Journal of Animal and Veterinary Advances 11 (24): 4693-4696, 2012
ISSN: 1680-5593
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The Influence of Polysaccharides from the Roots of *Achyranthes bidentata* on Biochemical Parameters Related to Oxidative Stress Induced by Exhaustive Exercise of Rats

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Abstract: The main purpose of this study was to investigate the effects of polysaccharides from the roots of *Achyranthes bidentata* (ABP) on exhaustive exercise-induced oxidative stress by measuring related biochemical parameters of rats. The animals were randomly divided into four groups (n = 10 in each group): one control group and three ABP administered groups. The control group was given saline solution and the administered groups were given different doses of ABP (50, 100, 200 mg kg^{-1}) by gavage once a day. After 21 days, the rats performed an exhaustive exercise test on a graded treadmill, along with the determination of related oxidative stress parameters. The results showed that ABP could significantly increase Superoxide Dismutase (SOD), Glutathione Peroxidase (GSH-Px) and Catalase (CAT) activities, decrease Malondialdehyde (MDA) contents in muscle of rats which meant that ABP effectively attenuates oxidative stress induced by exhaustive exercise.

Key words: Polysaccharides, *Achyranthes bidentata*, oxidative stress, biochemical parameters, exhaustive exercise, rats

INTRODUCTION

Achyranthes bidentata (Amaranthaceae) is an erect perennial herbaceous plant widely distributed and grown in hilly districts of China, Korea, Japan and India (Lin *et al*., 2010). The roots of *Achyranthes bidentata* named Niuxi in Chinese is an important medicinal herbal and documented in the Pharmacopeia of People's Republic of China (Wang *et al*., 2011). *Achyranthes bidentata* is one of the 50 fundamental herbs used in Traditional Chinese Medicine (TCM) which has anti-inflammatory activities and is used to nourish the kidney and liver, drain dampness and promote circulation and invigorate circulation (Ding *et al*., 2007; He *et al*., 2010). The phytochemical studies revealed that it contains various saponins, sterols, polysaccharides and alkaloids (Vetrichelvan and Jegadeesan, 2002). Polysaccharides is an main active component isolated from the root of *Achyranthes bidentata*. Many studies have shown that *Achyranthes bidentata* Polysaccharides (ABP) possess immunopotentiating, antioxidant, antisenile, antiradiation, antitumor and hypoglycemic effects (Jin *et al*., 2007; Zou *et al*., 2011; Zhu *et al*., 2012). The earlier study has also shown that ABP have anti-fatigue effects in exercise mice (Lin *et al*., 2010; Zhang and Lin, 2012). However, the effects of ABP on exhaustive exercise-induced oxidative stress have not been investigated thus far. Therefore, the present study is to investigate the effects of ABP on oxidative stress induced by exhaustive exercise in male rats.

MATERIALS AND METHODS

Plant material: The roots of *Achyranthes bidentata* were bought from from a local herbal market (Changsha, China) and identified by Professor Li Kejian of Central South University (Changsha, China) according to the identification standard of the eighth edition of Pharmacopeia of People's Republic of China (2005 PPRC). A voucher specimen (No. CST09-484)) is deposited in the Herbarium of the Central South University. The dried roots of *Achyranthes bidentata* was powdered with a blender to extraction.

Chemicals: The commercial assay kits for Superoxide Dismutase (SOD), Glutathione Peroxidase (GSH-Px), Catalase (CAT) and Malondialdehyde (MDA) were purchased from Jiancheng Biologic Project Company

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(Nanjing, China). All other chemicals and solvents used in this study were of analytical grade and obtained from Hunan Reagent Company (Changsh, China).

Experiment animal: Male Sprague-Dawley rats (weighing 160-180 g) were obtained from Laboratory Animal Center of Hunan (Changsha, China) and were bred in the animal facilities at Central South University (Changsha, China). The animals were housed in a temperature (23±2°C) regulated and humidity (55%) controlled room with a 12:12 h light-dark cycle. They were provided a standard pelleted diet (Zhengda Ltd. Changsha, China) and water *ad libitum*. This experiment was approved by the Institutional Animal Ethics Committee of Central South University and was conducted in accordance with the National Institutes of Health guidelines for the care and use in experimental animals.

