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Analysis of the anti-fatigue activity of polysaccharide from *Spirulina platensis*: Role of central 5-hydroxytryptamine mechanisms.

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ABSTRACT

This study is to evaluate the effects of polysaccharide from *Spirulina platensis* (PSP) on treadmill exercise endurance, the levels of some biochemical indicators as hemoglobin (Hb), Lactic acid (LA), creatine kinase (CK) and urea nitrogen (BUN) in the blood, 5-hydroxytryptamine (5-HT) concentrations, the second isoforms of tryptophan hydroxylase (TPH2) and serotonergic type 1B inhibitory autoreceptors (5-HT1B) expression in the caudate putamen of exercised rats. Sixty Sprague-Dawley male rats were randomly divided into six groups: control group, exercise group, exercise and PSP (50, 100,200 mg/kg)–treated groups, exercise and caffeine (10 mg/ kg)–treated group (positive control). In exercise groups, rats were put on treadmill and forced to run for 30 min once a day for 6 consecutive days. On the 7th day of the experiment, the time to exhaustion in treadmill exercise was determined for the trained groups. Immediately after the determination of the exhaustion time, all rats were sacrificed. The levels of Hb and LA were tested by the HiCN (Hemoglobin ferricyanide) colorimetry method and colorimetric assay method, respectively. The levels of CK and BUN were determined by automatic biochemical analyser. 5-HT concentrations were detected by HPLC analysis. TPH2 and 5-HT1B expression were measured by western blot analysis and real-time PCR. The results demonstrated that PSP could prolong the time to exhaustion in treadmill exercise, increase Hb levels and decrease LA, BUN and CK levels in the blood and suppress the exercise-induced increase of 5-HT concentrations and TPH2 expression and prevent the exercise-induced decrease of 5-HT1B expression in the caudate putamen. The most

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potent effects were observed at the dose of 200 mg/kg of PSP. It suggests that the mechanism of PSP's promoting physical performance might be related to increasing Hb levels and decreasing LA, BUN and CK levels in the blood and the inhibition of the exercise-induced synthesis of 5-HT and TPH2 expression, and the increase of the 5-HT1B expression in the caudate putamen of exercised rats.

Keywords: Spirulina,polysaccharide, 5-hydroxytryptamine, neurotransmitter, exercise, fatigue

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1. Introduction

Spirulina, a unicellular blue-green alga, is a popular nutritional supplement. Recent studies suggest that spirulina supplementation induced a significant increase in exercise performance [1], increasing people's ability to resist mental and physical fatigue [2], and finally improving locomotor behavior in Parkinson's disease [3]. Our research showed that spirulina supplement had a protective effect on the damaged nerve cells in hippocampal CA1 of rats with fatigue caused by incremental load exercise [4]. Polysaccharide is one of the main active components of spirulina. Anti-fatigue is one of the main activities of natural polysaccharides [5-8]. Polysaccharide of *Spirulina platensis* (PSP) can prolong the moving endurance time of mice and reduce the increase quantity of blood urea nitrogen of mice evidently [9], and extend climbing and weight-loaded swimming time of mice and increase the lactate dehydrogenase (LDH) activity of the serum and glycogen deposition in liver and muscle [10]. However, the effects of PSP on the endurance exercise in relation to central nervous system have not yet been clarified.

Fatigue occurring during prolonged physical activity has both peripheral and central origins [11]. Physical exercise is often terminated not due to muscle fatigue but to the increase in serotonin or 5-hydroxytryptamine (5-HT) concentrations in the brain during prolonged exercise [12-13]. Increased concentrations of brain 5-HT during sustained physical activity could hasten the onset of fatigue [14], while decreasing 5-HT concentrations could delay the time to fatigue [15]. Serotonin is modulated by many

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factors involved in intrinsic regulation of central 5-HT neurotransmission, which include tryptophan hydroxylase (TPH), serotonergic type 1B (5-HT1B) inhibitory autoreceptor [16]. The second isoform of tryptophan hydroxylase (TPH2) is the rate-limiting enzyme in 5-HT synthesis [17]. 5-HT1B inhibitory autoreceptor, upon stimulation, inhibits local synthesis and the release of 5-HT [18]. Polysaccharide fraction isolated from *passiflora edulis* could modulate the liberation or synthesis of serotonin [19]. Could polysaccharide of *Spirulina platensis* (PSP) improve the body's exercise endurance through affecting 5-HT ? To test this hypothesis, the effects of polysaccharide extracted from *Spirulina platensis* on treadmill running endurance, the levels of some biochemical indicators as hemoglobin (Hb), Lactic acid (LA), creatine kinase (CK) and urea nitrogen (BUN) in the blood, levels of 5-HT, mRNA and protein expression of TPH2 and 5-HT1B in the caudate putamen，which is one of the important areas in movement and fatigue [20]，of exercised rats were investigated.

2. Materials and methods

2.1 Materials and reagents and animals

2.1.1 Materials: Polysaccharide was isolated from *Spirulina platensis* and obtained from Wuhan Yuancheng Gongchuang Technology Co., Ltd (Wuhan, China) with a batch number 20171123, and the purity of PSP is 70.13%. The certificate of analysis for the test material is provided as a Supplementary document. Caffeine was purchased from Beijing Beina Chuanglian Biotechnology Institute (Beijing, China)

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with a batch number of the caffeine 19060310, and the purity of the caffeine is 98.66%.

