

EXPERIMENTAL STUDY

Effect of Inonotus Obliquus Polysaccharides on physical fatigue in mice

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RESULTS: IOP extended the swimming time of mice, and increased the glycogen content of liver and muscle, but decreased blood lactic acid and serum urea nitrogen levels. IOP had no toxic effects on major organs such as the liver as assessed by histopathological examinations.

CONCLUSION: IOP might be a potential anti-fatigue pharmacological agent.

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Key words: Polysaccharides; Fatigue; Blood urea nitrogen; Lactic Acid; Glycogen; Anatomy and histology; Pharmacological phenomena

Abstract

OBJECTIVE: To evaluate the potential beneficial effects of Inonotus obliquus polysaccharides (IOP) on the alleviation of physical fatigue in mice.

METHODS: Sixty-four male mice were randomly divided into four groups ($n = 16$ per group). Mice were orally administered IOP for a period of 14 days at 0, 100, 200 and 300 mg/kg/d, and were assigned to the control, IOP-100, IOP-200, and IOP-300 groups, respectively by the random number table method. Mice in the control group received an oral administration of sterile distilled water. A forced swimming test was performed for 8 mice per group at one hour after the last treatment. The other 8 mice in each group swam for 30 min. Blood, liver and muscle samples were taken after resting for 30 min. Levels of blood urea nitrogen and lactate, as well as glycogen contents of the liver and muscle were measured. Morphology of liver was observed by light microscopy.

INTRODUCTION

Fatigue is defined as physical and/or mental weariness resulting in negative impacts on exercise intensity, work performance, family life and social relationships.¹ Physical fatigue is accompanied by a deterioration in functional performance and exhaustive or intensive exercise can lead to the accumulation of excess reactive free radicals that result in tissue damage. Many studies have demonstrated that physical fatigue limits the ability to work and physical performance, and this has become a common problem worldwide.²

The medicinal mushroom *Inonotus obliquus* (commonly known as Chaga) belongs to the family Hymenochaetaceae of Basidiomycetes.^{3,4} It grows on the living trunks of mature birch trees and is mainly found at latitudes of 45° N-50° N.⁵ Traditionally, Chaga has been used as a folk remedy for the treatment of gastrointestinal cancer, cardiovascular disease and diabetes in Russia, Poland and most of the Baltic countries.⁴ Studies showed that polysaccharides from *Inonotus obliquus* possess a wide range of pharmacologic and

health-promoting properties including immune enhancement,⁶⁻⁷ anti-oxidation,⁴ anti-diabetes,⁸ and anti-tumor.⁹⁻¹⁰ However, little is known about the anti-fatigue effects of Chaga. In China, Japan, Korea, and other Asian countries, many edible and medicinal mushroom-derived preparations are widely applied for physical fatigue for either preventive or curative purposes.¹¹⁻¹³ The current study evaluated the anti-fatigue effects of *Inonotus obliquus* polysaccharides (IOP) in mice, using a forced swimming test and measuring changes of biochemical parameters to provide scientific evidence for the use of IOP for the prevention and treatment of diseases related to fatigue.

MATERIALS and METHODS

Plant materials and preparation of IOP

Commercially available dried *Inonotus obliquus* sam-

ples were purchased from the Natural Product Trading Company (Jilin, China) and identified by Professor Zhao Fenqin, College of Medicine, Henan University. Preparation of IOP is shown in Figure 1. HPLC analysis indicated that *Inonotus obliquus* extracts contained 13.3% polysaccharide.¹⁴

Animals

Sixty-four healthy Kunming male mice of SPF grade, four-weeks-old, weighing (20 ± 22) g, were supplied by the Jilin University Laboratory Animal Center (Certificate of quality No. SCXK [J] 2008-0005) and fed in the Laboratory Animal Center of Jilin Medical College. The study was approved by the experimental animal ethics committee of Jilin University.

