

## Original Research Article

# Evaluation of anti-fatigue property of *Porphyridium cruentum* in mice

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Sent for review: 25 October 2018

Revised accepted: 26 February 2019

### Abstract

**Purpose:** To evaluate the potential effects of *Porphyridium cruentum* (PC) on fatigue induced by forced swimming test in mice.

**Methods:** Mice were randomly divided into normal control group (NC, i.e., untreated non-swimming); model control group (MC, untreated swimming); *Spirulina* treated group (SP, 800 mg/kg); PC-treated groups (50, 100, and 200 mg/kg), respectively. After intragastric administration for 14 consecutive days, a weight-bearing swimming experiment was conducted for the mice, and the biochemical indicators related to fatigue were examined, including exhaustive swimming time, glucose levels (Glu), hepatic glycogen contents (HG), muscle glycogen contents (MG), glutathione peroxidase activities (GSH-Px), creatine kinase (CK), malondialdehyde (MDA), urea nitrogen levels (SUN), lactate dehydrogenase activities (LDH), lactic acid (LA) as well as superoxide dismutase (SOD).

**Results:** PC significantly prolonged the swimming endurance time compared to MC. After PC treatment, Glu, HG and MG were effectively increased dose-dependently, SUN, LA, LDH and CK levels in serum were significantly reduced. Moreover, PC treatment elevated the bioactivities of two antioxidant enzymes, namely, GSH-Px and SOD, while MDA content decreased when compared to MC group.

**Conclusion:** These results indicate that PC exhibits strong anti-fatigue effect. Thus, PC may be suitable for incorporation in functional food to counter fatigue.

**Keywords:** *Porphyridium cruentum*, Anti-fatigue, Energy metabolism, Detrimental metabolites, Oxidative stress

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## INTRODUCTION

*Porphyridium cruentum* (PC), a unicellular red microalgae mainly produced in tropical and subtropical regions, is a potential source of

biofuel, food and pharmaceutical [1]. Researches have shown that PC possesses abundant polysaccharides, polyunsaturated fatty acids (PUFAs), phycoerythrin, pigments and other bioactive substances [2]. Moreover, these

bioactive compounds from PC play a key role in clinical practice such as antioxidant, anti-tumour and immunomodulatory properties [3]. However, the potential anti-fatigue effect of PC has attracted limited attention. Therefore, our present study aimed to investigate the potential anti-fatigue activity and the possible mechanisms of PC in a load-induced endurance swimming mice model. Besides, *Spirulina* (SP) was proved to possess anti-fatigue efficacy according to previous studies and chosen as positive control in the present study [4].

## EXPERIMENTAL

### Materials and chemicals

PC and SP were obtained from Shanghai Guangyu Biological Technology Co. Ltd and identified by Prof. Ziren Su in June 2017. The commercial kits for biochemical analysis of hepatic glycogen (HG), muscle glycogen (MG), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), lactic acid (LA) and superoxide dismutase (SOD) were provided by Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China).

### Experimental animals

Male Kunming mice ( $20 \pm 2$  g) were purchased from Medical Experimental Animal Center of Guangdong Province (Foshan, China). All animals were housed and adapted for 7 days under standard surroundings (temperature  $22 \pm 2$  °C, humidity  $55 \pm 2$  %, and 12-h light/dark cycle). The whole experiment conducted was in accordance with the guidelines provided by the Animal Care and Welfare Committee [5] and approved by the Institutional Ethics Committee of Guangzhou University of Chinese Medicine (approval no. 2016047).

### Experimental design

After 7 days of acclimatization, mice were sorted into six different experimental groups by randomization ( $n=20$ ): normal control group (NC, normal saline), model control group (MC, normal saline), positive control group (SP, 800 mg/kg) and three PC intervention groups of low dose group (PC-L, 50 mg/kg), medium dose group (PC-M, 100 mg/kg) and high dose group (PC-H, 200 mg/kg), respectively. The doses of SP and PC were decided based on our preliminary tests and previous literatures [6]. SP and PC were suspended in distilled water and were orally administrated once daily for 14 days. Figure 1 is a schematic graph about this experimental design.