2.1.2 Main experimental reagents: Lactic acid and hemoglobin test kits (Nanjing jiancheng Bioengineering institute, Nanjing, China), creatine kinase and urea nitrogen test kits (Biosino Biotechnology Co., Ltd, Beijing, China), chloral hydrate (Sigma, USA), HClO4 (Sigma, USA), ethylenediamine tetraacetic acid (EDTA) (Sigma, USA), Sodium octaalkylsulfonate (OSA) (Sigma, USA), MeOH (Sigma, USA), orthophosphoric acid (Sigma, USA), trizol (Invitrogen, USA), DNA Free kit (Qiagen, Germany), Reverse Transcriptase M-MLV， (TaKaRa, Japan), SYBR Green master mix (TaKaRa, Japan) anti-TPH2 antibody (sc-134775， Santa Cruz, USA), anti-5-HT1B antibody (ab13896， Abcam, UK). Goat anti-rabbit IgG (ab97060, Abcam,UK), anti-GAPDH antibody (sc-25778,Santa Cruz,USA) and chemiluminescence detection kit (Amersham, Buckinghamshire, UK).

2.1.3 Animals

Adult male Sprague-Dawley rats of grade SPF, weighing 221.98±22.67 g were used in this study. They were obtained from Hunan Lake King of laboratory animal Co. Ltd, Changsha, Hunan, China. Ten animals were housed per cage under the controlled conditions of temperature (25℃-26℃), humidity (40%), a light/dark cycle (12 h/12 h) with the access to food and water at will. Animals were allowed to acclimatize to the laboratory before the commencement of the experiment. The rodent license of the laboratory (no.SCXK （ Xiang ） 2011-0003) was issued by the Hunan Province Laboratory Animal Care and Use Committee, and all the experiments were complied

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with the ARRIVE guidelines. All the experiments were carried out according to the general principles of laboratory animal care (NIH publication No. 8023, revised 1978) and the Guidance Suggestions for the the Hunan Province Laboratory Animal Care and Use Committee. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Jinggangshan University and approved by the Medical Ethics Committee of Jinggangshan University.

2.2 Experimental design

Sixty rats were randomly divided into six groups (n = 10 in each group): control group (A)， exercise group (B), exercise + PSP (50 mg/ kg)-treated group (C), exercise + PSP (100 mg/ kg)-treated group (D), exercise + PSP (200 mg/ kg)-treated group (E), and exercise + caffeine (10 mg/ kg)–treated group (F). The rats of PSP treated groups were injected by the intragastric gavage (ig) once per day with PSP at the respective one time dose. Rats of the caffeine-treated group received caffeine (10 mg/kg) by the same method. Rats of control group and exercise group were administrated with the same volume of saline by ig. The volume of each intragastric gavage was 2 ml per rat and the intragastric gavage was performed at 2 h before the start of treadmill running. Rats in the exercised groups (B, C, D, E and F) had been doing exercises through running on a treadmill (DSPT-202, HangZhouDuanShi, China) with 0° of inclination for 6 consecutive days. Rats in group A were left for 30 min on the treadmill without running. The exercise duration consisted of forced running at a speed of: 10 m/min for 10 min, 13 m/min for another 10 min, and 20 m/min for the last 10 min from the first day to the third day, and 18 m/min for 10 min,

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22 m/min for another 10 min, and 27 m/min for the last 10 min from the fourth day to the sixth day [21].

2.3 Determination of exhaustion time

Determination of exhaustion time was performed as previously described [21]. On the 7th day of the experiment, the time to exhaustion for treadmill exercise was determined for the trained groups. The time to exhaustion is defined as the time between the commencement of exercise and the first occurrence of the experimental animals failing to keep up with the treadmill machine for a period of 3 min or more. The speed used for measurement of the time to exhaustion was 18 m/min for the first 5 min, and after which the treadmill speed was incrementally increasing to 21 m/min for 5 min, 24 m/min for 5 min, 26 m/min for 5 min, 29 m/min for 5 min and 34 m/min until exhaustion.

2.4 Biochemical indicators (Hb, BUN, LA and CK) determination and collection of the caudate putamen sample

According to the method described in the literature [22,23], immediately after the determination of the exhaustion times, all rats were intraperitoneally injected by 10% chloral hydrate (0.3 mL/100 g body weight). The blood was collected from the left carotid artery in heparinized tubes and without anticoagulant tubes. The whole blood samples were used to determinate the hemoglobin and lactic acid according to the HiCN (Hemoglobin ferricyanide) colorimetry method and colorimetric assay method using the 721G visible spectrophotometer (Shanghai Yidian Analytical Instrument Co., Ltd, China), respectively. The serum was prepared by centrifugation at 1000 × g,

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4 ºC for 15 min and the levels of serum creatine kinase, urea nitrogen were determined by automatic biochemical analyser (Hitachi 7060, Hitachi, Ltd, Japan) with commercial kits (Biosino Biotechnology Co., Ltd, Beijing, China). After carotid artery blood collection, rats were sacrificed to remove the caudate putamen tissues (−0.92 to −1.80 mm posterior to bregma) for further processing.

2.5 HPLC analysis of 5-HT concentration in the caudate putamen tissues

HPLC analysis of 5-HT concentration was performed as a previously described method [21]. The caudate putamen tissues were sonicated in ice-cold 0.1 M HClO4 containing 0.01% EDTA. The supernatant collected after centrifugation at 10,000×g for 5 min was injected (10μl) into the HPLC system (Bioanalytical Systems Inc., West Lafayette, USA) equipped with a pump (PM80), ESA electrochemical detector (Coulochem III) with glassy carbon working electrode (5041 cell， 350mV), and a Rheodyne injector. A C18, ion pair, analytical column (2.1 mm x 250 mm; Zorbax Eclipse Plus; Agilent, USA), with a particle size of 5 μm and pore of 95 A° was used for separating 5-HT . The flow rate was 0.7 ml/min and the electrochemical detection was performed at + 0.35 V. The composition of the mobile phase was 200 mg OSA, 50 μl EDTA, 5% MeOH, and 0.1 M orthophosphoric acid.

