

Research Article

The Effect of *Limonium sinense* (Girard) Kuntze on Fatigue and Recovery after Exercise

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Abstract: To evaluate the physical and chemical properties of polysaccharides from selenium-rich tea and the effects of these polysaccharides on swimming performance and recovery from swimming-induced fatigue in mice. By setting up a 5-week endurance exercise model in mice and the mice were given by oral gavage different doses of *Limonium sinense* (Girard) Kuntze (LSK). The swimming exhaustion time of mice was recorded and the concentrations of blood glucose, Blood Urea nitrogen (BUN), Blood Lactic Acid (BLA) and the contents of hepatic glycogen and Malonaldehyde (MDA) as well as the activity of Hepatic Glutathione Peroxidase (GSH-Px) were measured immediately and at 24 h after exhaustive exercise. LSK were Se-binding glycoproteins and could prolong the swimming exhaustion time, improve the carbohydrate metabolism, significantly increase the activity of GSH-Px and reduce the content of MDA in liver tissue of mice. LSK have an anti-fatigue effect in mice and the underlying mechanism is related to regulating the carbohydrate metabolism and improving tissue lipid peroxidation caused by excessive exercise.

Keywords: Exercise-induced fatigue, exhaustive exercise, *Limonium sinense* (Girard) Kuntze (LSK)

INTRODUCTION

LSK, a traditional Chinese folk herbs, is a white Danko perennial herb. The roots or the whole plant can tonify the spleen, enrich the blood, invigorate the circulation of blood, stop bleeding and regulate the menstrual, mainly used for the treatment of colds, blood loss, blood heat and menorrhagia embolism (Jun and Binghua, 2008). LSK polyphenol extract has a strong capacity of scavenging free radical and the activity is stronger than the synthetic antioxidants-BHT which is of the same concentration (Bingha and Jing, 2013). The activity of scavenging free radicals in natural extract is often depended on its antioxidant capacity. For the further study of the antioxidant capacity of LSK and a comprehensive understanding and evaluation of LSK antioxidant potential of polyphenols extracted material, we base our theory on the special physiological phenomenon of endurance-sports fatigue and biological activity of polyphenol extract. In the experiment, we obtained LSK polyphenol extracts from the roots with ultrasonic assisted extraction-macroporous resin adsorption, established a five weeks model of mice endurance-sports, detected the effects of LSK on mice endurance exercise, behavioral manifestations and fatigue recovery by feeding different doses of LSK polyphenol solution, aimed to provide experimental support for the further development of natural antioxidants and sports supplements.

MATERIALS AND METHODS

Materials: LSK are collected from beach and wetlands, Chongwu town, Fujian, taken after washing the roots of the plants, 50°C drying, crushed through a 60 mesh sieve and stored for use at 4°C. Take appropriate amount of samples, with volume fraction of 45% ethanol as extracting agent, prepare polyphenol extract, respectively with ultrasonic assisted extraction-macroporous resin adsorption. For details see Ref (Jun *et al.*, 2009). Extracts stored at -20°C refrigerator for backup.

Kunming male mice 80, body mass 22~26 g, provided by the Experimental Animal Center of Fujian Medical University. Fed with National standard rodent dried food, free diet, animal room temperature (23±5)°C, relative humidity of 40~70%, divided cages. After one week's adaptive feeding, mice were randomly divided into four groups were tested: control group (A), positive control group (group B), administration of low-dose group (C1) and high-dose groups (C2 group), 20 mice in each group.

Basic model:

Establish motion model: Each group of mice are kept in a plastic pool (length and breadth: 80×60×50 cm, water temperature 30°C, depth 40 cm) for a period of five weeks of swimming training, tail-loaded swimming training (load of 5% of body weight) for 90 min at 8 every night from Monday to Saturday, take a rest on Sundays, load exhaustive swimming on the last week's Saturday (Exhaustive judging criteria: the mouse head

submerged in the water for more than five seconds). Gavage the mice Every time after exercise: C1 and C2 groups of mice were administered a dose of 100 and 200 mg/kg•day of LSK polyphenol solution; Group A of mice fed an equal volume of saline; group B as a positive control group, fed sucrose solution, as the reference for 6 g/kg•day (Haijun and Liang, 2009) Gavage for 30 days.

