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Full Length Research Paper

Spirulina platensis extract supplementation attenuates oxidative stress in acute exhaustive exercise: A pilot study

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This study was performed to investigate the protective effect of *Spirulina platensis* extract (SPE) supplementation on oxidative stress in acute exhaustive exercise. The experiment was carried out on 8 male Wistar rats distributed into 4 groups (A, B, C, and D) of 20 rats each. The A group designated as control group were administered with placebo (saline) water by gavage every day for 6 weeks. The B, C and D group designated as SPE group were administered with SPE of 50, 100, and 200 mg/kg body weight day for 6 weeks, respectively. After 6 weeks, the exhaustive exercise started at 10% grade, 15 m/min for 15 min followed by a gradual increase of treadmill speed and times as 25m/min for 15 min, 30m/min for 30 min, 35m/min for 60 min, 40 m/min for 30 min, 45 m/min for 30 min until exhaustion. The time of running to exhaustion, the BUN, glucose, lactate levels of blood, the MDA, SOD and GPX levels of muscle were determined after exhaustive exercise. Results of the above study showed that the time of running to exhaustion of rats in SPE groups were significantly prolonged compared with that in the control group (*P<0.05)*. Levels of the BUN, blood lactate and MDA of rats in SPE groups were significantly decreased compared with that in the control group (P<0.05) and levels of the blood glucose, SOD and GPx of rats in SPE groups were significantly increased compared with that in the control *group* (P<0.05). It was concluded that exhaustive exercise could result in oxidative stress. The SPE supplementation increased performance of exhaustive exercise, and it was beneficial in enhancing the antioxidant status and inhibiting oxidative stress induced by acute exhaustive exercise.

Key words: *Spirulina platensis*, oxidative stress, exhaustive exercise.

INTRODUCTION

Spirulina platensis, a blue green micro alga, is the nature's richest and most complete source of organic nutrition (Piñero Estrada et al., 2001). It contains a wide spectrum of nutrients that include B-complex vitamins, minerals, good quality proteins, gamma-linolenic acid and the super antioxidants, beta-carotene, vitamin E and trace elements (Liu and Zhang, 2002). Early interest in *S. platensis* focused mainly on its potential as a source of protein and vitamins but recently, more attention has been paid to its potential pharmacological properties, which include reports of the ability of preparations of this micro alga to prevent and inhibit cancers, to decrease blood cholesterol levels, to decrease blood glucose levels, free radical scavenging, stimulate the immunological system, to reduce the nephrotoxicity of pharmaceuticals and toxic metals and provide protection

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Abbreviations: SPE, *Spirulina platensis* extract; **BUN,** blood urea nitrogen; **MDA,** malondialdehyde; **SOD,** Superoxidase dismutase; GPx, Glutathione peroxidase; ROS, reactive oxygen species.

against the harmful effects of radiation (Guan and Guo, 2002; Xue et al., 2007; Ismail et al., 2009; Muthuraman et al., 2009; Nielsen et al., 2010). Although, many pharmacological properties of *S. platensis* have received a great deal of attention, researches in this area are to be continued. However, as far as we know, the role of *S. platensis* in exhaustive exercise-induced oxidative stress in human subjects or animal studies has not been investigated. Therefore, the present investigation was undertaken to assess the effect of *S. platensis* extract (SPE) on acute exhaustive exercise -induced oxidative stress in rats.

MATERIALS AND METHODS

Preparation of *S. platensis* extract (SPE)

S. platensis, a fine dark blue-green spray-dried powder, was purchased from DeKete Biological Engineering Co., Ltd. (Yunnan, China). *S. platensis* propagated under basic conditions (pH 11.0) in outdoor open tanks was extracted with water in an autoclave for 1 h at 120°C. Citric acid was added to the hot water extract to adjust the pH to 4.0 (Quoc and Dubacq, 1997). The water-soluble extract was prepared by removal of insoluble fractions by centrifugation. The soluble extract of *S. platensis* was condensed for oral administration as described previously (Hirahashi et al., 2002).

Animals

Male Wistar rats (general type, weight 180 to 220g) were supplied by animal experiment laboratory, Hangzhou Institute of Pharmaceutical Research. The animals were housed under conditions of controlled temperature and a 12 h lighting cycle and fed with standard diet ad libitum. All animal studies were performed in accordance with the guidelines and under approval of the Institutional Review Committee for the Animal Care and Use of the Hangzhou Normal University.