2.6 RNA isolation, generation of cDNA and real-time PCR

RNA isolation, generation of cDNA and real-time PCR were tested according to the method described in the literature [24].

2.6.1 RNA isolation

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RNA was obtained from the caudate putamen tissues. Approximately 10 mg of tissues were homogenized in 1 ml trizol and isolated according to the manufacturer's instructions. The concentration and purity of RNA were assessed by spectrophotometry.

2.6.2 Generation of cDNA

Ten micrograms of RNA were then treated with the DNA Free kit to remove genomic DNA. Aliquots of DNA free RNA were reversely transcribed using the TaKaRa reverse transcription kit according to the manufacturer's instructions. cDNA products were stored at -20℃for real-time PCR.

2.6.3 Real-time PCR

mRNA levels were determined by semiquantitative real-time PCR on a Lightcycler instrument (Bio-Rad IQ5). The PCR mix contained cDNA from an equivalent of 66 ng RNA, 0.55 mM of the appropriate forward and reverse primers, 2 SYBR Green master mix in a volume of 20 ml. The PCR programs involved 2 min at 95 ℃ and then 40 amplification cycles of denaturing at 95 ℃ for 20 s, 62 ℃ annealing for 20 s, and extension for 20 s at 72 ℃. The primers were selected by using Primer Express 2.0 software. The primer sequences used were shown in Table 1. Primer specificity was confirmed initially by PCR in a standard thermocycler. The consistency of target sequence produced by each sample in the Light cycler was assessed by melting curve analysis and agarose gel electrophoresis where required.

Table 1 The primer sequences used for real time PCR

Genes	Forward primer	Reverse primer	Accession no.

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TPH2	GTTGTCCTTGGATTCTGCTGTG	CCGCTGTCTTGCTGCTCTC	NM_173391
5-HT1B	GAACCAAGTCAAAGTGCGAGTC	CCAGGGAGATGATGAAGAAGGG	NM_010482

2.7 Western blot analysis

Western blot analysis was detected by previously described method [21]. The caudate putamen tissues homogenate was centrifuged at 13 000 g for 15 min at 4°C and the supernatant was collected. Protein concentration was measured by Bio-Rad Protein Assay (BioRad, Hercules, CA, USA). Sample proteins were separated on SDS-polyacrylamide gels, and then transferred to a polyvinylidine difluoride membrane, blocked by 5% non-fat milk and incubated overnight with the primary antibodies anti-TPH2 (1:1000) or anti- 5-HT1B (1:500). The membrane was washed twice and then incubated for 1 h with secondary antibodies (goat anti-rabbit IgG for TPH2 & 5-HT1B, 1:10,000), and the bound antibody was detected by using an enhanced chemiluminescence detection kit. For the gel loading control, membranes were re-probed with a monoclonal anti-GAPDH antibody (1:1000). Protein levels in the groups were expressed as a percentage of respective control values.

2.8 Statistical analysis

Statistical analysis was performed by the first author with SPSS 17.0 software (SPSS, Chicago, IL, USA). The results were expressed as mean ± SD. The data were assessed by the one-way analysis of variance (ANOVA). A level of $p<0.05$ was considered statistically significant.

3. Results

3.1 Effect of polysaccharide extracted from *Spirulina platensis* on the exhaustion

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time by treadmill exercise

The effect of the different doses of polysaccharide extracted from *Spirulina platensis* on the time to exhaustion of rats was presented in Fig. 1. The mean exhaustion time for forced treadmill exercise was 42.50 ± 4.97 min in the exercise group (B), the exercise and PSP (50 mg/kg)-treated group (C) was 60.80 ± 4.52 min, the exercise and PSP (100 mg/kg)-treated group (D) was 67.70 ± 5.44 min, the exercise and PSP (200 mg/kg)-treated group (E) was 85.80 ± 6.23 min. The results showed that polysaccharide extracted from *Spirulina platensis* prolonged the time to exhaustion by treadmill exercise in a dose-dependent way.

The time to exhaustion in treadmill exercise was also compared between the PSP (200 mg/kg)-treated group (E) and caffeine (10 mg/kg)-treated group (F). The mean exhaustion time for forced treadmill exercise was 85.80 ± 6.23 min in the exercise and PSP (200 mg/kg)-treated group (E), and it was 84.90 ± 5.47 min in the exercise and caffeine (10 mg/kg)-treated group (F) (Fig. 1). The results showed that polysaccharide extracted from *Spirulina platensis* (200 mg/kg) was just as effective as caffeine (10 mg/kg) in prolonging the time to exhaustion during treadmill exercise.

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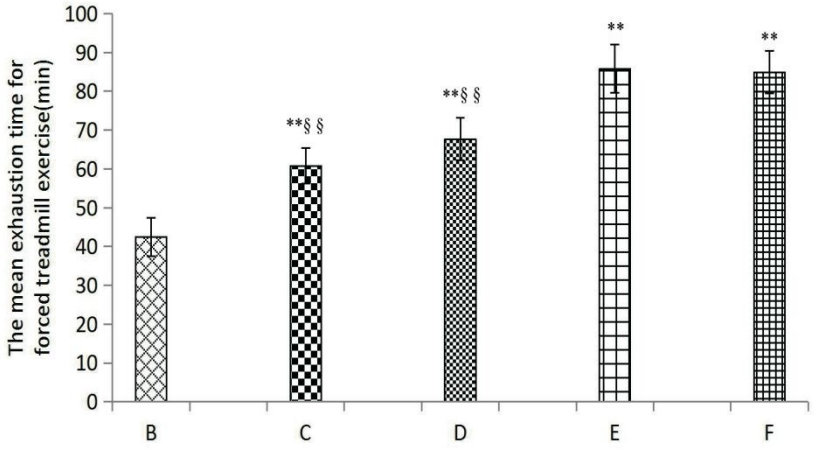

Fig.1 The effect of polysaccharide of *Spirulina platensis* on the mean exhaustion time for forced treadmill exercise.

