

Protection of Lotus Seedpod Proanthocyanidins on Organs and Tissues under High-intensity Exercise

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Abstract: Lotus seedpod proanthocyanidins (LSPC) as a kind of polyphenols is widely used in medicines, cosmetics, health products. High-intensity exercise can cause damage to the body's organs and tissues. Different doses of LSPC is given to mice to check the function of protect effect to the body's organs and tissues under high-intensity exercise. The hemoglobin (HB) content, red blood cell (RBC) number and white blood cell (WBC) number were tested for mice after exercise. The activity of superoxide dismutase (SOD) and the contents of glutathione (GSH) and malondialdehyde (MDA) in muscle and viscera were evaluated. The result showed that LSPC can effectively reduce inflammation reaction in the body of mice with high intensity exercise, alleviate oxidative stress-induced injury of tissues and organs, and execute protective function on skeletal muscle and cardiac muscle. And the LSPC could enhance myocardial anti-oxygen and enzymatic activity which suggests the protective effects of resveratrol against exercise-induced myocardial damage in mice.

Keywords: Exercise-induced myocardial damage, inflammation reaction, high-intensity exercise, lotus seedpod proanthocyanidins.

1. INTRODUCTION

People more and more attention to preserve one's health in the 21st century, nutrition care, fitness and sports issues such as how to better promote metabolism has been the attention of people. As is known to all, appropriate exercise can promote metabolism, improve blood circulation and respiratory function, to strengthen the body's adaptability and resistance to disease. However there are many people who do not adhere to appropriate intensity of exercise, and sports is becoming more and more interested in the strength of some big [1-3]. As early as 1982, some scholars pointed out that acute intensive sports make the body produces a large number of free radicals. Many studies have confirmed that the acute intensive games accelerate metabolism of the body, the body's oxygen consumption increased, make the body in a short period of time to generate the amount of free radicals, makes the body lipid peroxidation and cell membrane damage. Cause a certain degree of damage to the body [4, 5], a reduction in the ability to cause the body movement. At the same time, free radicals are involved in many pathological processes, such as inflammation, tumors, cardiovascular disease, aging, development of exercise-induced injury and fatigue, etc. And added antioxidants, the body's resistance to free radical damage has good effect, especially some natural antioxidants.

Procyanidins is the generic term of a large class of polyphenol compounds widespread in the plant kingdom, often extract from grape seed, Hindu lotus seedpod and so on.

Procyanidins have several pharmacological action including cardiovascular protection, prevention and treatment of cancer, anti-inflammatory action [1], anti-diabetic effect, anti-gastrelcosis, protect liver, ant radiation, elevate memory of learning, promote hair growth, and protect brain ischemia from damage. Because of such pharmacological effects, and also the characteristics of high efficiency and low toxicity, Procyanidins is the latest craze all over the world, widely used in medicines, cosmetics, health products, etc. Thus, procyanidins have enormous market potentiality and economic returns.

2. PROCYANIDINS AND LOTUS SEEDPOD PROANTHOCYANIDINS

Procyanidins chemically belong to polyphenols, they are oligomer and polymeric flavonoids comprised of flavan-3-ol monomeric subunits. These compounds are widely distributed in fruits, vegetables, seeds, flowers and bark. They have received considerable attention in recent years due to their excellent anti-oxidant activity and a broad spectrum of safety. Procyanidins possess significantly better free radical scavenging capability than vitamin C, E or β -carotene. In addition, these compounds have also been reported to exhibit a wide range of biological effects including antibacterial, antiviral, anti-inflammatory, and anti-carcinogen actions [6, 7].

Procyanidins are a class of polyphenol compounds in plants. More and more researchers in the fields of nutrition, health and medicine have paid more attention to procyanidins owing to its potent antioxidants, anti-inflammatory activities, and cardiovascular activities and so on. For a long time, the procyanidins of grape seed (GSOPC) have been a focus research in the world. With the further studies, many scientists turned their research on other plant species in order

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to explore new procyanidins' resources. It is well known that the seedpod of *Nebo nucifera* Gaertn. (Lotus Seedpod, LS) is a traditional medicine, which can remove blood stasis. It was preliminary proved that LSPC have the functions of anti-oxidation activities and anti-myocardial ischemia [3].

Through administration of lotus seedpod proanthocyanidins (LSPC) at different doses, the effect of LSPC on the protection of organ and tissue injuries in mice with high intensity exercise.