Experiment protocol

Following one week of acclimatization, the animals were divided into four groups (A, B, C, and D) of 20 animals each. The A group designated as control group were administered with placebo (saline) water by gavage every day for 6 weeks. The B, C and D group designated as SPE group were administered with SPE of 50, 100, and 200 mg/kg body weight day for 6 weeks (approximately 0.5 ml in volume), respectively. The dose was chosen based on estimates from prior studies. After 6 weeks, exhaustive exercise was performed on a rodent treadmill (model AS804, Tianjin Guoguang Instruments, Tianjin, China) with the following protocols. In the adaptive period, rats were accustomed to treadmill running for 1 week.

Then, the rats were subjected to graded treadmill running starting at 10% grade, 15 m/min for 15 min followed by a gradual increase in the treadmill speed and time to 25 for 15, 30 for 30, 35 for 60, 40 for 30 and 45 m/min for 30 min until exhaustive. Electrical shocks were used sparingly in exhaustive exercise groups to motivate the animals to run. Exhaustion was defined as the inability of the rats to run on the treadmills, despite electrical shock (Hsieh et al., 2006). In treadmill running exercise, intensity and duration are more easily manipulated and quantified compared with in voluntary wheel or swimming exercises (Rosa et al., 2008).

Reagents

BUN, lactate, MDA, SOD and GPX assay kits were purchased from the Nanjing Jiancheng Bioengineering Institute Nanjing, China. Other chemicals were of analytical-reagent grade and were purchased from Hangzhou Chemical Reagent Co. Hangzhou, China, unless otherwise stated.

Sampling and tissue preparation

All animals were anesthetized with ethyl ether and sacrificed immediately after the exhaustive exercise. Blood samples were collected from the abdominal aorta and the gastrocnemius muscle were carefully removed, rinsed in ice-cold normal saline, blotted dry and stored at 80°C for further analysis.

Biochemical analysis

Blood samples were centrifuged at 1,400 × g at 4°C for 10 min. The supernatants (plasma) were used to determine BUN, glucose, and lactate. Gastrocnemius muscle were homogenized in ice-cold buffer (0.25 M sucrose, 10 mM Tris-HCl, and 0.25 mM phenylmethylsulfonyl fluoride with pH 7.4), and a portion of the homogenate was measured immediately for MDA. Another portion of the homogenate was centrifuged at 10,000 × g for 20 min at 4°C, then SOD and GPX activities in the supernatant were measured.

Blood glucose was measured using an automatic analyzer (7170 model, Hitachi, Tokyo, Japan). BUN, lactate, MDA, SOD and GPX were measured using commercial diagnostic kits.

Statistical analysis

Data are presented as mean ± standard deviation (SD) of three replications and were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparison among all groups.

Differences below $P<0.05$ implied significance. Statistical package for social sciences (SPSS for windows, version 13.0) was used for this analysis.

RESULTS

Effects of SPE on the time of running to exhaustion

The results were shown in Figure 1. The time of running to exhaustion of rats in 50, 100, and 200 mg/kg SPE groups (groups B, C and D) was significantly prolonged compared with that in the control group (group A) ($P<0.05$). It was 1.39, 1.59 and 1.84 times longer than that in the control group, respectively.

Effects of SPE on the BUN, glucose, and lactate

The results were shown in Figure 2. The BUN levels of rats in 50, 100 and 200 mg/kg SPE groups were 6.25 ± 1.03, 6.01 ± 0.84, 5.84 ± 0.87 mmol/L, which were significantly decreased compared to that in the control group (8.68 ± 0.94 mmol/L) ($P<0.05$). The blood glucose levels of rats in 50, 100 and 200 mg/kg SPE groups were