Note: B: exercise group, C: exercise + PSP (50 mg/ kg)-treated group, D: exercise + PSP (100 mg/ kg)-treated group, E: exercise + PSP (200 mg/ kg)-treated group, F : exercise + caffeine (10 mg/ kg)–treated group. Values are mean±SD. ** $p<0.01$, compared with group B, §§ $p < 0.01$, compared with group E.

3.2 Effect of polysaccharide extracted from *Spirulina platensis* on Hb, BUN, LA and CK levels of trained rats

Several biochemical parameters were analyzed to elucidate metabolic alterations involved in anti-fatigue activity of polysaccharide extracted from *Spirulina platensis*. The CK, LA and BUN levels in exercise groups B, C, D, E and F were higher than those of the control group (A) ($P<0.01$). The CK and LA in the exercise group (B) were higher than those of the exercise and PSP -treated groups (D and E), but lower than those of the exercise and caffeine -treated group F ($P<0.01$). The BUN levels in the exercise group (B) were higher than those of the exercise and PSP -treated groups

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(D and E) and the exercise and caffeine -treated group (F)($P<0.01$). The CK, LA and BUN levels in the exercise and PSP (200 mg/ kg) -treated group (E) were lower than the exercise and PSP (50 and 100 mg/ kg) -treated groups (C, D) ($P<0.01$). The Hb concentrations in the exercise group (B) and the exercise and PSP (50 mg/ kg) -treated group (C) were lower than those of the control group (A) ($P<0.01$). There also was no significant differences on Hb concentrations among the exercise and PSP -treated groups (D and E) and the exercise and caffeine -treated group (F) ($P > 0.05$). See Table 2.

We also compared the effects of PSP and caffeine on Hb, BUN, LA and CK levels of trained rats. There also was no significant differences on Hb concentrations and BUN levels between the exercise and PSP (200mg/kg)-treated groups (E) and the exercise and caffeine-treated group (F). But The CK and LA levels in the exercise and PSP (200 mg/ kg) -treated group (E) were lower than the exercise and caffeine -treated group (F) ($P<0.01$). See Table 2.

Table 2 Effects of PSP on the biochemical indicators of trained rats

Groups / Parameters	Creatine kinase (CK) (U/mL)	Urea nitrogen (BUN) (mmol/L)	Lactic acid (LA) (mg/L)	Hemoglobin (Hb) (g/L)
A	1.47 ± 0.03	6.01±0.24	5.67±0.19	126.26±2.23
B	2.57±0.06††	9.30±0.34††	20.59±0.64††	124.06±0.78††

C	2.51±0.04††§§	9.25±0.37††§§	20.54±0.59††§§	124.68±1.10††§§
D	2.39±0.07††**§§	8.71±0.30††**§§	18.63±0.32††**§§	125.96±1.02**
E	2.15±0.06††**	7.56±0.34††**	17.34±0.31††**	126.48±0.96**
F	2.75±0.08††**§§	7.61±0.36††**	21.43±0.81††**§§	126.10±0.99**

Note: A: control group, B: exercise group, C: exercise + PSP (50 mg/ kg)-treated group, D: exercise + PSP (100 mg/ kg)-treated group, E: exercise + PSP (200 mg/ kg)-treated group, F : exercise + caffeine (10 mg/ kg)–treated group. Values are mean±SD. †† $p<0.01$, compared with group A, ** $p<0.01$, compared with group B, §§ $p < 0.01$, compared with group E.

3.3 Effect of polysaccharide extracted from *Spirulina platensis* on 5-HT concentrations in the caudate putamen of trained rats

The effect of polysaccharide extracted from *Spirulina platensis* on 5-HT concentrations in the caudate putamen was presented in Fig.2. The 5-HT concentration in the caudate putamen was 4.85 ± 0.58 nmol/g tissue in the control group (A), 24.76±1.15 nmol/g tissue in the exercise group (B), 20.80±1.17 nmol/g tissue in the exercise and PSP (50 mg/kg)-treated group (C), 16.61 ± 1.14 nmol/g tissue in the exercise and PSP (100 mg/kg)-treated group (D), 13.69±0.93 nmol/g tissue in the exercise and PSP (200 mg/kg)-treated group (E). The results showed that treadmill running increased the 5-HT levels in the caudate putamen, and PSP suppressed the exercise-induced increase of 5-HT levels in the caudate putamen in a dose-dependent way.

We also compared 5-HT concentrations in the caudate putamen between the PSP (200 mg/kg)-treated group (E) and caffeine (10 mg/kg)-treated group (F). The 5-HT levels in the caudate putamen were 13.69 ± 0.93 nmol/g tissue in the exercise and PSP (200 mg/kg)-treated group (E) , while the 5-HT levels stood at 13.79 ± 0.95 nmol/g tissue in the exercise and caffeine (10 mg/kg)-treated group (F). The results showed that PSP (200 mg/kg) was just as effective as caffeine (10 mg/kg) in suppressing the exercise-induced increase of 5-HT level in the caudate putamen.

