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## Effects of *Achyranthes bidentata* Polysaccharides on Physical Fatigue

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### ABSTRACT

The main purpose of this study was to examine the effects of *Achyranthes bidentata* Polysaccharides (ABP) on physical fatigue. One hundred and forty four mice were randomly divided into 4 groups: one control group and three ABP treatment groups. The control group was given isotonic sodium chloride solution and the treatment groups were given different doses of ABP (50, 100, 200 mg kg<sup>-1</sup>) by giving once a day for 28 days. After 28 days, the forced swimming test was performed and the biochemical parameters related to fatigue were examined. The results suggested that ABP had clear anti-physical fatigue effects which could extend the exhaustive swimming time of the mice, as well as increases the liver glycogen and muscle glycogen contents and decreases the blood lactic acid and blood urea nitrogen contents.

**Key words:** *Achyranthes bidentata*, polysaccharides, physical fatigue, mice, forced swimming test

### INTRODUCTION

Fatigue is best defined as difficulty in initiating or sustaining voluntary activities (Ataka *et al.*, 2007), it can be classified into physical and mental fatigue. Physical fatigue is thought to be accompanied by deterioration in performance (Chaudhuri and Behan, 2004). Since, the available therapies for fatigue in modern medicine are very limited, potential alternatives from traditional medicine and their respective mechanisms of action is worth investigating (Wang and Yan, 2010). Polysaccharides are known to have numerous biological activities, such as antitumor, antiinfection and immunity enhancing effects (Claver *et al.*, 2010; El-Enshasy *et al.*, 2010; Thetsrimuang *et al.*, 2011; Kone *et al.*, 2012). Plant polysaccharides with antifatigue functions have previously been reported from *Lycium barbarum*, *Saussurea involucrata*, *Morinda officinalis*, *Dimocarpus longan* Lour, *Panax ginseng* and *Radix rehmanniae* preparata (Zheng *et al.*, 2010; Tan *et al.*, 2012).

*Achyranthes bidentata* (Amaranthaceae, Chinese Phonetic Name-Niuxi) is an erect, annual herb distributed in hilly districts of China, Korea, Japan and India (Lin *et al.*, 2010). The plant has been proven to have wide application in traditional and folk medicines in these, as well as other eastern countries (Uma Devi *et al.*, 2007). The whole plant and particularly the roots, has been shown to contain various saponins, sterols, polysaccharides and alkaloids and has well-defined expectorant, anti-inflammatory, antipyretic, antirheumatic and diuretic activities (Li *et al.*, 2005; Ding *et al.*, 2007; He *et al.*, 2010). The bioactive polysaccharides isolated from its root is called

*Achyranthes bidentata* polysaccharides (ABP). ABP have been reported to possess immunopotentiating, anti-senile, antiradiation, antitumor and hypoglycemic effects (Lin *et al.*, 2010). However, no detailed study has been reported on the anti-physical fatigue activity of ABP. Therefore, the present study has been conducted to investigate the effects of ABP on physical fatigue using a forced swimming test in mice.

## MATERIALS AND METHODS

**Materials and chemicals:** The roots of *Achyranthes bidentata* (Voucher No. CST09-484) were bought from the market of traditional Chinese medicinal materials in Changsha, China and identified according to the identification standard of the 8th edition of Pharmacopeia of People's Republic of China (2005 PPRC). The assay kits for Blood Lactic Acid (BLA) and tissue glycogen were purchased from Jiancheng Biologic Project Company (Nanjing, China). The assay kit for Blood Urea Nitrogen (BUN) was purchased from Biosino Biotechnology and Science Company (Beijing, China). All other chemicals and solvents used in this study were of analytical grade and obtained from Hunan Reagent Company (Changsha, China).