3. EXTRACTION AND DETECTION OF LSPC

3.1. Extraction of LSPC

Lotus seedpod proanthocyanidins (LSPC) is well dissolved in acetone, ethanol, methanol, insoluble in benzene, chloroform, petroleum ether, etc. It is commonly used acetone-water, ethanol-water, and methanol-water in extraction of proanthocyanidins. The effect of extraction was optimized using orthogonal array experimental in water and ethanol respectively. The optimized process conditions by water were as follows: 4h extracting time, 30min for time interval, 100°C temperature and 18mL/g solvent to seedpod ratio, the extraction transfer rate was 81.1%. Compared with conventional water extraction method, the solvent consumption decreased by 43%, while the extraction transfer rate increased by 4%. The optimized process conditions by ethanol were as follows: 50% (V/V) ethanol, 2.5h extracting time, 30min for time interval, 12mL/g solvent to seedpod ratio, and the extraction transfer rate was 91.1%. Compared with conventional ethanol extraction method, the solvent consumption decreased by 25%; extraction time reduced by 37.5%, while the extraction transfer rate increased by 4.4%. The new extraction technology saved in time, energy and solvent consumption, which was appropriate to industrial production [8].

3.2. Purification of LSPC

The crude extracts were purified by resin, and the method was as follows: the solution in 0.1-0.2 crude drug g/mL was added 4 BV (BV, bed volume) into resin column in the speed of 1.5 BV/h. Then, 4 BV of water was added to wash away non-procyanidine in the speed of 4 BV/h. 40% ethanol eluted 6 BV in 2 BV/h, and the eluate was dried in vacuum. The resin column was cleaned and reused. The product yield was 3.8%, the purity of procyanidins was over 80%.

3.3. Detection of LSPC

Vanillin assay was carried out in HCl medium to determine the content of proanthocyanidins in the lotus seed peel (Sun, M. W. *et al.*, 2008). Several parameters affecting the precision and accuracy of vanillin assay including HCl concentration, vanillin concentration, reaction time, reaction temperature, sunlight were studied. The proper reaction conditions after testing were as the following: the medium comprised of 0.5 ml of sample, 3 ml of 4% vanillin solution in methanol, and 1.5 ml of concentrated HCl in methanol, and the reaction was carried out at 30°C for 20 min (measured at 500 nm). Assessment of the method by statistics proved its high stability, reproduction quality, and recovery.

Creat standard curve of Catechin as the standard sample, shown in Fig. (1), calculate concentration of LSPC.

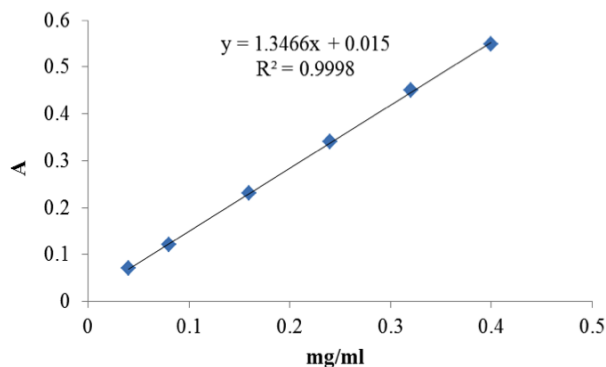


Fig. (1). The standard curve.

$$y = 1.3466x + 0.015 \quad (1)$$

$$C = (A - 0.015) / 1.3466 \quad (2)$$

C is the concentration of LSPC, and A is the absorbance. Through the formula, the concentration of LSPC is obtained [9, 10].

4. PROTECTIVE EFFECT OF LOTUS SEEDPOD PROANTHOCYANIDINS

Different groups of mouse are given different dosages of LSPC or water by gavage, for 30 days. From the 20th day, every mouse exercise 3 days in treadmill. After 2 days' rest, high intensity exhaustive exercise starts. The initial rate of each exercise is 10m / min, increasing to 28m / min in 10min. the mouse are stimulated to run 60min uninterrupted by weak current, once every day, ending the 5 day. Each group of mice is sacrificed immediately after exercise, getting blood, dissecting the heart, liver, kidney and quadriceps, preparing for biochemical tests and pathological analysis.