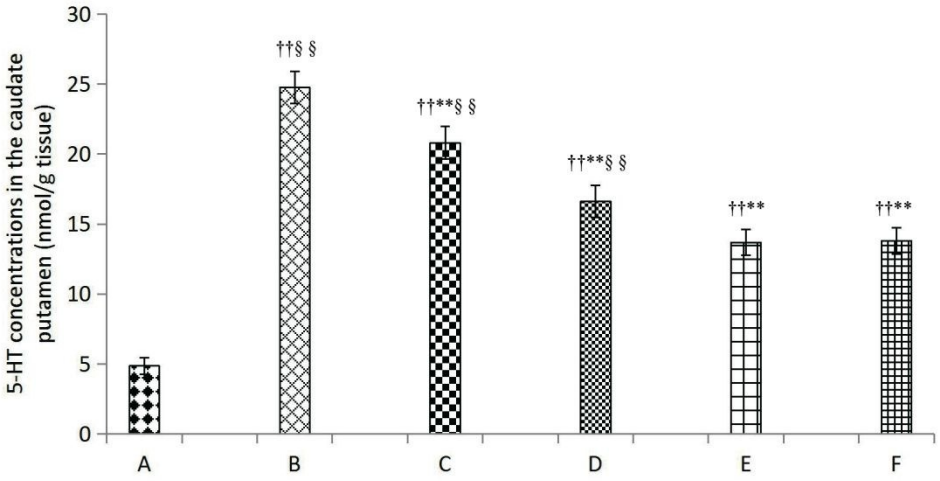

Fig.2 The effect of polysaccharide of *Spirulina platensis* on 5-HT concentrations in the caudate putamen of trained rats.

Note: A: control group, B: exercise group, C: exercise + PSP (50 mg/ kg)-treated group, D: exercise + PSP (100 mg/ kg)-treated group, E: exercise + PSP (200 mg/ kg)-treated group, F : exercise + caffeine (10 mg/ kg)–treated group. Values are mean±SD. †† $p<0.01$, compared with group A, ** $p<0.01$, compared with group B, §§ $p < 0.01$, compared with group E.

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3.4 Effect of polysaccharide extracted from *Spirulina platensis* on mRNA and protein expression of TPH2 in the caudate putamen of trained rats

The effect of polysaccharide extracted from *Spirulina platensis* on the levels of TPH2 mRNA expression in the caudate putamen for treadmill running was presented in Fig.3. The levels of TPH2 mRNA in the caudate putamen in the exercise groups (B, C, D, E and F) were significantly higher than those of the control group (A) (P<0.01). The rats of the exercise group (B) exhibited significantly higher levels of TPH2 mRNA in the caudate putamen than those of the exercise and PSP -treated groups (C, D and E) (P<0.01). The levels of TPH2 mRNA in the caudate putamen in the exercise and PSP (200 mg/kg)-treated group (E) were lower than those of the exercise and PSP(50 mg/kg) and (100 mg/kg)-treated groups (C and D) (P<0.01). No significant change was observed for the levels of TPH2 mRNA in the caudate putamen between the PSP (200 mg/ kg)-treated group (E) and caffeine (10 mg/kg)-treated group (F) (P>0.05). Similar results were obtained by using the method of western blot analysis to detect the level of TPH2 protein express in the caudate putamen (Fig.4). The results showed that treadmill running increased the levels of TPH2 mRNA and protein expression in the caudate putamen, and PSP suppressed the exercise-induced increase of the TPH2 mRNA and protein express levels in a dose-dependent way, and PSP (200 mg/ kg) was just as effective as caffeine (10 mg/ kg) in suppressing the exercise-induced increase of TPH2 mRNA and protein express levels.

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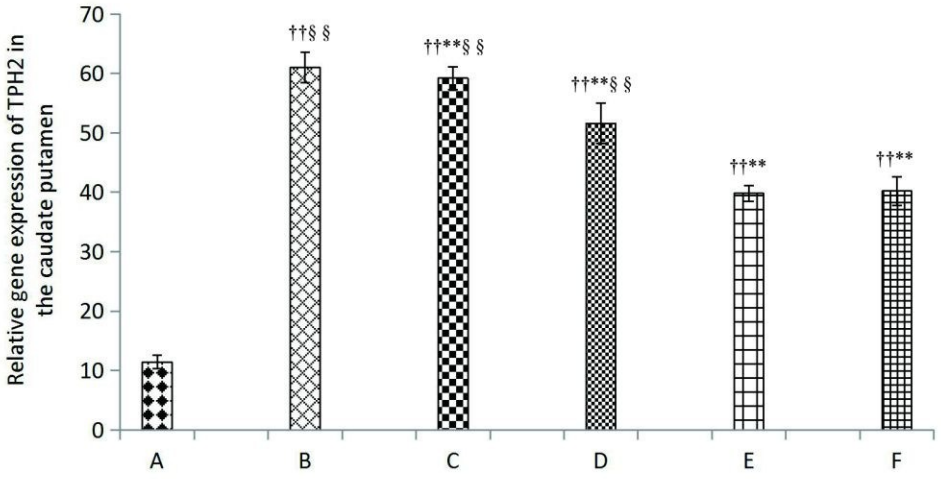

Fig.3 The effect of polysaccharide of *Spirulina platensis* on mRNA expression of TPH2 in the caudate putamen of trained rats.

