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Research on the Influence of Anti-fatigue Effect and Movement Ability of Blueberry Polysaccharides on Aged Mice

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Abstract: This topic introduces functions, extraction and purification of polysaccharides and focuses on the effect of blue polysaccharides (BPs) on sport capacity, anti-fatigue effect and related physiological indexes in aged mice induced by D-galactose was investigated. Water extract-alcohol precipitation method was used in the experiment to extract polysaccharides from blueberry, after preliminary purification, research on polysaccharide of its vitro antioxidant activity and bacteriostasis is taken, to provide a reference for the production and application of blueberry polysaccharides. Mice were randomly divided into normal control group, aged model group, low-dose BP group, middle-dose BP group, and high-dose BP group. After the administration of BPs for 20 consecutive days, the exhaustive swimming time and fatigue indexes were determined. The results showed that BPs could extend the exhaustive swimming time, decrease the content of BUN, BLA and MDA, and obviously increase the reservation of HG, MG, SOD and LDH. To conclude, BPs has good anti-fatigue effect. This study may provide theoretical evidence for the development of anti-fatigue drugs from BPs.

Keywords: Anti-fatigue effect, blueberry polysaccharides, exhaustive swimming, extraction, purification D-galactose.

1. INTRODUCTION

Blueberry is a kind of strong cold resistance of wild plants, native to North America, Scotland, and Russia. Blueberry fruit is not only sweet and sour moderate, good flavor, but also containing a lot of things that are good for human health, such as rich vitamins, dietary fiber, protein and mineral nutrient elements including antioxidants and tannic acid, folic acid, antibacterial ingredients, etc. Therefore, it is often known as the "king of berries".

Research abroad on the active ingredient in blueberry has focused on anthocyanins, various physiological functions of anthocyanins extract was studied, and some processing on its antioxidant capacity [1]. The functional sex of the flavonoids in blueberries is still studied. Studies on the activity of polysaccharide blueberries are not reported, water extract-alcohol precipitation method was used in the experiment to extract polysaccharides from blueberry, after preliminary purification, research on polysaccharide of its vitro antioxidant activity and bacteriostasis is taken, to provide a reference for the production and application of blueberry polysaccharides.

2. EXTRACTION AND PURIFICATION OF POLYSACCHARIDE

2.1. Extraction of Polysaccharide

Extraction and separation of polysaccharide including: Degrease~solvent extraction~deproteinization~decoloration processes, etc.

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Degrease: the polysaccharide extraction materials degreasing first, solvent extraction, general or dilute salt water, dilute alkali, dilute acid extraction under different temperatures.

Deproteinization: Through organic solvent precipitation polysaccharide, often contain more protein will need to be removed [2].

Decolorization: coarse polysaccharide often contains some pigment (free combination dyes and pigments), adopting different methods according to different properties.

2.2. Purification of Polysaccharides

Methods of polysaccharide purification are usually the following kinds:

(l) Division precipitation method: According to the polysaccharide in different concentrations of low alcohol, ketone with different solubility properties, from small to large in proportion to alcohol, ketone (such as methanol, ethanol and acetone) division of the precipitation.This method is suitable for the separation of various solubility large difference of the polysaccharide [3].

(2) Salt fractionation method: Different polysaccharide has different solubilities in different salt concentrations. Common salting-out agents are s sodium chloride, potassium chloride and ammonium sulfate etc., and sulfuric acid amine is the best.

(3) Metal complex method: polysaccharide can form complex ions and precipitation with copper, barium, calcium, lead sheath.

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3. MATERIALS AND METHODS

3.1. Materials and Reagents

Fresh blueberry without freezing injury, bought in the fruit supermarket. D-galactose Sigma-Aldrich companies in the United States; Blood urea nitrogen (BUN) kit, blood lactic acid (BLA) kit, liver glycogen, hepatic glycogen (HG) kit, muscle glycogen (MG) kit, malondialdehyde (MDA) kit, superoxide dismutase (SOD) kit, lactate dehydrogenase (LDH) kit, Nanjing building biological engineering research institute; Other reagents were pure homebred analysis. Kunming mice 50, SPF level, male and female are both half, 20~22g body quality.

