Journal of Medicinal Plants Research Vol. 4(10), pp. 982-986, 18 May, 2010
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR10.203
ISSN 1996-0875© 2010 Academic Journals

Full Length Research Paper

Effects on exercise endurance capacity and antioxidant properties of astragalus membranaceus polysaccharides (APS)

Sun Hao* and Wang Zhaobao

College of Physical Education, Dezhou University, Dezhou, Shandong Province, 253023, P. R. China.

Accepted 17 May, 2010

Astragalus membranaceus is a famous Chinese medicinal herb. Polysaccharides are the most important functional constituent in Astragalus membranaceus. In the present study, the effects of Astragalus membranaceus polysaccharides (APS) on exercise endurance capacity were evaluated in mice using swimming exercise. The antioxidant properties of APS were also investigated through the 1,1- diphenyl-2-picrylhydrazyl (DPPH) method. The results showed that APS supplementation could prolong swimming times of mice, and enhance exercise endurance capacity. APS possess the ability to retard and lower the production of blood lactate, and prevent the increase of serum blood urea nitrogen (BUN) after exercise. In addition, APS possess effective antioxidant potential.

Key words: Astragalus membranaceus polysaccharides, exercise endurance capacity, antioxidant, swimming exercise, DPPH.

INTRODUCTION

Astragalus membranaceus, a perennial belonging to the Fabaceae family delivers an important herbal drug in traditional Chinese medicine and it is considered to be one of the 50 fundamental herbs (Zhang et al., 1990; Matkowski et al., 2003). The root (Chinese name Huang-qi) has been used in treatment of common cold, diarrhea, fatigue, anorexia and cardiac diseases (Wang et al., 2002; Yuan et al., 2005; Sun et al., 2008; Wang et al., 2008; Yuan et al., 2008; Na et al., 2009) . It has also been used as an immunomodulating agent in treatment of immunodeficiency diseases, to alleviate the adverse effects of chemotherapeutic drugs and reduce myelosuppression in cancer patients (Zee-Cheng, 1992; Duan and Wang, 2002; Ma et al., 2002; Shao et al., 2004; Na et al., 2009). Moreover, several studies indicated that Astragalus membranaceus may prevent various types of injury resulting from exercise, or enhance physical strength (Lv et al., 2004; Han and Zhang, 2008; Zhang, 2009). Among constituents that contribute to its biological activity are steroid saponin glycosides (astragalosides), isoflavonoids as well as other polyphenolic compounds (flavonols, phenolic acids), essential oils and polysaccharides (He and Findlay, 1991; Ganzera et al., 2001; Duan and Xie, 2005; Huo, 2007). As an important bioactive component of Astragalus membranaceus, previous studies have shown that Astragalus membranaceus polysaccharides (APS) possess and exhibit immune-stimulatory, hypoglycemic, anti-hypertensive, anti-viral, anti-oxidation, anti-aging and anti-tumor effects (Dang et al., 2004; Yuan et al., 2008; He et al., 2008; Mao et al., 2009; Mao et al., 2007; Wang et al., 2004).

High Intensity exercise could increase reactive oxygen species (ROS) production, which might induce oxidative damage, include impair cell integrity and contractile function of muscle cells. The extent of oxidative damage is largely dependent on the ability of the body to defend against ROS production. This defense is collectively referred to as the antioxidant defense system, and includes both enzymatic and non-enzymatic antioxidants. Antioxidants intake has been widely used in an attempt to decrease oxidative stress resulting from exercise (Urso and Clarkson, 2003), which can contribute in reducing the

*Corresponding author. E-mail: DEZHW@msn.com. Tel: +86-0534-8985838. Fax: +86--0534-8985888

degree of exhaustion caused by high intensity exercise.

The present study had the purpose to study the effects of APS on exercise endurance capacity and evaluated the antioxidant properties of APS based on its ability to scavenge DPPH radical.

MATERIALS AND METHODS

Plant material

The roots of Astragalus membranaceus (Figure 1) were bought from the market of traditional Chinese medicinal materials in Dezhou, China and were identified according to the identification standard of the Pharmacopoeia of the People's Republic of China (PPRC).