### Forced swimming test

Firstly, forced swimming test was performed based on previous research [4] with modest modifications. Briefly, an hour after the final administration, 10 mice of each experimental group were used for forced swimming test and loaded by lead wires of 5 % body weights attached to the tails. Then experimental animals were put in a plastic water pool (50 cm  $\times$  35 cm  $\times$  30 cm) with water maintained at  $25 \pm 2$  °C. Meanwhile, the endurance time was immediately recorded when they were unable to coordinate movements and failed to come back to the water surface within 10 s [4,7].

### Measurement of biochemical indices

After final administration, the remaining 10 mice of each group were forced to swim for continuous 90 min without load except the NC group. Then mice were sacrificed under anesthesia with pentobarbital (60 mg/kg). Afterwards, blood samples were collected from the eyeball with heparinized capillary tube immediately. The liver and hind leg muscles were excised from the mice for the determination of HG and MG respectively.

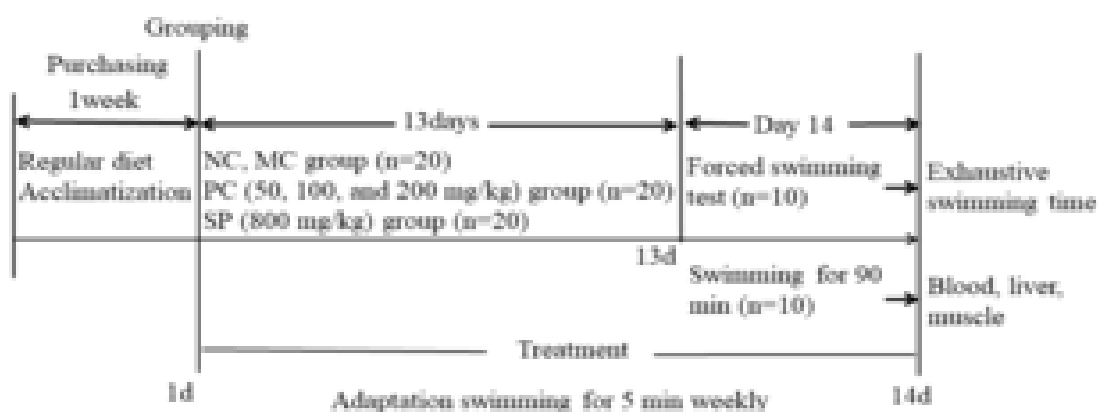


Figure 1: Experiment design

Blood was centrifuged at 3500 rpm, 4 °C for 10 min and plasma was obtained to measure the concentrations of SOD, GSH-Px, LA and MDA with Elisa kits, and the levels of SUN, LDH, CK and Glu were determined by Hitachi 7180 type biochemical analyzer.

### Statistical analysis

Values were expressed as mean  $\pm$  SD. Statistical test for differences were performed by one-way analysis of variance followed by LSD test and Duncan test using the SPSS statistics 23.0 (IBM Inc, New York, USA). Values of  $p < 0.05$  were considered as statistically significant.

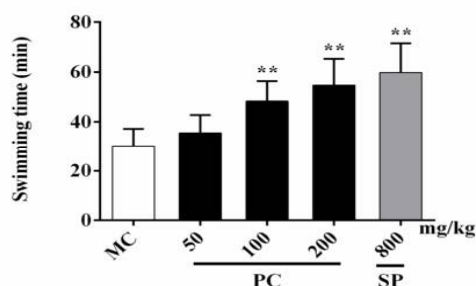
## RESULTS

### Effect of PC on body weight

Mouse weight, recorded daily throughout the experiment, is shown in Table 1. The data indicate that there was no remarkable difference in weight change among all the groups ( $p > 0.05$ ).

### Effect of PC on weight-bearing swimming capacity

To directly investigate whether PC exhibited anti-fatigue, the weight-bearing swimming time was recorded (Figure 2). As expected, compared with MC group, exhaustive swimming time was prolonged after PC treatment. Specifically, PC-M, PC-H and SP groups significantly prolonged the exhaustive swimming time by 59.3, 80.5 and 97.1 % ( $p < 0.01$ ), respectively. The results showed that PC could increase the exhaustive swimming capacity of mice.