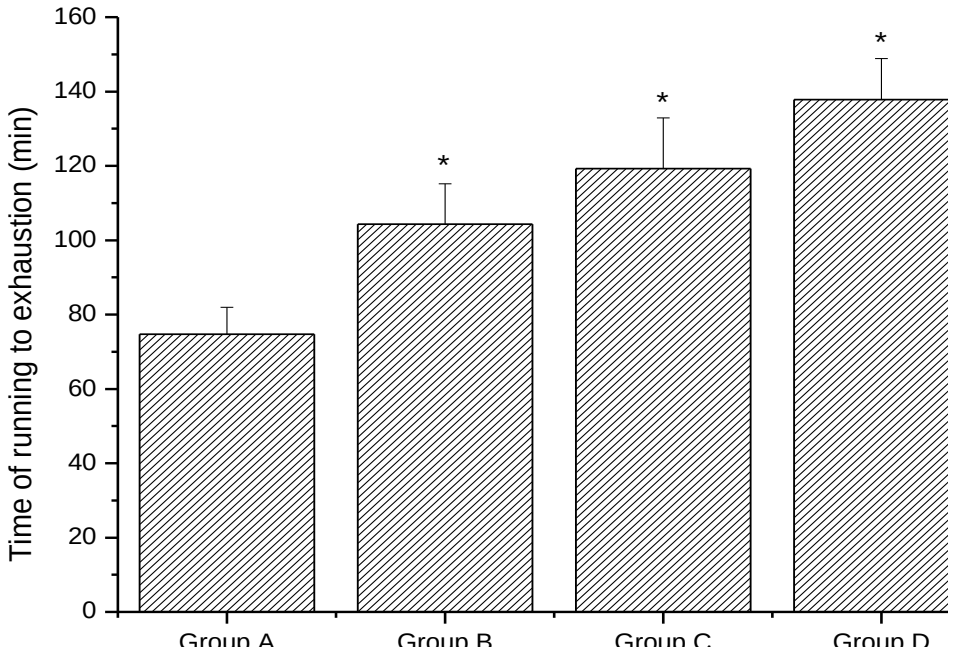

Figure 1. Effects of SPE on the time of running to exhaustion of rats.*$P<0.05$, compared with control group (group A). Twenty rats were observed and tested for each group during the experimental period.

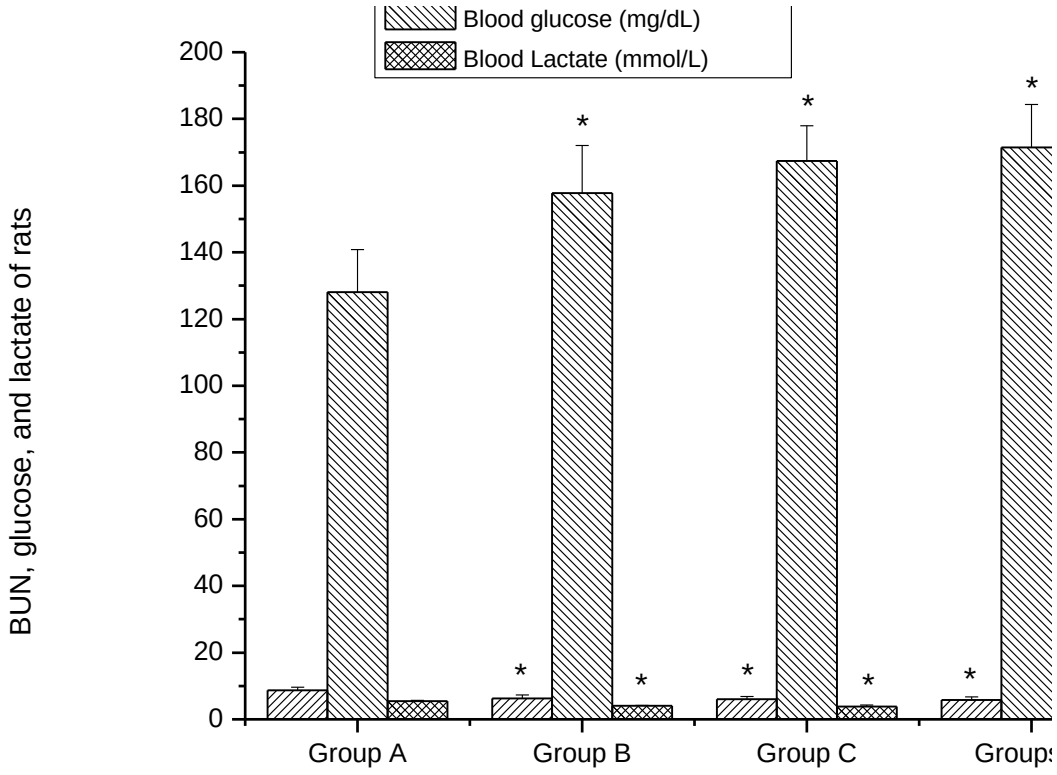

Figure 2. Effects of SPE on the BUN, glucose, and lactate of rats.*$P<0.05$, compared with control group (group A). Twenty rats were observed and tested for each group during the experimental period.