Preparation of APS

Preparation of APS was as described previously and was modified (Han et al., 2000; Ding and Wu, 2003; Xu et al., 2008). The roots of Astragalus membranaceus were grounded into powder, 500 g of which was added 20 times as much as distilled water and soaked for 10 h, then extracted for 30 min in ultrasonic wave extractor and filtrated for 3 times. Filtrated water centrifuged at 4800 rpm for 10 min at 55°C. Supernatant was collected and rotary evaporated to condense it. When it was condensed to 500 ml, extraction was poured into a beaker and 2000 ml ethanol (95%) was added slowly and stirred without stopping in case of the emergence of lamellar precipitation. It was left overnight at 4°C. The next day, super-layer ethanol was slowly poured out. Same step was repeated the next day as well. On the third day, the settling was vacuum filtrated and dried in the vacuum drying box and weighed. 37.96 g of the APS was obtained.

Animals and grouping

This experiment was approved by the Institutional animal Care and Use Committee at Dezhou University and was conducted in accordance with the National Institutes of Health guidelines for the care and use in experimental animals.

Male Kunming mice (weighing 20 ± 2 g) were purchased from the Laboratory Animal Center of Dezhou University (Dezhou, China). They were housed in standard cages (35 × 25 × 15 cm) under controlled conditions of temperature (22 ± 1°C), humidity (50%), and lighting (lights on daily from 06:00 - 18:00) . They were provided pelleted food and fresh water *ad libitum*. In addition, they were housed under these conditions for 1 week prior to the start of the experiments.

Forty Male mice were randomly divided into four groups, each group consisting of 10 animals. One group was the control group (I). The others were APS treated groups (α, β and χ):

Group I (n = 10): Mice orally administrated with the volume (0.02 ml) of physiological saline for 28 consecutive days.
Group α (n = 10): Mice were treated by oral infusion with the same volume of APS at a dose of 100 mg/kg BW/day dissolved in physiological saline for that same period.
Groupβ (n = 10): Mice were treated by oral infusion with the same volume of APS at a dose of 200 mg/kg BW/day dissolved in physiological saline for that same period.
Groupχ (n = 10): Mice were treated by oral infusion with APS at a dose of 400 mg/kg BW/day dissolved in physiological saline for that same period.

The doses used in this study were confirmed to be suitable and effective in tested mice according to preliminary experiments.

Swimming exercise

The mice were made to swim for 15 min three times a week to accustom them to swimming. After 28 days, the mice were made to swim to exhaustion exercise. The swimming exercise was carried out in an acrylic plastic tank (25 × 20 × 30 cm) filled with water to a depth of 25 cm at a temperature of 23°C. Each of the mice had a weight attached (10% body weight) to the tail for the duration of the swimming to exhaustion exercise. The mice were assessed to be exhausted when they failed to rise to the surface of water to breathe within a 5-s period.

Blood samples were collected from the veins on the tails of individual mice, and the lactate, serum BUN were determined using a commercial diagnostic kit (Jiancheng Diagnostic Inc., Nanjing, China).

The DPPH radical scavenging activity of APS

The DPPH radical scavenging activity of APS was as described previously and was modified (Yu et al., 2007). A 1.5 ml of ethanolic solution of DPPH (2×10^{-4} mol/l) was mixed with equivalent aliquot of different concentrations of sample in a tube. Absorbance at 517 nm was measured at 2 min intervals by the use of spectrophotometer. After standing in dark for 30 min when the absorbance reached a plateau, it was measured against ethanol. Controls containing ethanol instead of the antioxidant solution and blanks containing ethanol instead of DPPH solution were also made. DPPH scavenging activity was calculated with the equation:

(1- As of sample/ As of control) × 100%

The percentage of scavenging activity was plotted against the sample concentration to obtain the IC50, defined as the concentration of sample necessary to cause 50% inhibition (Chen et al., 2009). The DPPH radical scavenging activity of BHT was also assayed for comparison.

Statistical analysis

All values are expressed as mean ± SD. Statistical significance was determined using analysis of variance (ANOVA) followed by Tukey's test. $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effect of APS supplementation on exercise endurance capacity

Many systems for the determination of exercise endurance capacity in mice have been employed, including treadmill running, spontaneous wheel running and swimming (Fushiki et al., 1995). In this study, the method of swimming exercise was used. We selected this method because exhaustion is more clearly observed than during treadmill running or spontaneous wheel running. Exercise endurance capacity was evaluated by measuring the swimming time to exhaustion of the mice. To standardize the workload and reduce the swimming

Figure 1. The roots of Astragalus membranaceus (Chinese name Huang-qi).