Extraction of *Achyranthes bidentata* polysaccharides: *Achyranthes bidentata* Polysaccharides (ABP) were extracted following the methods described earlier (Lin *et al*., 2010; Zhang and Lin, 2012). In brief, the powder samples were moistened with water and refluxed with 80% ethanol for 1 h twice to remove impurity. The volume of ethanol used every time was five times that of the plant material. The residue was dried and then extracted with boiling water. The filtrate was filtered and condensed under ordinary pressure. The concentrated solution was deproteined with 3% trichloroacetic acid three times. The supematant was concentrated again and its pH value was adjusted to 10 with 10% NaOH. Pre-cooled 95% ethanol was added into the concentrated supematant till the final concentration of ethanol was 80% and kept 4-5 h until the precipitate settled and the precipitate was dissolved in water, then the polysaccharides was precipitated again with ethanol at the concentration of 80%. The precipitate was then dehydrated with 90, 95 and 100% ethanol in that order. The crude polysaccharides (ABP) were dried at 80°C in vacuum.

Animals grouping and treatment: After an adaptation period of a week, the rats were randomly divided into four groups (n = 10 in each group): the control (C) group was administered 2.0 mL saline solution by gavage every day. The low dose ABP (LA) group were administered ABP at 50 mg kg^{-1} body weight by gavage every day. The intermediate ABP (MA) group were administered ABP at 100 mg kg^{-1} body weight by gavage every day. The high-dose ABP (HA) group were administered ABP at 200 mg kg^{-1} body weight by gavage every day. ABP was dissolved in 2.0 mL of saline solution. After 21 days, the rats were submitted to exhaustive exercise test to determine the effect of ABP on exhaustive exercise-induced oxidative stress.

Exhaustive exercise test: Before the exhaustive exercise test, the rats were acclimated to the treadmill running starting at 10% grade, 10 m min^{-1} for 10 min day^{-1} on a graded treadmill (TSE Treadmill System, QiChi Instrument, Shanghai, China). On the day of the exhaustive exercise test, the rats were subjected to treadmill running starting at 10% grade, 15 m min^{-1} for 15 min followed by a gradual increase in the treadmill speed and time to 25 m min^{-1} for 15 min, 30 m min^{-1} for 30 min, 35 m min^{-1} for 60 min, 40 m min^{-1} for 30 min, 45 m min^{-1} for 30 min until exhaustion (Lin *et al*., 2010). Exhaustion was defined as the rat being unable to upright itself when placed on its back (Lira *et al*., 2010; Liu *et al*., 2011).

Determination of biochemical parameters related to oxidative stress: After the exhaustive exercise test, the rats were anesthetized with ether (an anesthesia chamber was utilized as the induction method of delivering volatile anesthetic agent to the rats), ether was volatilized by placing it on cotton balls at the bottom of the jar. The rats after anesthetization were killed by decapitation. Then, the gastrocnemius muscle tissue were dissected out quickly and homogenized in ice-cold 0.15M Tris-KCl buffer (pH 7.4) to yield a 10% (w/v) homogenate. SOD, GSH-Px, CAT activities and MDA contents were estimated using the commercial assay kits according to product instructions.

Statistical analysis: Results were expressed as means±Standard Deviations (SD). Data were analyzed by using Analysis of Variance (ANOVA) and t-test to the statistical significance ($p<0.05$).

RESULTS

As shown in Fig. 1, exhaustive exercise time in the LA, MA and HA groups were significantly longer compared with that of the C group ($p<0.05$).

As shown in Fig. 2, SOD and GSH-Px activities in the LA, MA and HA groups were significantly higher compared with that of the C group ($p<0.05$). CAT activities in the MA and HA groups were significantly higher compared with that of the C group ($p<0.05$); CAT activities in the LA group were also higher but not significantly ($p>0.05$).

As shown in Fig. 3, MDA contents in the LA, MA and HA groups were significantly lower compared with that of the C group ($p<0.05$).