Modeling and grouping

Overall, 64 mice were randomly divided into 4 groups

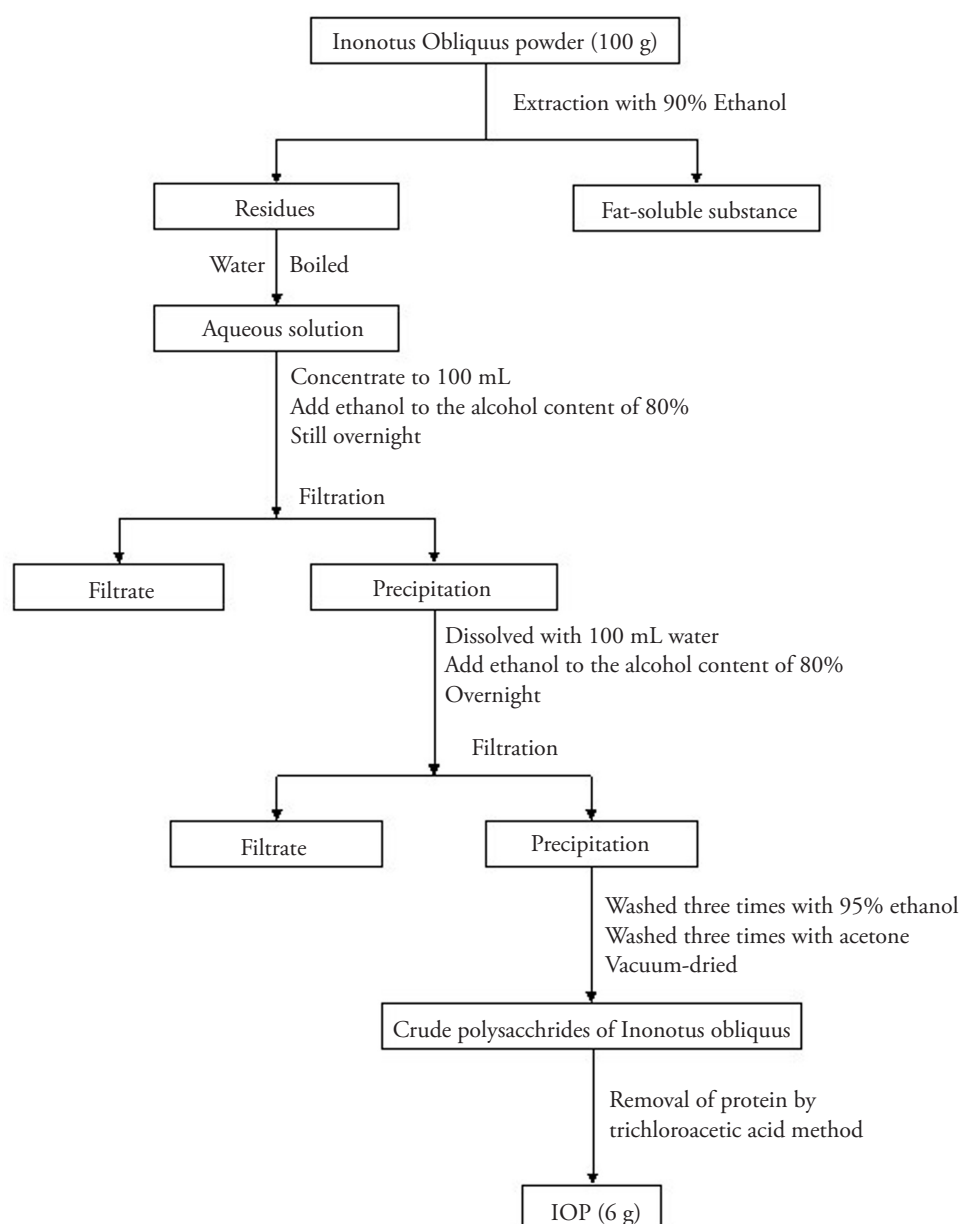


Figure 1 Fractionation scheme for polysaccharides from *Inonotus obliquus*
IOP: *Inonotus obliquus* polysaccharides.

by the random number table method: a control group and three treated groups receiving different doses of IOP (IOP-100, IOP-200 and IOP-300), with 16 mice per group. The treatment groups were orally administered with 100, 200 or 300 mg/kg IOP per day by gavage for a period of 14 days. The rationale for the selection of the doses was based on our previous experiments and some early literature.¹⁵ Mice in the control group were administered a corresponding volume of sterile distilled water.

Equipments and reagents

Mouse heated swimming pool equipment: 722 UV Spectrophotometer was purchased from Shanghai Xin Mao Instrument Co., Ltd. (Shanghai, China); Ag135 electronic balance was purchased from Shanghai Scientific Instrument Co, Ltd. (Shanghai, China); BA300 Digital Biological Microscope Mike Audi was purchased from China Industrial Group Co., Ltd. (Guangdong, China). Lactate assay kit, muscle glycogen and liver glycogen assay kits and blood urea nitrogen (BUN) test kit were purchased from Nanjing Ji-an Cheng Bioengineering Institute (Jiangsu, China).

Forced swimming test

Mice were administered with sterile distilled water, or 100, 200, or 300 mg/kg IOP of 0.4 mL volume once per day for 14 continuous days, followed by an exhaustive swimming test which began 1 h after the last administration. The forced swimming test in mice was measured in a swimming tank (90 cm × 45 cm × 45 cm) filled with fresh water to a depth of 35 cm. Water temperature was maintained at (24 ± 1) °C. A loaded swimming test was carried on 8 mice per group at 1 h after the last administration. A lead block (5% of body weight) was loaded on the tail root of the mice. The endurance of each mouse was recorded as the time from the beginning to exhaustion, which was determined by observing loss of coordinated movements and failure to return to the surface within 10 s.¹⁵ Swimming time was recorded in minutes for each mouse.

