; Rinard et al., 2000). This damage is manifested by delayed-onset pain and soreness, weakness, and increases in the circulation of such muscle proteins as create kinase (CK), lactate dehydrogenase (LDH), and myoglobin (Mb)(Sayers and Clarkson, 2001). Myocardial infarction and sudden death during intense physical exercise have been reported (Mittleman et al., 1993; Willich et al., 1993), whereas moderate exercise is recognized as beneficial in reducing the risk of heart disease and is therefore recommended (Pate et al., 1995).

Compared with normal control rats, serum CK activity (893.59±37.23 vs 163.51±11.73) was markedly (P<0.01) increased in exhausted swimming model rats. Pretreatment of LBE dose-dependently significantly reduced serum CK activity compared to exhausted swimming model rats. Minimum values for CK activity (353.19±25.03) were observed in group LBE (III) (Table 2).

Typically, CK-MB increases above normal within the first 4 to 8 h following an AMI, reaching maximum concentrations between 12 and 24 h. CK-MB should be evaluated in context to the total CK (relative index). If the relative index is more than 5, the heart is the likely muscle damaged. A high CK with a very low relative index suggests that other muscles were damaged. Blood concentrations of CK-MB can be elevated as a result of acute or chronic muscle damage, including strenuous exercise and trauma (Storm, 1984). Table 3 represents the activities of CK-MB in blood of rats after supplementation with LBE extract. The status of blood CK-MB activity was found to be significantly (P<0.01) increased in blood of exhausted swimming rats compared to normal control rats. Pre-administration of LBE for 30 days significantly (P<0.01) reduced the CK-MB activity in rats in a dose-dependent manner when compared to exhausted swimming model control rats.

Previous studies have shown that *L. barbarum* polysaccharides (LBP) can enhance exercise endurance

capacity, reduce fatigue and exhibit antioxidant activity *in vitro* and *in vivo* (Zhang, 1993; Lin et al., 2009; Luo et al., 2000; Chen et al., 2009; Yao and Li, 2010). In exhausted swimming model rats, the myocyte apoptosis occurred after T lymphocyte infiltration in myocardium. A large areas of myocytes lytic necrosis was detected. However, in LBE pre-treatment rats, some symptom was markedly improved. Our result indicated that *L. barbarum* extract could reduced serum CK and CK-MB activities and reduce fatigue in exhausted swimming rats.

DISCUSSION

In the exhausted swimming rats, exhausted exercise resulted in an increased growth in serum CK activity, accompanied by increases in serum CK-MB activity. This increase, however, was markedly reduced in the exhausted exercise rats after pre-administration of LBE. Moreover, it has been observed that LBE can alleviate myocardium injury in exhausted swimming rats.

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