Determination of indicators and methods:

Measurement of blood and some biochemical indicators of liver tissue will record the exhaustive time of mice. After exhaustive swimming, randomly select half of the mice in each group and kill for material. Determination of the project include: blood glucose, Blood Lactate (BLA), Blood Urea Nitrogen (BUN) concentration; glycogen, liver tissue Malondialdehyde (MDA) content and GSH-Px activity of liver tissue. Twenty four hour after recovery, sacrifice the remaining half of the mice in each group to collect blood and material and measure the same projects. All the kits purchased from Nanjing Jiancheng biologics company and all the instructions followed. Use SPSS 20.0 software for statistical analysis, use the t test to compare the difference between the two groups, experimental data expressed with $\bar{x}\pm s$.

RESULTS AND DISCUSSION

Results: Comparing each group of mice swimming time. Table 1 shows that the exhaustive time of the sucrose group was significantly prolonged compared ($p<0.05$) with the control group, the exhaustive time of the high LSK polyphenols dose group was significantly prolonged compared with the control group ($p<0.01$). This showed that blood sugar and high doses of polyphenols grass could delay the exhaustive fatigue in mice, but there was no significant difference between the two.

Measurement results of some biochemical indicators in each group of mice blood. As can be shown in Table 2, judging from the comparison of the blood glucose concentration immediately after exercise in mice, only the high LSK polyphenols dose group was significantly higher than the control group ($p<0.05$), 24 h recovery after exercise, there was no significant difference between the groups of mice blood glucose concentration, but significantly increased compared with the same set of exercise immediate group ($p<0.05$,

$p<0.01$). Since the energy requirements reduced the blood glucose levels in mice during exercise. After 24 h recovery, blood glucose in mice in each group recovered well, but there was no significant difference between the groups, which is related to the body glucose homeostasis. In the comparison of nitrogen concentration in blood and urea, both the sucrose group and the high or low LSK polyphenols dose groups showed different degrees of decrease compared with the control group. This showed that after the sucrose and LSK polyphenols participated in the energy supplying, the protein catabolism was significantly saved in mice. In contrast to the same group before and after restoration, in addition to the control group, the nitrogen in other groups of mice blood or urea have significantly reduced ($p<0.05$). Comparison between the groups in blood lactic acid, the blood lactic acid concentration of both the sucrose group and the high or low LSK polyphenols group was significantly lower than the control group ($p<0.05$); in the comparison between the same group, 24 h recovery after exercise group compared with the immediate exercise group, each group of mice blood lactate concentrations were reduced very significantly ($p<0.01$). Description of blood lactate can be restored in a relatively short time.

Measurement results of some biochemical indicators in each group of mice liver tissues. The results in Table 3 show that, compare the glycogen content in each immediate group after exercise, there was no significant difference, which means that exhaustive exercise cause the body's glycogen depletion in each group of mice. But after 24 h of recovery, there was a significantly glycogen recovery in each group mice ($p<0.05$), the recovery of sucrose group and LSK polyphenols high-dose group was significantly better than the control group ($p<0.05$). In contrast to liver tissue GSH-Px activity, whether it is immediate group or recovery group after exercise, the GSH-Px activity of LSK polyphenol group were higher than the control group and the positive control group in varying degrees ($p<0.05$, $p<0.01$) and compared to the same group, the mice GSH-Px activity was significantly decreased

Table 1: Comparison of swimming exhaustion time in mice (n = 20)

Group	A	B	C1	C2
Time (min)	246	285*	270	293**

*: Significant difference ($p<0.05$) in group A; **: Highly significant difference from group A ($p<0.01$); (the next table is the same)

Table 2: Comparison of blood biochemical indexes in mice ($\bar{x}\pm s$, n = 10)

Group	Blood glucose concentration (mmol/L)		Blood uric acid nitrogen concentration (mmol/L)		Blood lactate concentration (mmol/L)	
	Group after training	After training recovery group	Group after training	After training recovery group	Group after training	After training recovery group
A	4.19±1.01	7.07±0.95 ^{###}	14.08±2.16	12.69±0.12	369.91±25.98	217.03±30.02 ^{###}
B	5.26±1.58	6.21±1.09 [#]	11.23±1.03*	9.85±1.42 [#]	309.96±36.69*	263.21±37.89 ^{##}
C1	5.26±1.96	7.28±1.21 [#]	12.73±2.09	10.59±0.53 [#]	334.12±25.36*	287.01±45.26 ^{##}
C2	5.89±1.48*	7.41±1.52 [#]	11.69±2.05*	10.47±0.51 [#]	300.01±30.97*	231.02±63.93 ^{###}

[#]: Significant difference with the same group compared with group after training ($p<0.05$); ^{##}: Highly significant difference with the same group compared with group after training ($p<0.01$); (the next table is the same)