Analysis of blood urea nitrogen and lactate

The other 8 mice in each group swam for 30 min and blood samples were collected after resting for 30 min. Blood samples were taken by capillary glass tubes from the eye venous pool of mice to examine lactate and BUN levels.

Analysis of tissue glycogen contents and histological staining of tissues

After blood samples were taken, all mice were sacrificed and then examined for glycogen levels in the gastrocnemius muscle and liver tissues to determine whether IOP administration increased the content of glycogen deposition. The contents of glycogen in the liver and muscle were determined according to the recommended procedures provided by the commercial di-

agnostic kit. All mice were sacrificed and examined for the morphology of liver at the end of experiment. Livers were cut transversely or longitudinally to obtain ventricular sections or four-chamber cross-sections, respectively. Tissues were then embedded in paraffin and cut into 4-µm thick slices for morphological and pathological evaluation. Tissue sections underwent hematoxylin and eosin (HE) staining and observation by light microscopy.

Statistical analyses

All data were expressed as the mean ± standard deviation for eight mice per group. All statistical analyses were performed using SPSS 13.0 statistical software (SPSS Inc., Chicago, IL, USA). Statistical differences among groups were analyzed by a one-way analysis of variance. Values of $P < 0.05$ indicated statistical significance.

RESULTS

Effect of IOP on the forced swimming capacity in mice

Swimming-to-exhaustion is an experimental exercise model to evaluate the anti-fatigue effect of medicines.¹⁶ In this study, the forced swimming test was employed to evaluate the effect of IOP on exercise tolerance of mice. The swimming time to exhaustion was significantly longer in the IOP-treated groups [IOP-100, (154 ± 18) min; IOP-200, (210 ± 15) min; IOP-300, (241 ± 15) min] compared with the control group [(115 ± 18) min, $P < 0.05$]. This indicated IOP significantly elevated the exercise tolerance of mice and had an anti-fatigue effect that was dose-dependent.

Effect of IOP on blood lactate and serum urea nitrogen levels in mice

The swimming exercise is known to induce blood biochemical changes. Fatigue is related to the accumulation of metabolites such as lactic acid after high-intensity or prolonged exercise. Results of physical fatigue tests showed that creatine phosphate and ATP are mainly consumed in the first 10 s of exercise. Then glycolysis is the main energy source for intense exercise over a short time. Blood lactate is the glycolysis product of carbohydrate under an anaerobic condition. Therefore, lactate is an important indicator to determine the degree of fatigue after exercise and the condition of recovery.¹⁷ Blood lactate was significantly lower in the IOP-treated groups [IOP-100, (3.72 ± 0.18) mmol/L; IOP-200, (3.48 ± 0.17) mmol/L; IOP-300, (3.47 ± 0.17) mmol/L] compared with the control group [(4.41 ± 0.22) mmol/L, $P < 0.05$]. This indicated IOP delayed the increase of lactic acid in the blood and postponed the appearance of physical fatigue. During normal physiological activity, urea formation and excretion are in equilibrium and the content of

urea nitrogen is stable. Therefore, BUN, the metabolic outcome of proteins and amino acids, is a sensitive index to evaluate the bearing ability during physical load.¹⁸ BUN levels were lower in the IOP-treated groups [IOP-100, (6.57 ± 0.31) mmol/L; IOP-200, (5.84 ± 0.48) mmol/L; IOP-300, (5.07 ± 0.42) mmol/L] compared with the control group [(7.39 ± 0.25) mmol/L].

Effect of IOP on liver and muscle glycogen level

Endurance capacity is markedly decreased when energy is exhausted.¹⁹ Improved glucose storage in the muscles and liver is particularly important for endurance exercise. Glycogen is synthesized from glucose during glycogenesis and converted to glucose supply via glycogenolysis, a reconversion process, to provide a rapid supply of extramuscular glucose.²⁰ Therefore, glycogen content is a sensitive parameter related to fatigue. Liver glycogen contents were significantly higher in IOP-treated groups in the liver [IOP-100, (13.52 ± 1.97) mg/g; IOP-200, (17.19 ± 2.31) mg/g; IOP-300, (22.91 ± 2.40) mg/g] and muscle [IOP-100, (1.70 ± 1.16) mg/g; IOP-200, (1.95 ± 0.12) mg/g; IOP-300, (2.10 ± 0.11) mg/g] compared with controls [liver: control group (7.22 ± 0.54) mg/g; muscle: (1.25 ± 0.12) mg/g] (all $P < 0.05$ between all IOP groups and controls in liver and muscle). Furthermore, the IOP-induced increase in glycogen levels in liver and muscle was dose-dependent.