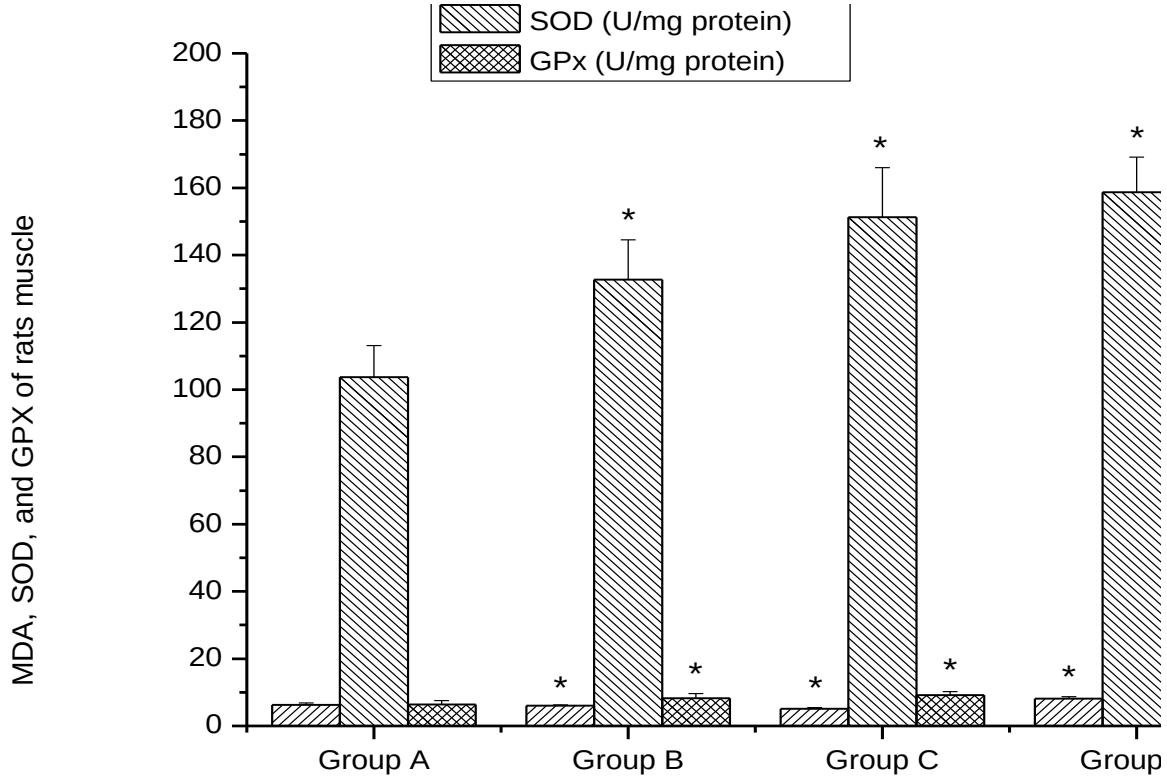

Figure 3. Effects of SPE on MDA, SOD, and GPX of rats muscle. *$P<0.05$, compared with control group (group A). Twenty rats were observed and tested for each group during the experimental period.

157.8 ± 14.2, 167.4 ± 10.6 and 171.5 ± 12.8 mg/dL, respectively, which were significantly increased compared to that in the control group (128.1 ± 12.7 mg/dL) ($P<0.05$). The blood lactate levels of rats in 50, 100 and 200 mg/kg SPE groups were 4.01 ± 0.16, 3.88 ± 0.47, 3.74 ± 0.39 mmol/L, which were significantly decreased compared to that in the control group (5.42 ± 0.32 mmol/L) ($P<0.05$).

Effects of SPE on MDA, SOD and GPX

The results were shown in Figure 3. The MDA levels of rats in 50, 100 and 200 mg/kg SPE groups were 6.23 ± 0.63, 5.98 ± 0.28 and 5.07 ± 0.41 nmol/mg, which were significantly decreased compared to that in the control group (8.16 ± 0.49 nmol/mg) ($P<0.05$). The SOD levels of rats in 50, 100 and 200 mg/kg SPE groups were 132.69 ± 11.84, 151.23 ± 14.75 and 158.67 ± 10.47 U/mg, respectively, which were significantly increased compared to that in the control group (103.68 ± 9.46 U/mg) ($P<0.05$). The GPx levels of rats in 50, 100 and 200 mg/kg SPE groups were 8.27 ± 1.36, 9.12 ± 1.05 and 12.34 ± 1.47 U/mg, respectively, which were significantly increased compared to that in the control group (6.34 ± 1.19 U/mg) ($P<0.05$).

