Polysaccharides from *Morinda officinalis How* Protect Liver from Oxidative Stress Induced by Exhaustive Exercise in Mice

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ABSTRACT

The present study is designed to investigate the effects of polysaccharides from *Morinda officinalis How* (PMO) on exhaustive exercise-induced oxidative stress in liver of mice. The animals were divided into four groups, i.e. the control (C), low-dose PMO-treated (DPT, 100mg/kg), moderate-dose PMO-treated (MPT, 200 mg/kg) and high-dose PMO-treated (HPT, 400 mg/kg) groups. PMO were administered orally by gavag once a day for 28 days. After 28 days, the mice performed an exhaustive swimming exercise and the livers were collected to measure malondialdehyde (MDA) levels, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), xanthine oxidase (XO) and reduced glutathione (GSH) activities. The data obtained showed that MDA levels were significantly lower in the DPT, MPT and HPT groups than those in the C group ($P < 0.05$). SOD, GPx and GSH activities were significantly higher in the DPT, MPT and HPT groups than those in the C group ($P < 0.05$). CAT activities were significantly higher in the MPT and HPT groups than those in the C group ($P < 0.05$). XO activities were significantly lower in the MPT and HPT groups than those in the C group ($P < 0.05$). The results revealed that PMO can effectively attenuate the exhaustive exercise-induced oxidative stress in liver.

INTRODUCTION

It is well established that regular physical exercise has many beneficial effects, and it is an effective means for preventing chronic diseases. However, these beneficial effects are lost with exhaustive exercise (Araújo et al., 2011). Numerous studies have demonstrated that exercise is often associated with an increase in the production of reactive oxygen species (ROS) in various tissues, which may overwhelm the capacity of the antioxidant defense systems, and the result can lead to increased oxidative stress (Yan et al., 2014). Specific sources of ROS during exhaustive exercise include leakage of electrons from the mitochondrial electron transport chain, xanthine oxidase reaction, haemoglobin oxidation and activated neutrophils (Aguilóet al., 2005). In particular, liver cells need to generate high amounts of en-

ergy to perform their various functions. The high metabolic rate of the liver is directly associated with a high flow of electrons in the mitochondrial respiratory chain, some of these electrons are deflected, producing additional ROS (Araújo et al., 2011). As a result, accumulated excessive ROS can attack the vital biomolecules, such as plasma membrane lipids and proteins, and therefore deteriorates normal cellular functions (Yu et al., 2012). It has also been suggested that exhaustive exercise-induced oxidative stress may be associated with physical fatigue, muscle damage and a decrease in physical performance (Xu and Li, 2012). Recently, several studies have indicated that exogenous antioxidants, primarily obtained as nutrients or nutritional supplements, could attenuate oxidative stress and some of the detrimental effects associated with ROS production (Síktar et al., 2011).

Morinda officinalis How (*M. officinalis*), family Rubiaceae, is a small vine that grows widely in tropical and subtropical regions (Wu et al., 2009). The dried roots of this plant, Bajitian in Chinese, are a famous traditional Chinese medicine and are listed in the Pharmacopoeia of the People's Republic of China (PPRC). It has been used to treat a wide range of symptoms, including depression, inflammation, and osteoporosis in China since ancient times (Yang et al., 2010). Recently, studies on *M. officinalis* including biologically activity components and relevant pharmacological properties have been performed, and polysaccharides are the major biologically activities components of *M. officinalis*. Pharmacological studies showed that polysaccharides from *M. officinalis* (PMO) have many pharmacological activities, including protective effect on bone loss and ageinduced bone degeneration, anti-fatigue, hypoglycemic and immunomodulation agents (Zhang et al., 2009). In addition, a series of studies have confirmed that PMO is a free radical scavenger and possesses considerable antioxidant activity (Zhang et al., 2013), which suggests that PMO might be beneficial to exhaustive exercise-induced oxidative stress. However, few studies have examined the effects of PMO on exhaustive exercise-induced oxidative stress. Hence, the present study was designed to investigate the protective effects of PMO against exhaustive exercise-induced oxidative stress in liver of mice.

MATERIAL AND METHODS

Plant Material and Reagents

Dried roots of *M. officinalis* were purchased from a local drug store (Hangzhou, China) and were identified according to the identification standard of the Pharmacopoeia of the People's Republic of China (PPRC). The voucher specimen is available in the herbarium of Hangzhou Normal University (Hangzhou, China). Dried roots of *M. officinalis* were ground to pass through 1 mm screen, and the powder was kept in sealed polyethylene bags at 4 °C for further use. Commercial kits used for superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), xanthine oxidase (XO), and malondialdehyde (MDA) were purchased form Jiancheng Institute of Biotechnology (Nanjing, China). All other chemical reagents used in the study were of the highest analytical grade.