3.2. Instrument and Equipment

DHG-9240A thermostatic drum wind drying oven, DK-8D three holes thermostatic water tank, Shanghai Qixin scientific instrument co. LTD. Ultrasonic cleaning machine, ningbo xingzhi biological technology co. LTD. Refrigerated centrifuge Beckman companies in the United States; UV-2450 ultraviolet-visible spectrophotometer Shimadzu.

3.3. Preparation of Blueberry Polysaccharides

Weigh and blueberry fruit 120g, pulping after quick-freeze in -80 ℃ for 10 min after cleaning the materials, add a small amount of distilled water after using microwave assisted extraction. After the suction filtration filtrate is made to centrifuge at 5 000 r/min in 15 min. Add a small amount of water dissolving pulp after repeated extraction twice, extracted the supernatant fluid of merger blending, rotary evaporation solution all moisture evaporation. Add 95% ethanol, 100% ethanol and acetone and process respectively, placed in 4℃ for 24h, centrifuge at 3000 r/min in 10 min, use Sevag method in addition to protein, which will get the blueberry precipitation after freeze drying coarse polysaccharide [4].

3.4. Conditions and Grouping of Experimental Animals

Before the trial, 50 mice will be in the experimental environment with based feed for 1 week, and then they are randomly divided into 5 groups according to body quality (male and female half), they are respectively the normal control group, model control group, low-dose, medium-dose and high-dose BPs groups. Intraperitoneal injection of normal control group with 100 mg/ (kg·d) (with body quality meter, similarly hereinafter) physiological saline, intraperitoneal injection of the rest of the four groups of 100 mg/ (kg·d) D-galactose, cycle for 60d. At the 41th days, normal control group is gavaged with gastric distilled water, model control group are injected of D-galactose in intraperitoneal and lavage distilled water at the same time, the low, medium and high dose groups of BPs are injected of D-galactose in intraperitoneal and lavage distilled water at the same time in 100, 200, 400 mg/ (kg·D) dose of BPs, continuous dosing 20d. Groups are given basic feed and free drinking water.

3.5. Exhaustion Swimming Time of Mice

30 min after the last treatment experiment. After each mice are weighed, the rat tail root are born load of 5% weight lead sheath skin, the degree of tightness of fixation should be appropriate, put groups of mice respectively in 10 thermostatic water box, with the water temperature 25℃ and the water depth (20+5) cm. Record the mice swimming time with a stopwatch when mice began to sink under the water after 10 s not surfaced as swimming exhaustion time/min. Some mice floating in the water not to swim, which will make swimming significantly prolonged, therefore, in order to get reliable results, every mice must be made continued to swim, stir gently around sticks available in mice.

3.6. Preparation and Testing Test Sample

After exhaustion swimming experiment, pull eyeball and draw blood, centrifuge at 4000 r/min in 10 min, and take supernatant on standby. All processing are completed under the condition of 4℃. The BUN, BLA, HG, MG, MDA, SOD and LDH kit description combined with ultraviolet-visible spectrophotometer determination of each index respectively.

4. RESULTS AND ANALYSIS

Test sports endurance of mice with swimming method. The effects of blueberry polysaccharides on sports endurance of mice are shown in Fig. (**1**).

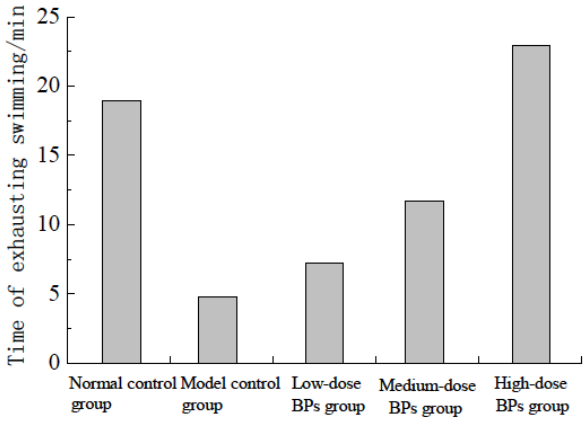

Fig. (1). Result of exhaustive swim of aged mice.