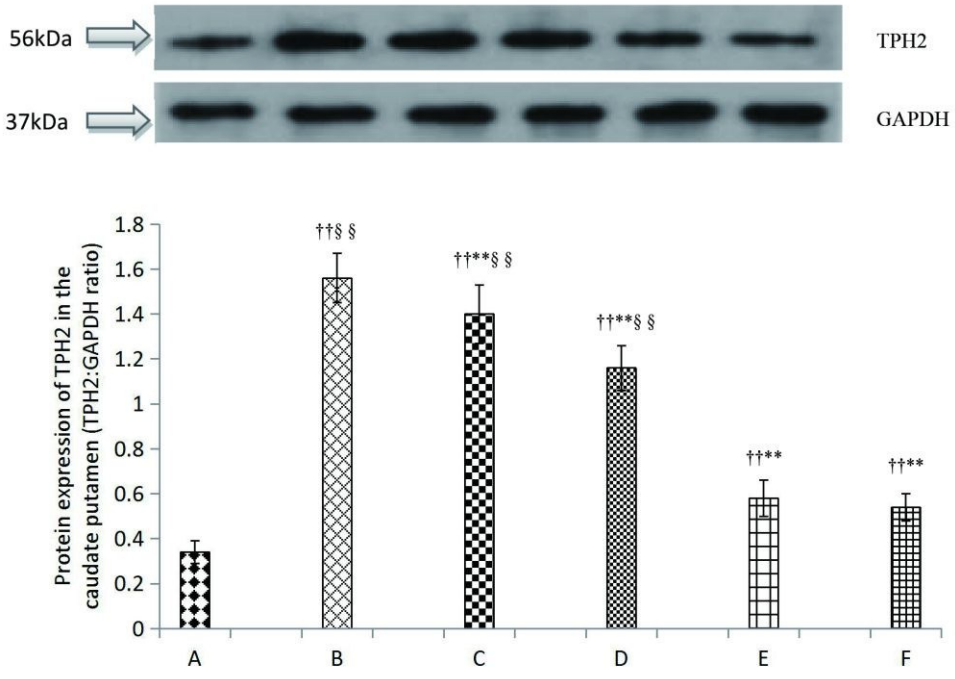

Fig.4 The effect of polysaccharide of *Spirulina platensis* on protein expression of TPH2 in the caudate putamen of trained rats.

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Note: A: control group, B: exercise group, C: exercise + PSP (50 mg/ kg)-treated group, D: exercise + PSP (100 mg/ kg)-treated group, E: exercise + PSP (200 mg/ kg)-treated group, F : exercise + caffeine (10 mg/ kg)–treated group. Values are mean ±SD. †† $p<0.01$, compared with group A, ** $p<0.01$, compared with group B, §§ $p < 0.01$, compared with group E.

3.5 Effect of polysaccharide extracted from *Spirulina platensis* on mRNA and protein expression of 5-HT1B in the caudate putamen of trained rats

The effects of polysaccharide extracted from *Spirulina platensis* on the levels of 5-HT1B mRNA and protein expression in the caudate putamen for treadmill exercise were presented, respectively in Fig.5 and Fig. 6. The levels of 5-HT1B mRNA in the caudate putamen in the exercise groups (B, C, D, E and F) were significantly lower than those of the control group (A) ($P<0.01$ or $P<0.05$). The levels of 5-HT1B mRNA in the caudate putamen in the exercise group (B) were lower than those of the exercise and PSP -treated groups (C, D and E) ($P<0.01$). The levels of 5-HT1B mRNA in the the caudate putamen in the exercise and PSP (200 mg/kg)-treated group (E) were higher than those of the exercise and PSP (50 and 100 mg/kg) -treated groups (C and D) ($P<0.01$). No significant change was observed in the levels of 5-HT1B mRNA in the caudate putamen between the PSP (200 mg/kg)-treated group (E) and caffeine (10 mg/kg)-treated group (F) ($P<0.05$). Similar results were obtained by using the method of western blot to detect the level of 5-HT1B protein expression in the caudate putamen. The results showed that treadmill exercise decreased the levels of 5-HT1B mRNA and protein express in the caudate putamen, and PSP resisted the

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exercise-induced decrease of the 5-HT1B mRNA and protein express levels in a dose-dependent way, and PSP (200 mg/kg) was just as effective as caffeine (10 mg/kg) in resisting the exercise-induced decrease of 5-HT1B mRNA and protein express levels.

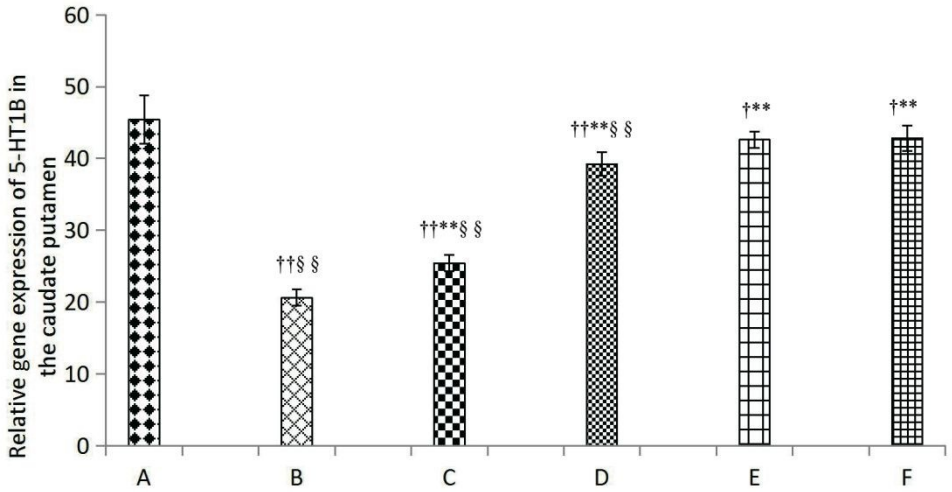

Fig.5 The effect of polysaccharide of *Spirulina platensis* on mRNA expression of 5-HT1B in the caudate putamen of trained rats.