In analysis, p value is needed.

$$\bar{X} = \frac{1}{n} \sum_{i=1}^n X_i \quad (3)$$

$$s = \sqrt{\frac{1}{n} \sum_{i=1}^n X_i^2 - \bar{X}^2} \quad (4)$$

$$t = \frac{\bar{X} - \mu}{\frac{s}{\sqrt{n-1}}} \quad (5)$$

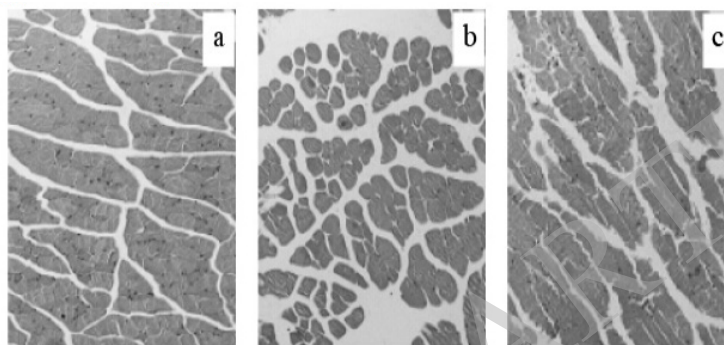
n is the number of mice. Through checking t, p value can be obtained.

4.1. Blood Indexes in Mice with High Intensity Exercise

Under high-intensity exercise, active free radicals generate in body attacking organelles and subcellular organelles, and inflammation occurs in sports body tissues, even a series of pathological damage. After high-intensity exercise, RBC and Hb decrease; WBC and the number of lymphocytes increase in Group B, compared with Group A. it means that immune function in mice start decreasing because of high intensity exercise, shown in Table 1. After the pre-treated with different doses of LSPC, there are different levels of

Table 1. Effect of LSPC on blood indexes in mice with high intensity.

Group	Hb/(g/L)	RBC/(10 ¹² /L)	WBC/(10 ⁹ /L)	WBC sort /(10 ⁹ /L)	
				Lymph	Mononuclear
A	112.4±8.7	2.02±0.22	3.11±0.33	3.02±0.27	0.09±0.06
B	80.3±5.0	1.54±0.12	5.76±1.18	5.54±1.04	0.22±0.14
C	85.7±7.9	2.12±0.31	4.42±0.99	4.25±0.89	0.17±0.10
D	95.2±10.3	1.84±0.19	4.19±1.07	4.03±0.98	0.16±0.09
E	90.2±7.6	2.11±0.24	4.89±0.96	4.69±0.89	0.20±0.07

**Fig. (2).** Skeletal Muscle Sections of the Mice in Each Group. a: Group A with no exercise; b: Group B with high-intensity exercise; c: Group D with high-intensity exercise and LSPC given.

recovery of these indicators in mice. And moderate-dose group was significant in intervention, related to strong free radical scavenging activity of LSPC.

4.2. Effect of LSPC on the Protection of Oxidative Stress-Induced Organ Damage

Dissecting each group of mice, the heart, liver, kidney and quadriceps are obtained. Determine the SOD, GSH, MDA content and liver glycogen and muscle glycogen content.

Liver and kidneys are usually damaged as the main target organ during high-intensity exercise training organ. Muscle tissue and skeletal muscle is vulnerable parts in excessive movement. Under the strong role of oxidative stress in the high-intensity exhaustive exercise, anti-free radical damage ability in mice the body is disturbed. A large number of free radicals sustained attack biofilms, resulting in lipid peroxidation, SOD vitality decreasing significantly, and MDA levels significantly increasing. At the same time, a lot of GSH and glycogen are consumed, it shows that High intensity exhaustive exercise leads to immune function and energy metabolism changing. After the pre-treated with different doses of LSPC, there are different levels of recovery of these indicators in mice. And moderate-dose group was significant in intervention, explained that LSPC can protect organs and tissues from oxidation in high-intensity exercise. And it can also maintain liver glycogen and muscle glycogen in normal levels, reducing fatigue damage caused by excessive movement, enhancing the effect of exercise training.

4.3. Effects of LSPC on Skeletal Muscle Pathology Under High-Intensity Exercise

As Fig. (2) shows, skeletal muscle fibers of Group B mice exhibit extensive swelling, shape distortion or fracture, there is muscle dissolution phenomenon. Skeletal muscle cell structure remains regular in Group D which is given LSPC, with a little slight swelling. There is wide distortion, fracture, muscle dissolution and interstitial hemorrhage, myocardial focal necrosis in mice muscle fibers of Group B. Although the existence of local myocardial fibers are twisted or broken, but the muscle fibers arranged regularly, and there are slight abnormalities in Group D with LSPC, shown in Fig. (3).