4.1. Effects of BPs on Aged Mice of Exhaustion Swimming Time

The time of continuous movement until exhausting and survival time of hypoxia condition can reflect the body's endurance, which is the relatively objective index to evaluate fatigue state. Methods building aging animal models at present mainly include injection of D-galactose, ozone damage, removing thymus, using older animals, etc. As shown in Fig. (**1**), normal control group and different exhaustion swimming time in mice BPs dose group are higher than that of model control group, the BPs low dose group compared with model control group has no significant difference ($P > 0.05$), and the rest of the group rre significantly higher than that of model control group ($P<0.05$). The experimental results show that exhaustion swimming time in mice and BPs dose were positively correlated, showing that blueberry polysaccharides can enhance the endurance of the movement of mice, has the anti-fatigue function. BPs high dose group had a significantly higher exhaustion swimming time of mice in BPs, low dose group ($P<0.05$). Results show that blueberries

Table 1. Effect of BPs on BUN and BLA of Aged Mice.

Group	Dose(mg/（kg·d）)	BUN content /（m mol/L）	BLA content (mg/l00ML)
Normal control group	100 normal saline	13.26±1.78	14.02±0.66
Model control group	0	15.32±2.05	19.76±1.53
Low-dose BPs group	200	13.75±1.96	18.01±0.94
Medium-dose BPs group	300	13.70±2.36	15.97±0.27
High-dose BPs group	500	13.62±1.84	14.15±0.99

Table 2. Effect of BPs on HG and MG of Aged Mice.

Group	Dose(mg/kg·d)	HG content /(mg/g)	MG content（mg/g)
Normal control group	100 normal saline	30.28±4.87	1.80±0.21
Model control group	0	21.16±3.16	0.95±0.08
Low-dose BPs group	200	34.15±4.88	1.22±0.07
Medium-dose BPs group	300	36.13±3.91	1.57±0.17
High-dose BPs group	500	35.59±4.69	1.81±0.10

polysaccharide can significantly improve the sport ability of aging mice, delay the onset of fatigue.

4.2. Effect of BPs on Aging Mice of Related Physiological Indexes like BUN and BLA

After the experimental group rats were filled and fed with blueberry polysaccharides 15 d. The results of BUN and BLA are shown in Table **1**. The results shows that the content of serum urea nitrogen dose group were lower than the control group, and extremely significant difference ($P<0.01$), suggesting the blueberry polysaccharides have lower serum urea nitrogen.

When glycogen is in a large amount of consumption, biological activity reduced, resulting in the slow and tired of body. Therefore reserve original conducive to reduce the amount of protein and sugar catabolism of nitrogen compounds, improve the body's endurance. Lack of oxygen supply relatively caused by strenuous exercise of body will increase metabolites of lactic acid etc., leading to muscle contraction efficiency drop. Therefore, the changing rule of the BLA content also appears similar, Contents of BLA of low-dose, medium-dose and high-dose BPs groups compared with model control group reduced by significant differences ($P<0.05$).

4.3. Determination Results of Liver Glycogen and Muscle Glycogen

It can be seen from Table **2** that after the rats of all experimental groups were filled and fed with blueberry polysaccharides 15 for days. Liver glycogen content increased significantly, it is extremely significant difference compared with the control group ($P<0.01$).

Table **2** shows that different BPs dose group of HG content is higher than that of normal control group. Compared with the normal control group, the HG content in three dose group were significantly increased ($P<0.05$). There is evidences that, after high intensity exercise of 2h, MG is almost exhausted, because MG reduced gradually, the HG hydrolysis increased to maintain the stability of blood sugar.From the results, each BPs dose group can effectively enhance the ability of continuous movement in mice.