**Preparation of *Achyranthes bidentata* polysaccharides:** The roots of *Achyranthes bidentata* 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 deproteinized with 3% trichloroacetic acid three times. The supernatant was concentrated again and its pH value was adjusted to 10 with 10% NaOH. Pre-cooled 95% ethanol was added into the concentrated supernatant till the final concentration of ethanol was 80% and kept 4 to 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 was dried at 80°C in vacuum. The contents of polysaccharides were measured by the phenol sulphuric acid method by using glucose as standard.

**Animals and grouping:** Male Kunming mice (weighing 18 to 22 g) were obtained from Hunan biological supplier (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*. The approval of this experiment was obtained from the Institutional Animal Ethics Committee of Central South University. Mice were trained to accustom themselves to swimming twice (10 min per time) in the first week. During the period, the mice which could not learn to swim were screened out. Then, 144 mice were randomly divided into 4 groups equally based on body weight: Control Group (CG), ABP Low dose Treatment Group (ALTG), ABP Middle dose Treatment Group (AMTG) and ABP High Dose Treatment Group (AHTG). The control group was given isotonic sodium chloride solution and ABP treatment groups were given different doses of ABP (50, 100, 200 mg kg<sup>-1</sup>) by giving once a day for 28 days.

**Forced swimming test:** The forced swimming test was used as described previously with some modifications (Ikeuchi *et al.*, 2006; Ishola and Ashorobi, 2007; Lu *et al.*, 2009; Liu *et al.*, 2011;

Prasad and Khanum, 2012). After 28 days, 12 mice were taken out from each group for swimming exercise supporting constant loads (lead fish sinkers, attached to the tail) corresponding to 5% of their body weight. The swimming exercise was carried out in an acrylic plastic pool (50×50 ×40 cm) 30 cm deep with water maintained at 25±0.5°C. Exhaustion was determined by observing loss of coordinated movements and failure to return to the surface within 10 sec and the exhaustive swimming time was immediately recorded.

**Assay of blood lactic acid:** After 28 days, 12 mice were taken out from each group for Blood Lactic Acid (BLA) analysis. The mice were forced to swim with a load (2% body weight) for 30 min and the blood was collected before and after forced swimming with a capillary tube by using the retro-orbital bleeding method, respectively. Then BLA was tested according to the recommended procedures provided by the commercial assay kit.

**Assay of blood urea nitrogen and tissue glycogen:** After 28 days, 12 mice were taken out from each group for Blood Urea Nitrogen (BUN) and tissue glycogen analyses. The mice were forced to swim for 90 min without weight loading, after 60 min of recess, the mice were anesthetized with ether and blood was collected in tubes by heart puncture. In addition, immediately after the blood had been collected, the liver and gastrocnemius muscle were dissected out quickly from the mice, washed with physiological saline and dried with absorbent paper. Then BUN and tissue glycogen were tested according to the recommended procedures provided by the commercial assay kit.

**Statistical analysis of the data:** 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 AND DISCUSSION

**Effects of ABP on the exhaustive swimming time of mice:** The forced swimming test was employed in this study to evaluate the effects of ABP on physical fatigue. It is commonly accepted that swimming is an experimental exercise model. Other methods of forced exercise such as the motor driven treadmill or wheel can cause injury of animals and may not be routinely acceptable (Wu *et al.*, 1998). As shown in Fig. 1, there were significant differences in the exhaustive

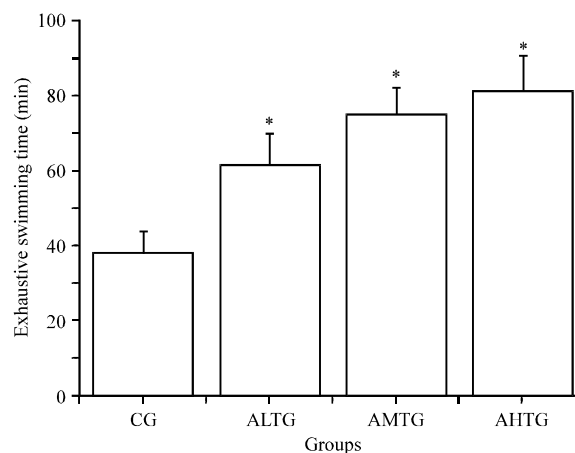


Fig. 1: Effects of ABP on the exhaustive swimming time of mice, Values are means±SD. Each group contains 12 mice, \* $p<0.05$  when compared to the control group (CG)