Preparation of Polysaccharides from *M. officinalis*

Polysaccharides from *M. officinalis* (PMO) were prepared according to the published methods (Zhu et al., 2009; Chen et al., 2011) with minor modification. Briefly, the powder of *M. officinalis* was soaked with 95% ethanol to remove the pigments and small lipophilic molecules. The organic solvent was volatilized and pretreated dry powder was obtained. The pretreated powder was extracted with boiling water three times under reflux. The aqueous extract was filtered through Whatman filter paper. The filtrate was concentrated in a rotary evaporator under reduced pressure, and centrifuged at 3000 rpm for 15min. Then treated with Sevag reagent [V(n-butanol): V(chloroform) = 1:4] to remove the proteins, centrifuged at 6000 rpm for 15 min to separate the super-

natant and the residue. The supernatant was precipitated with three volumes of 95% ethanol, and stored overnight at 4 °C. The precipitate were collected by centrifugation and lyophilized to get the crude polysaccharides.

Experimental Animals

All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the Chinese National Institutes of Health. Male Kunming mice, weighing 20 ± 2 g, were purchased from Zhejiang Animal Husbandry Center (Hangzhou, China). Animals were housed in individual cages with free access to water and diet in temperature-controlled animal facility under a12h light–dark cycle at 20 ± 1 °C and 60 ± 10% humidity. Animals were acclimatised for a period of three days in their new environment before the initiation of the experiment. The study was approved by the Institutional Ethical Committee of Hangzhou Normal University.

Experimental Design and Treatment

The mice were divided into four groups and each group consisted of eight mice.

(i) control (C) group: mice were allowed free access to a normal diet and treated with 2.0 ml of distilled water.

(ii) low-dose PMO-treated (DPT) group: mice were allowed free access to a normal diet and treated with PMO at a dose of 100 mg/kg.bw.

(iii) moderate-dose PMO-treated (MPT) group: mice were allowed free access to a normal diet and treated with PMO at a dose of 200 mg/kg.bw.

(iv) high-dose PMO-treated (HPT) group: mice were allowed free access to a normal diet and treated with PMO at a dose of 400 mg/kg.bw

PMO were dissolved in 2.5 mL of distilled water, and treatments were administered orally by gavage once a day for 28 days. After 28 days, exhaustive swimming exercise was carried out in a plastic pool (50 cm × 50 cm × 40 cm). The water depth and temperature were 30 cm and 25 ± 1 °C, respectively. Mice were loaded 5% of the body weight of lead threads at the bases of the tails. They were then forced to swim and continued to exhaustion (Xu and Zhang., 2013). Exhaustion was determined by observing loss of coordinated movements and failure to return to the surface within 10 sec (Zhang et al., 2010).

Tissue Preparations

Immediately after exhaustive swimming exercise, the mice were anesthetized with ethyl ether and sacrificed by decapitation. The liver were quickly excised and frozen into liquid nitrogen, and then stored at - 80 °C until biochemical analyses. The liver were homogenized in Tris buffer (50 mM, pH 7.5). The suspension was centrifuged at 3000 rpm for 10 min at 4 °C, and clear supernatant was used for the following estimations of MDA levels, SOD, CAT, GPx, XO and GSH activities using detection kits according to the manufacturers' instructions

Statistical Analysis

All results are expressed as mean ± SD. A Computer program (SPSS version 15.0) was used to analyze the obtained results. Student-Newman-Keuls test for multiple comparisons, which was used to evaluate the difference between two groups. Significance was set at an alpha level of 0.05..

RESULTS AND DISCUSSION

Effect of PMO on Malondialdehyde Levels in Liver of Mice

Exhaustive exercise-induced oxidative stress, leading to lipid peroxidation (LPO), has been well documented over the last decade (Bahman et al., 2013). LPO may be due to the oxidation of molecular oxygen to produce superoxide radicals. This reaction is also the source of H2O2, initiating the peroxidation of unsaturated fatty acids in the membrane. Both H2O2 and O2- produced highly reactive hydroxyl radical with Haber-Weiss reaction. The hydroxyl radical can initiate LPO, which is a free radical chain leading to loss of membrane structure and function (Barbara et al., 2006). The LPO leads to the formation of a wide array of pri-

mary oxidation products and secondary oxidation products. Malondialdehyde (MDA) is secondary oxidation product generated during the oxidation of polyunsaturated fatty acids, which has been routinely used as an index of LPO (Wu et al., 2008). MDA levels in liver of control and treated mice are clearly depicted in Fig. 1. As shown in Fig. 1, MDA levels were significantly lower in the DPT, MPT and HPT groups than those in the C group ($P < 0.05$). As per the above findings, it is suggested that PMO have the effect of reducing LPO and preventing muscle damage caused by exhaustive exercise.