Numerous studies have found that [5, 6] short time high strength caused by muscle fatigue is because the energy storage materials ATP in muscle are in short supply, when ATP decreased to a certain level, the glycolysis of MG will be carried out in ATP synthesis, the process of which will be accompanied by the accumulation of lactic acid, causing the rise of hydrogen ion concentration in the body, drop of muscle pH, eventually lead to the decrease of muscle contraction ability. The experiment confirmed that the low-dose BP group, middle-dose BP group, and high-dose BP group three dose groups of HG content were significantly higher than that of normal control group ($P<0.05$), suggesting BPs can increase reserve of glycogen, provide the body with energy supplies, relieve physical fatigue and enhance the effect of exercise endurance.

4.4. Determination Results of MDA and Enzymes Dehydrogenase Activity

The experimental group rats were fed blueberriesg polysaccharide for 15 days, test results of MDA, SOD and LDH activities are shown in Table **3**.

The results showed that blueberry polysaccharides lactate dehydrogenase activity of each dose group were higher than

Table 3. Effect of BPs on MDA, SOD and LDH of Aged Mice.

Group	Dose(mg/kg·d)	LDH vitality/ (U/mL)	SOD vitality/ (U/mL)	MDA content (mg/g)
Normal control group	100 normal saline	597.55. ±42.15	302.32±19.32a	22.24±1.10
Model control group	0	539.55. ±37.30	250.01±13.74d	26.35±1.58a
Low-dose BPs group	200	585.09. ±42.66	311.27±24.47a	25.40±1.32ab
Medium-dose BPs group	300	741.24. ±76.62	338.65±19.00b	23.29±1.04
High-dose BPs group	500	1027.61±98.84	364.07±21.14c	22.33±1.02c

that of normal control group, medium-dose group significant difference ($P<0.05$), high-dose group of extremely significant difference ($P<0.01$), suggesting the blueberry polysaccharides can increase the activity of MDA, SOD and LDH. Compared with the serum SOD activity of model control group, low- dose, medium-dose and high-dose BPs groups had increased by 24.5%, 35.4% and 45.6% respectively ($P<0.05$), suggesting that BPs to adjust the serum SOD effect is very obvious. In addition, the serum LDH activity also significantly increased with the increase of the BPs dose, but compared with BPs, high dose group is no significantly different from serum LDH, which reflecting the effect of BPs dose on the activity of LDH. The above results show that the BPs has good antioxidant activity of body, not only significantly increase the antioxidant activity of the enzyme system in the body, but also effectively remove free radicals and prevent cell membrane lipid peroxidation, perhaps this is one of the key mechanism of BPs has the anti-fatigue function.

CONCLUSION

Fatigue (fatigue) refers to the ability to work due to the activity and the phenomenon of body function temporarily reduce, the improvement of sports endurance is the ability to resist fatigue strengthen the strong macro performance. The premise of mice exercise endurance is enhancing energy reserves is enough, the more energy supply, the more durable endurance will be. BPs can reduce the content of BLA, BUN and increase HG, MG content in different degrees, these changes may be the important material base of its role of expressing anti-fatigue functions. Based on this, the time of exercise endurance of high-dose BPs group is extended than that of the normal control group at 14.3% ($P<0.05$), high-dose BPs group also effectively increased the SOD and LDH activity in mice, and significantly reduced BUN, BLA and MDA content in mice after strenuous exercise, which showed that high doses of BPs have strong ability to remove free radicals. Blueberry polysaccharides can increase the ability of aerobic metabolism and exercise endurance in mice, so after completing the same exercise load, blood lactic acid content is relatively lower than the control group, thus able to resist the role of sports fatigue strength.

CONFLICT OF INTEREST

The author confirms that this article content has no conflict of interest.

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