swimming time between the Control Group (CG) and each ABP treatment group (ALTG, AMTG and AHTG). The swimming time to exhaustion in the CG, ALTG, AMTG and AHTG were  $38.4 \pm 5.6$ ,  $61.7 \pm 8.4$ ,  $75.2 \pm 7.3$  and  $81.3 \pm 9.7$  min, respectively. Thus, the exhaustive swimming time in the ALTG, AMTG and AHTG were significantly longer than that of the CG ( $p < 0.05$ ). This result suggested that ABP had significant anti-physical fatigue effects.

**Effect of ABP on the blood lactic acid of mice:** Blood Lactic Acid (BLA) is the glycolysis product of carbohydrate under anaerobic conditions and glycolysis is the main energy source for intense exercise over a short time. The accumulation of BLA is a reason for fatigue during physical exercise (Cairns, 2006; Prasad and Khanum, 2012) and rapid removal of lactic acid is beneficial to relieving fatigue (Li *et al.*, 2008). As shown in Fig. 2, there was no significant change in the BLA contents among all the groups before the forced swimming ( $p > 0.05$ ). After swimming, the BLA contents in the ALTG, AMTG and AHTG were  $9.52 \pm 1.03$ ,  $8.49 \pm 1.06$  and  $7.51 \pm 0.84$  mmol L<sup>-1</sup>, respectively which were lower than that of the control group ( $12.86 \pm 1.38$  mmol L<sup>-1</sup>) ( $p < 0.05$ ). This result suggested that ABP could effectively retard and lower the BLA produced and postpone the appearance of fatigue.

**Effects of ABP on the blood urea nitrogen of mice:** Blood Urea Nitrogen (BUN) 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 (Zhang *et al.*, 2006; Tajik *et al.*, 2011; Huang *et al.*, 2011). Protein and amino acids have a stronger catabolic metabolism when body can not 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 (Wei *et al.*, 2010). As shown in Fig. 3, after swimming, the BUN contents in the ALTG, AMTG and AHTG were  $8.15 \pm 1.14$ ,  $7.53 \pm 1.02$  and  $7.41 \pm 0.92$  mmol L<sup>-1</sup>, respectively which were lower than that of the control group ( $10.36 \pm 1.23$  mmol L<sup>-1</sup>) ( $p < 0.05$ ). This result suggested that ABP might reduce catabolic decomposition of protein for energy.

**Effects of ABP on the liver glycogen and muscle glycogen of mice:** Sugar is an important energy during sports, after strenuous exercise for 2 h muscle glycogen will exhaust. Body depletion

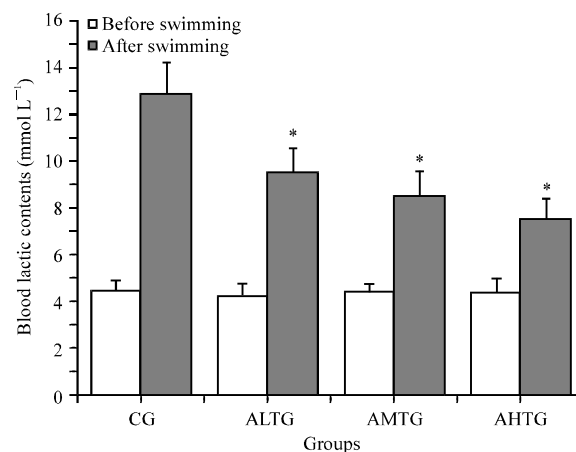


Fig. 2: Effects of ABP on the blood lactic acid of mice. Values are means $\pm$ SD. Each group contains 12 mice. \* $p < 0.05$  when compared to the Control Group (CG)