Table 3: Comparison of liver tissue biochemical indexes in mice ($\bar{x}\pm s$, n = 10)

Group	Hepatic glycogen content/ (mg/g)		GSH-Px activity/ (U/mg pro)		MDA content/ (mmol/mL)	
	Group after training	After training recovery group	Group after training	After training recovery group	Group after training	After training recovery group
A	16.04±6.17	22.62±1.35 [#]	51.77±11.26	47.51±5.92 [#]	9.03±1.86	5.16±0.42 [#]
B	18.16±8.03	25.43±2.97* [#]	51.69±12.85	44.51±10.32 [#]	9.35±4.01	93.02±1.45 ^{##}
C1	17.02±5.03	26.11±5.45 [#]	60.14±11.27* [▲]	47.06±5.25 ^{▲#}	7.32±1.47*	4.67±0.36* [▲]
C2	19.15±2.96	30.22±3.19* [#]	61.23±15.24** [▲]	51.19±6.03* ^{▲#}	8.11±2.21*	4.35±0.16* [▲]

▲: Indicates a significant difference (p<0.05) compared with the positive control group (group B)

(p<0.05). In comparison of MDA in the same group, the MDA value of each recovery group were significantly lowered than the immediate group (p<0.05, p<0.01), but between the two groups, the MDA content of LSK polyphenol group was significantly lower than the control group and sucrose group immediately after exercise or 24 h after exercise, which means the LSK polyphenol may enhanced the GSH-Px activity in mice liver tissues.

Discussion: Studies have shown that in the long high-intensity exercise, glycogen depletion, lowering blood glucose concentration, accumulation of lactic acid can induce the occurrence of exercise-induced fatigue (Aoi *et al.*, 2011). Monosaccharides and disaccharides are easily absorbed by the digestive system that can quickly recover and improve the body's glycogen reserves, thereby improving recovery after exercise fatigue. Currently in sports fatigue and carbohydrate supplement study, sucrose sugar supplement is one of the main species, so the sucrose complementary group was taken as a positive control group. The generation of fatigue after exercise is also related to oxidative injury, environmental changes, decreased immune function and the body's protective inhibition and other multiple factors, so different factors should be taken into consideration in terms of sports supplements study. Because of high molecular weight polysaccharides, it cannot be easily absorbed by the digestive system, but numerous studies have shown that polysaccharide can improve the intestinal mucosal immune system and indirectly enhance the body's immune system (Jentjens *et al.*, 2005). In addition, there are also a number of studies showing that polysaccharide can improve oxidative damage caused by the exercise. This shows that the mechanism of action sports supplement polysaccharide fatigue may be related to improved immune function and antioxidant capacity of the body. The results confirm that high doses of polyphenols LSK may delay the exhaustive time of mice, which is similar to the positive control group. However, both the pathway and mechanism may be different.

In this experiment, three blood biochemical parameters: Blood glucose, blood urea nitrogen, blood lactate, are able to indirectly reflect the movement of blood biochemical parameters fatigue (Chen *et al.*, 2014). The results show no significant change in blood glucose, which may be related to blood glucose homeostasis. BUN is an indicator of body protein

metabolism. Usually under the circumstance of a long high-intensity exercise, insufficient glycogen reserves, protein will involve more in metabolism. This experiment proved that the protein metabolism can be saved by complementing sucrose and LSK polyphenols. Lactic acid content reflects the extent of anaerobic glycolysis sugar in another aspect and it is also an indicator of the body's aerobic capacity. The experimental results show that lactate content is reduced compared with the control group after complementing LSK polyphenols and sucrose, indicating that both the LSK polyphenols and sucrose will promote the body's aerobic capacity of sugar. Glycogen reserves is an important indicator of the level of aerobic capacity and body's fatigue level. The experimental results show that the restoration of glycogen is significantly enhanced by the sucrose supplement and high doses of LSK polyphenols.

MDA may reflect the degree of tissue lipid peroxidation, GSH-Px can remove lipids hydroxide and organic hydroperoxide, reducing body injury (Zhonghui *et al.*, 2014). Experimental results show that after taking LSK polyphenols, whether in immediate group or recovery group after exercise, the GSH-Px activity were significantly increased in mice liver tissue, while the content of MDA decreased significantly. Although sucrose and LSK polyphenols can also delay the fatigue in mice, but LSK polyphenol is significant in enhancing the antioxidant enzymes and protecting the body from oxidative damage. It remains to be confirmed whether LSK polyphenols have an impact on exercise-induced fatigue immunologically.

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