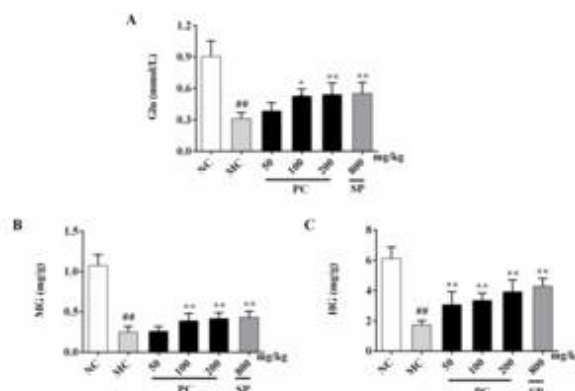


**Figure 2:** Effect of PC on exhaustive swimming time in mice. Data were expressed as mean  $\pm$  SD ( $n = 10$ ). \*\*  $p < 0.01$  compared to MC group

### Effect of PC on energy consumption

After swimming test without load for 90 min, the level of Glu was measured (Figure 3 A). Compared with non-swimming mice, the level of Glu in MC group remarkably decreased ( $p <$

0.01). Nevertheless, Glu level in PC-L, PC-M, PC-H and SP group was increased by 23.4, 68.4, 73.6 and 76.8 %, respectively compared to MC group. Among the treatment groups, PC-M, PC-H and SP exhibit significant changes (all  $p < 0.01$ ). As the major source of energy consumption, glycogen in muscle and liver were also determined. As Figure 3 B shows, MG content of MC group declined significantly when compared with NC group ( $p < 0.01$ ). However, the MG content of PC-M, PC-H and SP groups significantly increased by 56.3, 66.9 and 72.8 % ( $p < 0.01$ ) compared with MC group. In addition, the HG content of MC group declined notably (Figure 3C,  $p < 0.01$ ), the HG content of three PC-treated groups and SP group significantly improved by 77.2, 93.2, 126.0 and 147.3 %, respectively ( $p < 0.01$ ).



**Figure 3:** Effects of PC on Glu (A), MG (B) and HG (C) in mice. Data were expressed as means  $\pm$  SD ( $n=10$ ); ##  $p < 0.01$  compared to NC group; \* $p < 0.05$  or \*\* $p < 0.01$  compared to MC group

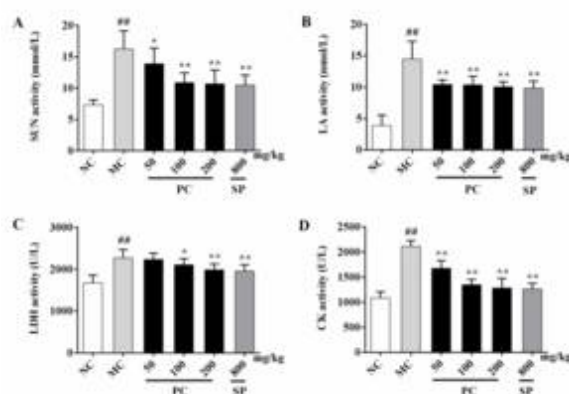
### Effect of PC on SUN, LA, LDH and CK activities

SUN, LA, LDH, CK are sensitive indicators of exercise capacity. As shown in Figure 4 A, after PC administration for 14 days, SUN activity in MC group was higher than that of NC group ( $7.32 \pm 0.80$  versus  $16.25 \pm 2.91$  mmol/L,  $p < 0.01$ ). After treatment, SUN activity in PC or SP groups were remarkably decreased ( $13.89 \pm 2.51$ ,  $10.87 \pm 1.59$ ,  $10.68 \pm 2.21$  and  $10.48 \pm 1.62$  mmol/L, respectively, versus  $16.25 \pm 2.91$  mmol/L,  $p < 0.05$  or  $p < 0.01$ ). As shown in Figure 4 B, the level of LA in three doses of PC groups and SP group significantly decreased by 28.1, 28.5, 30.7 and 32.1 %, respectively, versus MC group ( $p < 0.01$ ). In Figure 4 C, the effects of PC and SP on LDH activities were presented. The activity of LDH was significantly inhibited in PC-M, PC-H and SP groups by 7.2 % ( $p < 0.05$ ), 12.6 % ( $p < 0.01$ ) and 13.8 % ( $p < 0.01$ ), compared with MC group.