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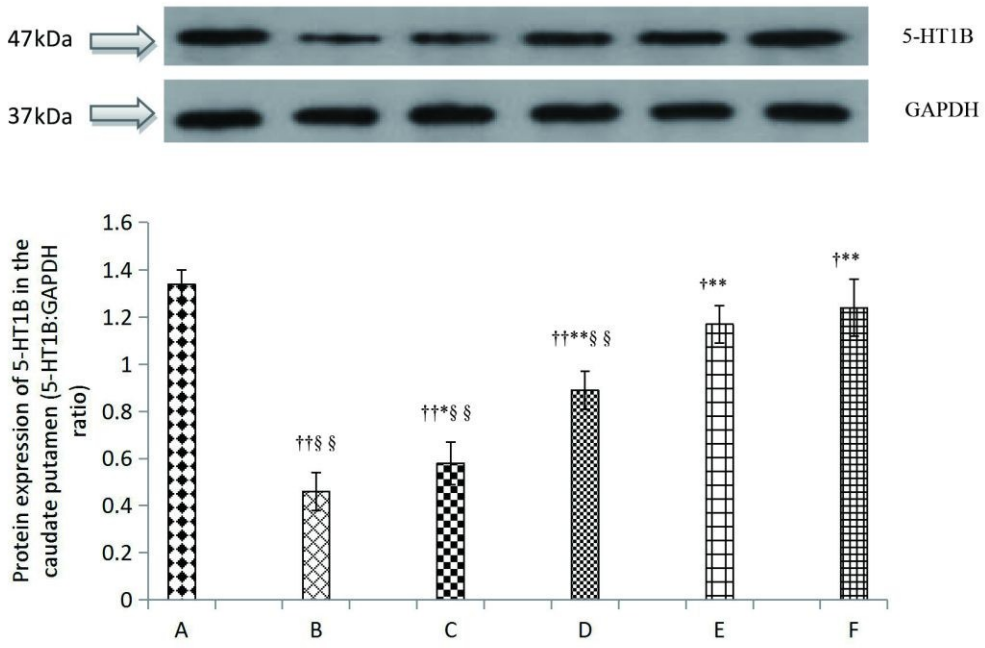

Fig.6 The effect of polysaccharide of *Spirulina platensis* on protein expression of 5-HT1B in the caudate putamen of trained rats.

Note: A: control group, B: exercise group, C: exercise + PSP (50 mg/ kg)-treated group, D: exercise + PSP (100 mg/ kg)-treated group, E: exercise + PSP (200 mg/ kg)-treated group, F : exercise + caffeine (10 mg/ kg)–treated group. Values are mean ± SD. †† $p<0.01$, † $p<0.05$, compared with group A, * $p<0.05$, ** $p<0.01$, compared with group B, §§ $p < 0.01$, compared with group E.

4. Discussion

The present data revealed that polysaccharide from *Spirulina platensis* (PSP) treatment significantly promoted physical performance, increased Hb levels and decreased LA, BUN and CK levels in the blood and inhibited the exercise-induced synthesis of 5-HT and TPH2 protein expression and improved 5-HT1B protein expression in the caudate putamen. Furthermore, the most potent effects of PSP were

observed at the dose of 200 mg/kg of PSP. Three different gavage doses of PSP (50, 100 and 200 mg/ kg) used were based on the literature [11] and on the preliminary experiment results.

The time of forced treadmill exercise to exhaustion is used as an indicator of anti-fatigue activity [21,24]. Hb is commonly stated to be one of the major factors that can improve endurance capability by carrying oxygen to the tissues [22]. After the onset of exercise-induced fatigue, decrease of Hb can be regarded as a biochemical marker to judge whether there is exercise-induced fatigue in rats [25]. The occurrence of fatigue can be determined by measuring the blood LA increase [25]. BUN is generally considered as one of the important indicators that reflect the degree of body fatigue and the assessment of functional status [26]. Blood CK activity is an important sensitive biochemical indicator in assessing the body fatigue and understanding the adaptation and recovery of muscle [27-28]. The present data revealed that three doses of PSP treatment could significantly prolong the time to exhaustion by treadmill exercise, indicating that PSP has obvious anti-fatigue activity. Meanwhile, the results also show that PSP treatment (100mg, 200 mg/kg) can significantly increase Hb levels and decrease LA, BUN, CK levels in the blood. It is suggested that the anti-fatigue effect of PSP may be related to the increase of hemoglobin concentration and decrease of LA, BUN, CK levels in blood of exercised rats.

Given that the muscle contraction is initiated by the central nervous system, which is reasonable to assume that the alterations in CNS may contribute to the progress of fatigue[29.] Serotonin is an important determinant of performance during both forced

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and voluntary exercise [30]. Stronger release of 5-HT can inhibit rhythmic activity and motoneuron firing which is responsible for central fatigue [31], which is the term for fatigue caused by factors residing within the central nervous system, brain, spinal cord and motor neurons [32]. Increased concentrations of brain 5-HT during sustained physical activity could hasten the onset of fatigue [14], while decreasing 5-HT concentrations could delay the time to fatigue [15]. Significantly increased 5-HT levels were detected in the frontal cortex and hippocampus in a rat model of exercise-induced chronic fatigue [33]. Polysaccharide fraction isolated from *passiflora edulis* could modulate the liberation or synthesis of serotonin [19]. Polysaccharide fraction isolated from *Polygonatum sibiricum Red* significantly decreased the production of 5-HT in the brain of exercise fatigue rats [34]. In this study, 5-HT concentrations in the caudate putamen in exercise group were significantly higher than control group, indicating that 5-HT plays an important role in central fatigue [35] PSP treatment was proved to be able to inhibit exercise-induced increase in 5-HT concentrations in the caudate putamen. The most potent inhibition of PSP on 5-HT concentrations was observed at the dose of 200 mg/kg of PSP. It suggests that noticeable anti-fatigue effect of PSP might be related to inhibiting the increase of 5-HT concentration induced by exercise.