Effect of PMO on Antioxidant Enzyme Activities in Liver of Mice

The antioxidant defense systems of the living body consist of antioxidant enzymes (i.e. SOD, CAT and GPx) and antioxidant nutrients, which may be involved in reducing oxidative stress. As 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 (Lee et al., 2009). SOD resolves O2- into H2O2 and O2, whereas CAT and GPx catalyze the reduction of H2O2 to H2O (Jeon et al., 2001). The significant decrease in the activities of SOD, CAT and GPx in the blood and tissues after exhaustive exercise may be an indication of oxidative stress (Li et al., 2014). Antioxidant enzyme activities in liver of control and treated mice are clearly depicted in Fig. 2. As shown in Fig. 2, SOD and GPx activities were significantly higher in the DPT, MPT and HPT groups than those in the C group ($P < 0.05$). CAT activities were significantly higher in the MPT and HPT groups than those in the C group ($P < 0.05$). DPT group was observed to have no significant effect on the CAT activities compared with the C group ($P > 0.05$). The increase in antioxidant enzyme activities may be related to the ability of PMO to scavenge ROS and enhancement of several antioxidant proteins (Huang et al., 2009). Based on these results, it could be concluded that PMO possessed protective effects against exhaustive exercise-induced oxidative stress in liver.

Effect of PMO on Xanthine Oxidase Activities in Liver of Mice

It is known that xanthine oxidase (XO) is thought to be one of the key enzymes producing reactive oxygen species. Xanthine dehydrogenase (XDH) can be converted reversibly to XO by oxidation of cysteine residues or irreversibly by limited proteolysis. XO has high reactivity toward O2 but negligible reactivity toward NAD+ (Sokolovic et al., 2008). Several studies have demonstrated that elevated XO activity in liver after exhaustive exercise is considered as a main intracellular source for O2- production and XO-derived free radicals exerted some deleterious effect on liver tissue associated with oxidative stress (Huang et al., 2009). XO activities in liver of control and treated mice are clearly depicted in Fig. 3. As shown in Fig. 3, XO activities were significantly lower in the MPT and HPT groups than those in the C group ($P < 0.05$). DPT group was observed to have no significant effect on the XO activities compared with the C group ($P > 0.05$). Findings from the present investigation indicate that PMO affords protection against free radical production and against oxidative damage by exhaustive exercise.

Effect of PMO on Reduced Glutathione Activities in Liver of Mice

Reduced glutathione (GSH), one of the most important endogenous antioxidants, exists in both the cytosol and mitochondria. Liver is the major organ for de novo GSH synthesis, and it supplies 90% of the circulating GSH and exports GSH into plasma during prolonged exercise (Sun et al., 2010). A tendency to a decrease of GSH levels in liver after exhaustive exercise has been observed in many studies (Cholewa et al, 2008; Polotow et al, 2014). The decrease may be due to the diminishing GSH pool and regulation of redox balance and may generally be expected due to a possible increase in formation of oxidized glutathione (GSSG) and subsequent export of GSSG out

of cell (Aydin et al, 2007). GSH activities in liver of control and treated mice are clearly depicted in Fig. 4. As shown in Fig.4, GSH activities were significantly higher in the LPT, MPT and HPT groups than those in the C group ($P < 0.05$). Thus it is reasonable to think of increasing of GSH might contribute to antagonize the exhaustive exercise-induced oxidative stress effects.

CONCLUSION

In conclusion, the results presented here indicated that PMO can effectively attenuate the exhaustive exercise-induced oxidative stress in liver. The mechanism underlying these effects is based on a decrease in the MDA levels and XO activities, and an increase in the SOD, CAT, GPx and GSH activities. The finding of the study suggests that PMO can be used as an antioxidant supplement for competing athletes, who participates in exhaustive endurance events.

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CONFLICT OF INTEREST

No authors in this study have any conflict of interest.

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