**Table 1:** Change in body weight (mean ± SD, n = 10)

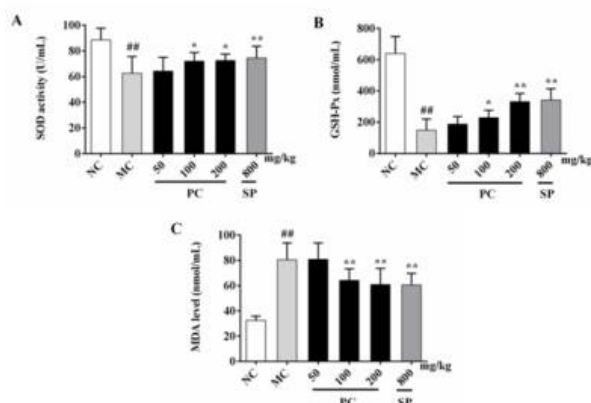
Group	Initial weight (g)	Final weight (g)	Weight increase (g)
NC	21.00 ± 1.06	26.21 ± 1.11	5.21 ± 1.31
MC	20.97 ± 0.65	26.40 ± 1.09	5.43 ± 1.06
SP	21.38 ± 0.73	26.34 ± 1.33	4.96 ± 1.02
PC-L	20.55 ± 1.03	25.67 ± 0.97	5.12 ± 1.24
PC-M	20.45 ± 0.74	25.81 ± 1.28	5.37 ± 1.15
PC-H	20.56 ± 1.12	25.48 ± 1.39	4.92 ± 0.82

Besides, significant decrease in the CK activity was observed in PC- and SP-treated groups in comparison with MC group (Figure 4 D). Particularly, CK concentration were decreased by 20.7, 36.1, 39.3 and 40.1 % ( $p < 0.01$ ), respectively, indicating that PC treatment lead to a reduction in CK activity in a dose-dependent manner.



**Figure 4:** Effects of PC on serum levels of SUN (A), LA (B), LDH (C) and CK (D) in mice. Data were illustrated by means ± SD (n = 10). ##  $p < 0.01$  in comparison with NC group; \*  $p < 0.05$  or \*\*  $p < 0.01$  when compared with MC group

**Effects of PC on oxidative stress index**



**Figure 5:** Effects of PC on oxidative stress index (SOD (A), GSH-Px (B) and MDA (C)) in mice. Data were illustrated by means ± SD (n=10). ##  $p < 0.01$  in comparison with the NC group, \*\*  $p < 0.01$  versus the MC group

To evaluate the effects of PC on fatigue-induced oxidative stress index, the SOD, GSH-Px and MDA levels were measured (Figure 5 A). There was remarkable difference between MC and NC groups on SOD ( $p < 0.01$ ), which were notably improved by PC- and SP- treatment than those in MC group ( $p < 0.05$  or  $p < 0.01$ ). Additionally, SOD level in PC-M, PC-H and SP groups were significantly elevated by 15.0, 15.9 and 19.0 % respectively. Similarly, the concentration of GSH-Px in PC-M, PC-H and SP groups displayed significant increase of 53.7, 121.9 and 129.3 % (Figure 5 B,  $p < 0.05$  or  $p < 0.01$ )

Moreover, serum MDA level in PC-M, PC-H and SP groups descend significantly when compared to that of MC group (20.2, 24.4 and 24.8 %, respectively,  $p < 0.01$ ) in Figure 5 C. More specifically, PC-H group showed a similar reduction after SP treatment.

**DISCUSSION**

Nowadays, weight-loaded forced swimming test is widely applied to evaluate anti-fatigue effect and endurance capacity for exercise performance in mice [8]. In this study, we first investigated the influence of PC on the body weight and physiological status of mice. The results suggest that PC had no toxic effect on the animals and no negative effect on their body weight after 14-day consecutive treatment, which also indicates that the PC doses used were safe for mice. We also found that PC groups extended exhausting time, especially PC-M and PC-H groups, indicating the potential anti-fatigue effect of PC on mice.