Inhibition of tryptophan hydroxylase (TPH) abolishes fatigue induced by central tryptophan in exercising rats [36]. Increased TPH2 expression has been shown to be a good predictor of increased TPH activity and 5-HT synthesis [37]. The expressions of TPH2 significant increased in chronic fatigue syndrome rats induced by forced

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treadmill running [38]. Our previous reports have shown exercise-induced increased TPH2 expression [21, 24]. In this study, the data showed that mRNA and protein expression of TPH2 in the caudate putamen in exercise group were significantly higher than control group, which was consistent with the literature. PSP treatment could decrease mRNA and protein expression of TPH2 in the caudate putamen of exercised rats. The most potent inhibition of PSP on expression of TPH2 was observed at the dose of 200 mg/kg of PSP. It suggests that polysaccharide of *Spirulina platensis* can inhibit exercise-induced increase in 5-HT levels possibly by decreasing TPH2 expression in the caudate putamen of exercised rats.

Higher levels of 5-HT1B autoreceptor mRNA in the raphe nuclei may be involved in delaying onset of exercise-induced fatigue [39]. The acute treatment with 5-HT(1B) agonist (CP-94253) significantly decreased the 5-HT synthesis in both the Flinders Sensitive Line (FSL) and Flinders Resistant Line (FRL) rats [40]. Our previous reports have also shown exercise-induced decreased 5-HT1B protein expression of treadmill trained rats [21,24]. This study showed that intensity incremental treadmill exercise produced a reduction of 5-HT1B mRNA and protein expression in the caudate putamen, and polysaccharide from *Spirulina platensis* inhibited exercise-induced decreases of 5-HT1B mRNA and protein expression in the caudate putamen. The most potent augmentation of 5-HT1B expression was observed at the dose of 200 mg/kg of PSP. These data suggest that polysaccharide of *Spirulina platensis* could constrain the increase in 5-HT levels produced by physical activity also through increasing local 5-HT1B autoinhibition of 5-HT neurons in the caudate putamen of

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exercised rats.

The ergogenic effect of caffeine on endurance exercise is commonly accepted [41]. Caffeine used as a supplement has been shown to improve physical performance due to its effects on the central nervous system [42]. So caffeine was selected as the positive control drug. The gavage dose of caffeine (10 mg/ kg) used were based on the literature [24]. Our previous research showed that caffeine increased all-out time in exercised rats, and decreased 5-HT concentrations, and inhibited the exercise-induced elevation of TPH2 expression, and reduction of 5-HT1B protein expression [21, 24]. In this study, we found that PSP (200 mg/kg) was just as effective as caffeine (10 mg/kg) on prolonging the exhaustion-time by treadmill running, reducing serum BUN levels, caudate putamen's 5-HT contents and TPH2 mRNA and protein expression, enhancing Hb concentrations in the blood and 5-HT1B mRNA and protein expression in the caudate putamen of exercised rats. And PSP (200 mg/kg) can significantly decrease serum LA, CK levels, but caffeine (10 mg/kg) can significantly increase serum CK and LA levels. The elevated LA levels are more likely due to a reduced clearance by the exercising muscle and a greater release by non exercising tissues[43], and the elevated CK levels are resulted from caffeine increasing the exercise induced muscle injury[44]. It is suggested that the anti-fatigue of PSP might be better than caffeine.

5. Conclusions

Polysaccharide of *Spirulina platensis* at 200 mg/kg has shown a good effect on improving endurance of exercised rats. The mechanism of polysaccharide of *Spirulina*

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platensis's promoting physical performance might be related to increasing Hb levels and decreasing LA, BUN and CK levels in the blood and the inhibition of the exercise-induced synthesis of 5-HT and TPH2 expression, and the increase of the 5-HT1B expression in the caudate putamen of exercised rats. Results from this study has not only suggested that polysaccharide of *Spirulina platensis* might be the active fraction of spirulina' strong effect of promoting physical performance[1-2], but also could provide partial experimental basis for the use of polysaccharide of *Spirulina platensis* in sports nutrition, which its wide application is limited because of the fishy smell of spirulina.

Conflicts of interest

There are no conflicts to declare.

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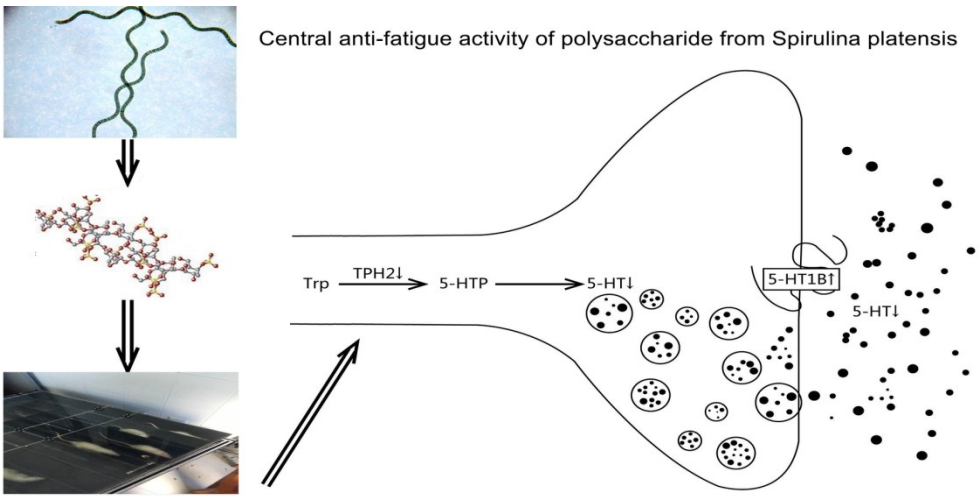

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