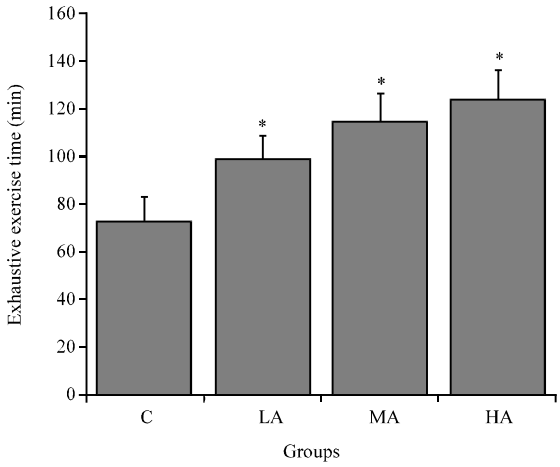

Fig. 1: Effect of ABP on exhaustive exercise time of rats

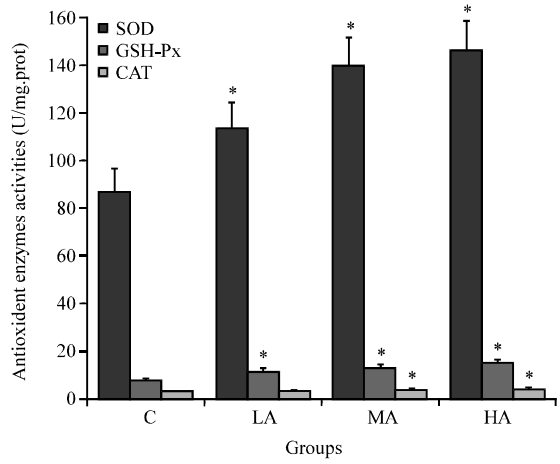

Fig. 2: Effect of ABP on SOD, GSH-Px and CAT activities in the muscle of rats

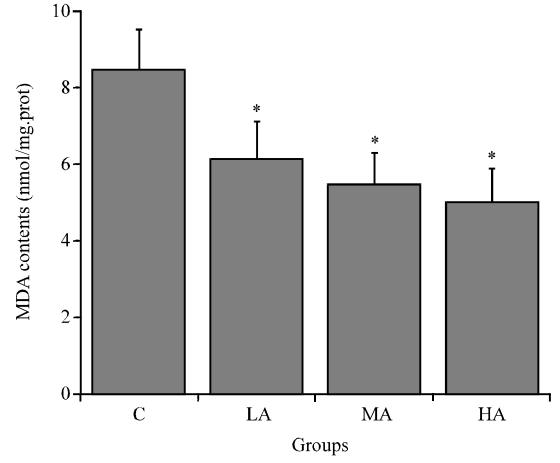

Fig. 3: Effect of ABP on MDA contents in the muscle of rats

DISCUSSION

The well-documented benefits of regular physical exercise include reduced risk of cardiovascular disease, cancer, osteoporosis and diabetes. However, strenuous physical exercise with dramatically increased oxygen uptake is associated with the generation of free radicals and ROS (Kruk, 2007; Shan *et al*., 2011; Li *et al*., 2012, 2013). When ROS levels exceed the normal physiological coping range during strenuous physical exercise, the accumulation of ROS and a reduction in antioxidant status may result (Xu and Li, 2012). This scenario increases oxidative stress and leads to modifications of lipid and protein structures that consequently compromise the cellular functions in tissue. During the past three decades, exhaustive exercise-induced muscle damage has been widely reported (Aoi *et al*., 2003; Lu *et al*., 2006; Korivi *et al*., 2012).

SOD, GSH-Px and CAT are regarded as the first line of defense of the antioxidant enzyme system against ROS generated during strenuous physical exercise (Deng and Hu, 2011). The increase in antioxidant enzymes in muscle would indicate an up-regulation of the defense mechanism to try to cope with an enhanced production of superoxide anion radicals. This in turn might help to down-regulate the production of lipid peroxides or oxidative stress (Liu *et al*., 2011). In the present study, researchers found that ABP could significantly increase SOD, GSH-Px and CAT activities which indicate that ABP were able to up-regulate antioxidant enzyme activities to protect against oxidative stress induced by exhaustive exercise.

Oxidative stress induced by exhaustive exercise can significantly elevate markers of tissue per-oxidative damage because physical exercise promotes the production of ROS due to a substantial increase in oxygen consumption (Liu *et al*., 2011). MDA is a secondary product generated during the oxidation of polyunsaturated fatty acids which has been frequently measured as an indicator of lipid peroxidation and oxidative stress *in vivo* (Dalle-Donne *et al*., 2006; Xu and Li, 2012). In the present study, researchers found that ABP could significantly decrease MDA contents which indicates that ABP could reduce lipid per-oxidation during exhaustive exercise.

CONCLUSION

The present investigation showed that ABP could significantly increase SOD, GSH-Px and CAT activities, decrease MDA contents in muscle in rats. This meant that ABP effectively attenuates oxidative stress induced by exhaustive exercise.

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