Effect of IOP on liver morphology

In the control group, the hepatic lobule was normal, but some liver cells around the center vein were degenerated and the sinusoidal was narrow. In the IOP groups, the structure of liver tissue was clear, and the size of liver cells was uniform. Liver cells were arranged radially around the central vein and no degeneration or necrosis of liver cells was observed (Figure 2).

DISCUSSION

Physical fatigue is also called peripheral fatigue, which derive from the action of muscles and may be accompanied by a deterioration in functional performance.²¹ Overloading work or exhaustive exercise can lead to the accumulation of excess reactive free radicals that result in tissue damage.²² Exercise endurance is an impor-

tant variable in evaluating anti-fatigue treatment. In this study, IOP increased the swimming time-to-exhaustion of test mice, which indicated that IOP significantly elevated exercise tolerance in mice and had an anti-fatigue effect that was dose-dependent.

The exhaustion theory suggests that energy source depletion and excess metabolite accumulation lead to fatigue.²³ Energy storage and supply is an important factor related to exercise performance.²² Energy expenditure during exercise leads to physical fatigue and is mainly caused by rapid ATP consumption and energy deficiency.²⁴ Glycogen is the predominant source of glycolysis for ATP production.²⁵ Therefore, glycogen storage directly affects exercise ability.²⁵ In this study, the liver and muscle glycogen content of mice in the IOP experimental groups were significantly higher than those in the control group. This indicated that IOP might allow the slower utilization of glycogen, which is important role in situations requiring extended periods of prolonged exercise endurance. With a prolonged exercise time, energy substances including creatine phosphate, ATP and glycogen decrease gradually; therefore, fat in the body starts to catabolize as fatty acids to provide energy. However, during exercise for a long time (30 min or more), the energy generated by catabolism of glucose and fat is no longer sufficient for the demand. Thus, the catabolism of proteins and amino acids is enhanced to overcome the deficiency of energy substances while exercising, which increases the BUN content. The current study showed that BUN levels in the IOP groups were decreased significantly compared with the control group ($P < 0.05$), indicating that IOP may delay the generation of fatigue by reducing the production of serum urea nitrogen after exercise.

The muscle produces high levels of lactate when it obtains sufficient energy from anaerobic glycolysis during high-intensity exercise. The increased lactate levels further reduce pH, which could induce various biochemical and physiological side effects, including glycolysis, as well as phosphofructokinase and calcium ion release, through muscular contraction.²⁶ Blood lactate is the glycolysis product of carbohydrates under anaerobic conditions and glycolysis is the main energy source for short-term intensive exercise. Therefore, removing lactate rapidly is beneficial to relieve fatigue.²⁶ In this study, serum lactate levels in the experimental groups were lower than in the control group after the adminis-

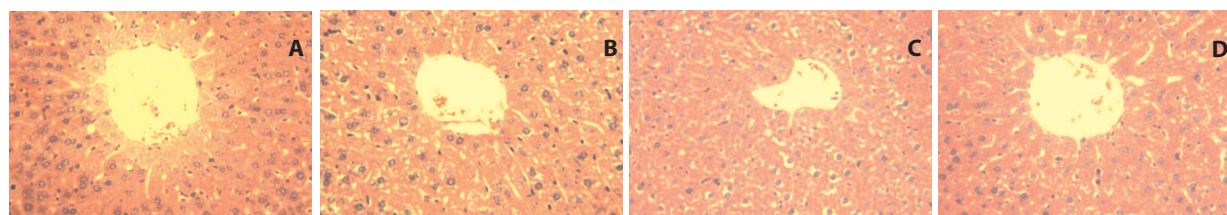


Figure 2 Effect of IOP on liver morphology (HE staining, ×20)

A: control; B: low dose IOP (100 mg/kg); C: mid dose IOP (200 mg/kg); and D: high dose IOP (300 mg/kg) groups. Control group was administered a corresponding volume of sterile distilled water. The treatment groups were orally administered with 100, 200 or 300 mg/kg IOP per day by gavage for a period of 14 days. IOP: inonotus obliquus polysaccharides. HE: hematoxylin and eosin.

tration of IOP to mice for 14 days. Therefore, IOP enhanced lactate elimination.

In conclusion, our results indicated that IOP had a significant role in alleviating fatigue and accelerating the elimination of fatigue substances in mice. Furthermore, IOP had no toxic effects on major organs such as the liver as assessed by histopathological examinations. Therefore, IOP could be a potential anti-fatigue agent. However, further study is needed to elucidate the cellular and molecular mechanisms involved in the anti-fatigue effects of IOP.

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