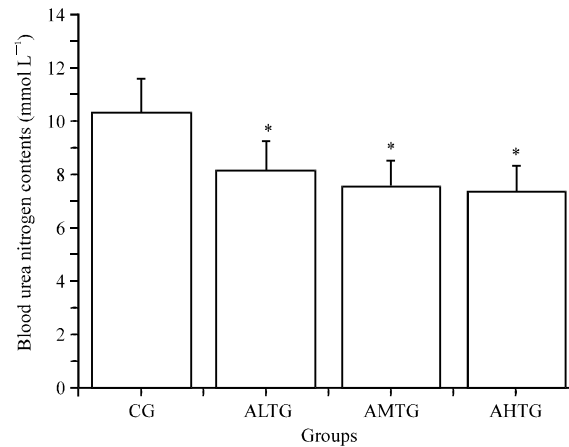


Fig. 3: Effects of ABP on the blood urea nitrogen of mice, Values are means±SD, Each group contains 12 mice, \*p<0.05 when compared to the control group (CG)

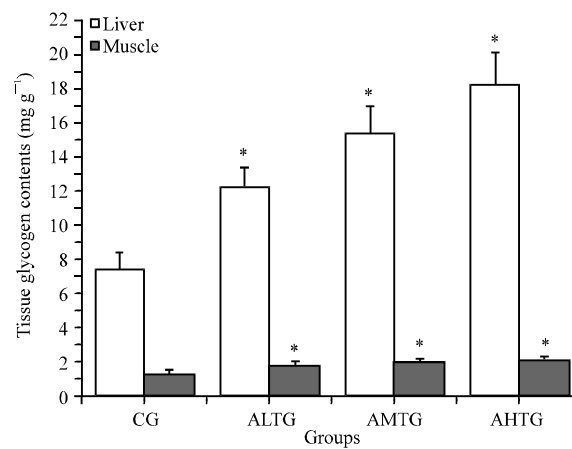


Fig. 4: Effects of ABP on the liver glycogen and muscle glycogen of mice. Values are means±SD. Each group contains 12 mice, \*p<0.05 when compared to the Control Group (CG)

often happens with the exhaustion of glycogen during strenuous exercise. Thus, the content of glycogen can illustrate the speed and degree of the development of fatigue. In order to maintain the blood glucose the reservation of liver starch will reduce when muscle glycogen is exhausted. So, glycogen is a sensitive index to test physical fatigue (Li *et al.*, 2008; Yan and Wang, 2010). As shown in Fig. 4, after swimming, the liver glycogen contents in the CG, ALTG, AMTG and AHTG were  $7.48 \pm 0.93$ ,  $12.26 \pm 1.17$ ,  $15.39 \pm 1.61$  and  $18.25 \pm 1.88$  mg g<sup>-1</sup>, respectively. The muscle glycogen contents in the CG, ALTG, AMTG and AHTG were  $1.36 \pm 0.22$ ,  $1.84 \pm 0.19$ ,  $2.03 \pm 0.24$  and  $2.16 \pm 0.21$  mg g<sup>-1</sup>, respectively. Thus, the liver glycogen and muscle glycogen contents in the ABP treatment group were significantly higher than that of the CG (p<0.05). This result suggested that ABP might increase tissue glycogen contents of mice after exercise by improving glycogen reserve or by reducing the glycogen consumption during exercise or both. However, the detailed mechanism of this phenomenon is not clear and needs further study.

## CONCLUSION

In conclusion, the present study demonstrated that ABP had clear anti-physical fatigue effects which could extend the exhaustive swimming time of the mice, increase the liver glycogen and muscle glycogen contents and decrease the blood lactic acid and blood urea nitrogen contents. However, further studies to clarify the detailed mechanisms involved in the anti-fatigue effects of ABP are necessary.

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