DISCUSSION

Cells continuously produce free radicals and reactive oxygen species (ROS) as part of metabolic processes. These free radicals are neutralized by an elaborate antioxidant defense system consisting of enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and numerous non-enzymatic antioxidants, including vitamins A, E and C, glutathione, ubiquinone, and flavonoids (Urso and Clarkson, 2003). Exercise can cause an imbalance between ROS and antioxidants, which is referred to as oxidative stress (Aoi et al., 2004; Muaz and Hakki, 2006). However, during increased oxygen utilization, as happens during strenuous exercise, the rate of ROS production may overwhelm the body's capacity to detoxify them, which can lead to increased oxidative stress and subsequent lipid peroxidation (Close et al., 2004; Reid, 2008). Numerous studies have shown that Free radical production reaches the highest level when exercise is exhaustive (Yu et al., 2006; Hwang et al., 2007). Oxidative stress may progress to oxidative damage involving cellular proteins (contractile, structural,

and enzymatic), lipids, DNA, and other molecules in ways that might lead to abnormal cellular function (Lakatta, 1980; Davydov and Shvets, 2001; Nayanatara et al., 2005). The present study aimed to examine the effect of *S. platensis* extract (SPE) on acute exercise-induced oxidative stress in rats.

The laboratory rat is a commonly used model for the evaluation of exhaustive exercise effects on biochemical changes in man (Huang et al., 2009). In this study, the rats were subjected to graded treadmill running until exhaustion. The time of running to exhaustion of rats in SPE groups were significantly prolonged compared with that in the control group (P<0.05), which showed that SPE supplementation increased performance of exhaustive exercise in rats. In addition, it has been reported that exercise intensity could be 92.3% VO_2 max, when rat at 26.8 m/min and 10% grade (Bedford et al., 1979). Obviously, the intensity of exhaustive exercise in this study was very strenuous.

Previous studies indicated that exhaustive exercise increases BUN and blood lactate levels, decreases blood glucose levels (Antunes-Neto et al., 2006), which causes significant skeletal muscle, liver, and kidney damage. SUN is a sensitive index to evaluate the bearing capability when human bodies suffer from a physical load and caused by catabolism of proteins and amino acids. Protein and amino acids have a stronger katabolic metabolism when body cannot obtain enough energy by sugar and fat catabolic metabolism. Therefore, there is a positive correlation between the urea nitrogen in vivo and the exercise tolerance (Sun and Wang, 2010). In this study, the BUN levels of rats in SPE groups were significantly decreased compared with that in the control group (P<0.05), which showed that SPE supplementation may reduce catabolic decomposition of protein for energy.

Homeostasis of blood glucose is important for the prolongation of endurance exercise (Abe et al., 1995; Astorino et al., 2000). It is known that running endurance capacity is markedly decreased by the inhibition of gluconeogenesis. If phosphoenolpyruvate carboxylase, a key enzyme in gluconeogenesis, is inhibited by mercaptopicolinic acid, gluconeogenesis significantly decreases. Gluconeogenesis carries out the major role of glucose homeostasis in endurance exercise (Abe et al., 1995). In this study, the blood glucose levels of rats in SPE groups were significantly increased compared with that in the control group (*P<0.05*) , which showed that SPE supplementation must be brought about by an improvement in the physiological function or metabolic control of exercise as well as by an activation of energy metabolism.

Blood lactate acid is the glycolysis product of carbohydrate under an anaerobic condition, and glycolysis is the main energy source for intense exercise in a short time. Many organs such as liver and heart, and tissues such as skeletal muscle, help to remove lactate from the blood, but intense exercise can increase lactate production (Wei et al., 2010). In this study, the blood lactate levels of rats in SPE groups were significantly decreased compared with that in the control group (*P<0.05*), which showed that SPE supplementation could inhibit the production of blood lactate during acute exhaustive exercise.

Oxidative stress is characterized by ROS-induced lipid peroxidation, DNA damage and protein degradation. MDA is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA. MDA level is commonly known as a marker of oxidative stress (Gaweł et al., 2004; Misra et al., 2009). In this study, the MDA levels of rats SPE groups were significantly decreased compared with that in the control group (*P<0.05*), which showed that SPE supplementation could effectively reduce lipid peroxidation.

Antioxidant enzymes play an important role in the protection against free radical damage, a decrease in the activities or expressions of these enzymes may predispose tissues to the free radical damage. Physical exercise is known to have differential effects on antioxidant enzymes (Salminen and Vihko, 1983). In humans, Regular exercise was shown to increase activities of SOD and GPx in muscle (Higuchi et al., 1985; Teixeira et al., 2009). However, strenuous exercise causes a dramatic drop in antioxidant enzymes (Lee et al., 2009; Shan et al. 2010). In this study, the SOD and GPx levels of rats in SPE groups were significantly increased compared with that in the control group (*P<0.05*), which showed that SPE supplementation could up-regulate antioxidant enzymes to protect against oxidative stress-induced injury during exercise.

To conclude, the results in this study demonstrated that exhaustive exercise could result in oxidative stress. SPE supplementation increased performance of exhaustive exercise and it was beneficial in enhancing the antioxidant status and inhibiting oxidative stress induced by acute exhaustive exercise.

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