Several relevant biochemical indicators closely related to fatigue were determined to further investigate PC's anti-fatigue property. As reported previously, intense exercise leads to rapid ATP depletion and energy shortage, which is a contributing factor to fatigue [9]. During physical exercise, Glu is first oxidized to generate ATP to supply the energy requirements of the muscles [10]. Besides, the body's energy can also be derived from glycogenolysis, which is an important secondary long-term energy storage that provides enough energy for muscle

contraction and be used to meet an urgent need for glucose [11].

It has been reported that restoring HG and MG is beneficial to improve performance endurance in sports like swimming [12]. In the present study, our result indicated that PC-M and PC-H treatment significantly increased Glu level. Also, PC significantly increased HG and MG in a dose dependent manner during swimming exercise. Moreover, the effects of PC-H treatment on contents of HG and MG was comparable to the effect of the positive drug SP. These results suggested that PC makes a positive effect to enhance exercise tolerance and delay the occurrence of physical fatigue.

It is well-known that when body could not acquire sufficient energy from aerobic metabolism, anaerobic glycolysis becomes an alternative source to meet the energy requirement. Therefore, LA, the metabolites of glycolysis, was determined as one of the biochemical parameters of muscle fatigue [13]. As exercise intensity increases, the content of SUN rises correspondingly, due to the catabolism of proteins and amino acids, to compensate for the catabolism of sugar and fat and meet the energy consumption requirements of the body [14]. As the results obtained in this study indicate, PC treatment significantly decreased the accumulation of SUN and LA in mice. The results suggest that PC exerted its anti-fatigue effects by reducing the accumulation of exhaust inducing metabolic substances during exhaustive swimming experiment.

Simultaneously, as an enzyme catalyzing the oxidation-reduction reaction of pyruvate, LDH plays an important role in anaerobic glycolysis. It is a sensitive index related to fatigue, and its level influences endurance capacity during the swimming test [7]. As an cytosolic enzyme abundantly stored in the cells of the body, CK is a clinical biomarker related to fatigue mainly due to its reflection of the damage caused to muscle and cell membrane structures [15]. The results showed that CK and LDH levels in PC-treated groups were markedly reduced when compared with MC group. These data indicate that PC probably exerts its anti-fatigue property by facilitating the elimination of lactic acid and reduction in the damage to muscle and cell membrane structures.

Furthermore, oxidative stress is closely related to physical fatigue [16]. As previous studies revealed, exhaustive exercise produces abundant ROS [17]. At the same time, antioxidant enzymes such as SOD and GSH-Px

have the function of scavenging ROS [7]. Moreover, SOD mainly catalyzes superoxide radicals to generate  $H_2O_2$  and  $O_2$ . GSH-Px catalyzes the decomposition of  $H_2O_2$  to  $H_2O$  and  $O_2$  [18].

Thus, SOD and GSH-Px are well-known fatigue-relevant enzymes that resist oxidative damage. In addition, MDA, the final product of lipid peroxidation, is an indicator of oxidative damage in anti-fatigue experiments [13]. In this study, PC treatment promoted SOD and GSH-PX bioactivities in plasma than that of MC group, especially in PC-H group. Besides, MDA level declined significantly after PC treatment. Thus, these results indicate that PC has effective anti-fatigue performance in mice by enhancing anti-oxidation function and preventing lipid peroxidation after exhaustive exercise.

## CONCLUSION

The findings of this study demonstrate that PC possesses anti-fatigue properties and accelerates recovery from fatigue, especially in PC-H, and compares well with the positive (reference) control, SP. Thus, PC may be suitable for inclusion in functional foods to minimize fatigue.

## DECLARATIONS

### Acknowledgement

This work was supported by grants from the key lab of Zhanjiang for R&D marine microbial resources in the Beibu Gulf Rim (no. 2012E02), the natural science foundation of Guangdong Province (no. 2015A030310121), and the public service platform of south China Sea for R&D marine biomedicine resources (no. 2017C8A).

### Conflict of interest

No conflict of interest is associated with this work.

### Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Yongmei Huang, Wenjie Li and Yongfu Wang designed the study. Hui Luo ran and financed the study. Jingting Zhan and Lihua Tan performed ELISA analyses blinded. Jinli Lei, Ning Li and Jiannan Chen performed the statistical analyses. Yongfu Wang and Chang Qu wrote the manuscript. All

authors contributed to the writing of the manuscript by constructive criticism and the final version was approved by all authors.

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