4.4. Effect of LSPC on the Protection of Exercise-Induced Myocardial Damage

As the Table 2 shows, TSOD and CuZn-SOD are significantly higher in Group D compared with Group A and Group B ($P < 0.01$ or $P < 0.05$), and there was no significant difference with CAT activity ($P > 0.05$), indicating that LSPC can effectively reduce myocardial damage caused by high-intensity exercise.

NO content and TNOS, iNOS activity in Group B was significantly higher than that in Group A and Group D ($P < 0.05$), and Group D was restored, close to the A group level. cNOS activity in Group B was significantly lower than that in Group A and Group D ($P < 0.05$), and Group D was restored, with no significant difference compared with group

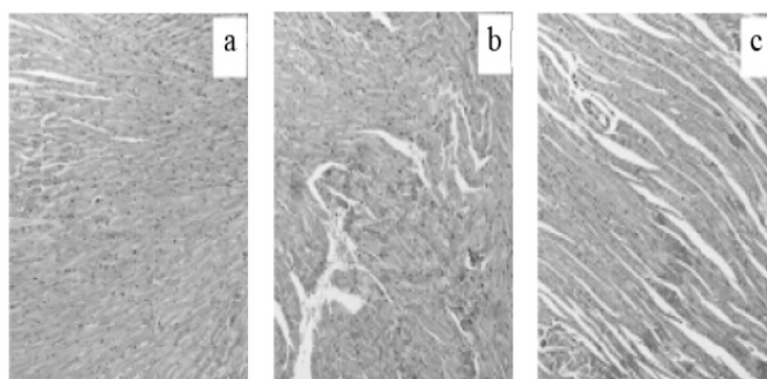


Fig. (3). Cardiac Muscle Sections of The Mice in Each Group. a: Group A with no exercise; b: Group B with high-intensity exercise; c: Group D with high-intensity exercise and LSPC given.

Table 2. Impact On NO, NOS of High-intensity Exercise.

	Group A	Group B	Group D
NO/($\mu\text{mol} \cdot \text{g}^{-1} \text{ prot}$)	0.52 \pm 0.12	0.74 \pm 0.06	0.51 \pm 0.11
TNOS/($\text{u} \cdot \text{mg}^{-1} \text{ prot}$)	0.82 \pm 0.08	1.13 \pm 0.07	0.94 \pm 0.07
iNOS/($\text{u} \cdot \text{mg}^{-1} \text{ prot}$)	0.23 \pm 0.08	0.65 \pm 0.04	0.42 \pm 0.06
eNOS/($\text{u} \cdot \text{mg}^{-1} \text{ prot}$)	0.61 \pm 0.05	0.43 \pm 0.06	0.59 \pm 0.03

Table 3. Impact On Na⁺, K⁺-ATPase, Ca²⁺-aATPase activity of High-intensity Exercise

	Group A	Group B	Group D
Na ⁺ , K ⁺ -ATPase/ ($\mu\text{molpi}/\text{mg prot}/\text{hour}$)	55.91 \pm 7.12	61.23 \pm 5.99	68.98 \pm 4.87
Ca ²⁺ -aATPase/ ($\mu\text{molpi}/\text{mg prot}/\text{hour}$)	40.12 \pm 6.98	43.26 \pm 6.93	53.67 \pm 3.84

A, as the Table 2 shows, indicating that LSPC can effectively reduce myocardial damage caused by high-intensity exercise.

Exercise caused a significant decrease of Group B in Na⁺, K⁺-ATPase, Ca²⁺-aATPase activity ($P < 0.01$). ATP activity in Group D significantly increased compared with exercise Group B ($P < 0.01$), close to group A, as the Table 3 shows.

CONCLUSION

High-intensity exercise can cause damage to the body's organs and tissues. And LSPC as a kind of polyphenols, is widely used in medicines, cosmetics, health products. Different doses of LSPC is given to mice to check the function of protect effect to the body's organs and tissues under high-intensity exercise. The result showed that LSPC can effectively reduce inflammation reaction in the body of mice with high intensity exercise, alleviate oxidative stress-induced injury of tissues and organs, and execute protective function on skeletal muscle and cardiac muscle. And the LSPC could enhance myocardial antioxidant and enzymatic activity which

suggests the protective effects of resveratrol against exercise-induced myocardial damage in mice.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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