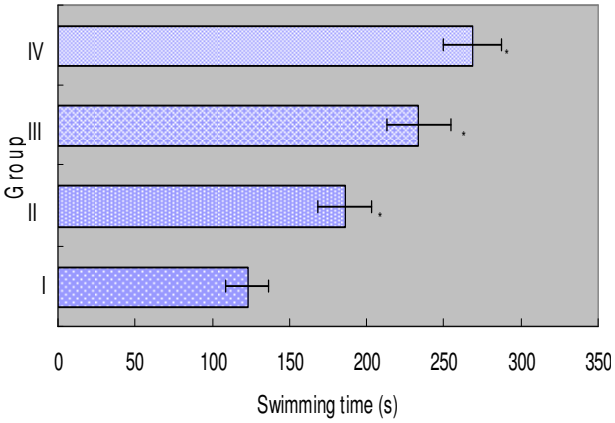

Figure 2. Effect of APS supplementation on exercise endurance capacity.
*P < 0.05 indicates significant difference from the group.

time, weights at specific body weight percentages were added to the chest or tail of the animal (Matsumoto et al., 1996). In this study, the mice had a weight attached (10% body weight) to the tails during the swimming based on the above reason. As shown in Figure 2, the swimming time of each APS treated groups (α, β and χ) increased significantly ($p < 0.05$) when compared with that of the control group (I). The results indicated that APS supplementation could prolong the swimming times. It is thus considered that APS supplementation could enhance exercise endurance capacity.

Effect of APS supplementation on blood lactate and serum BUN of mice

The concentration of is insufficient. Many organs, especially the liver, heart and skeletal muscle, help remove lactate from the blood (Bonen, 2000; Van Hall et al., 2001), but intense exercise can increase lactate production to a point that exceeds the rate of lactate

Table 1. Effect of APS supplementation on blood lactate and serum blood urea nitrogen.

Group	Blood lactate (mmol/l)	Serum BUN (mmol/l)
I	10.19 ± 1.17	8.11 ± 0.86
α	7.96 ± 1.26*	7.93 ± 1.01
β	6.88 ± 1.43*	6.54 ± 0.94*
χ	6.21 ± 1.38*	6.17 ± 0.91*

*p < 0.05 indicates significant difference from the group.

removal, which results in fatigue (You et al., 2006). Blood urea nitrogen (BUN) is a sensitive index to evaluate the bearing capability when the body suffer from a physical load. There is a positive correlation between the urea nitrogen *in vivo* and the exercise tolerance (Tsopanakis and Tsopanakis, 1998; Tang et al., 2008). lactate circulating in blood is a biochemical indicator of anaerobic metabolism, which occurs when oxygen delivery to the tissues The effect of APS on blood lactate and blood urea nitrogen is shown in Table 1. The blood lactate concentrations of each APS treated groups (α, β and χ) decreased significantly ($p < 0.05$) when compared with that of the control group (I). The serum blood urea nitrogen concentrations of APS treated groups (β and χ) decreased significantly ($p < 0.05$) when compared with that of the control group (I). The results indicated that APS possess the ability to retard and lower the blood lactate produced, and prevent the increase of serum BUN after exercise. Such effects might enhance exercise endurance capacity mechanism of APS supplementation.

DPPH radical scavenging activity of APS

Intense or Prolonged aerobic exercise has been found greatly to increase oxidative stress (Nieman et al., 2002; You et al., 2006). Exercise endurance capacity can be improved by supplementation with an antioxidant. A similar increase in exercise endurance capacity was observed in mice fed *Panax japonicus* polysaccharides, *Porphyra yezoensis* polysaccharides and Longan Polysaccharide, which act as antioxidants and can enhance endurance performance (Gu et al., 2007; Wang et al., 2007; Nie et al., 2009). Based on these observations, the improved exercise endurance capacity by APS might be at least in part due to a protective effect against the oxidative stress induced by exercise. The free radical scavenging activity of APS was tested through the DPPH method and the results were compared with BHT. As shown in Figure 3, APS exhibited a concentration-dependent antiradical activity by inhibiting the DPPH radical and it showed higher scavenging activity than BHT ($p < 0.05$). The results indicated that APS possess effective antioxidative potential. It is thus considered that APS as antioxidants could play a role in enhancing

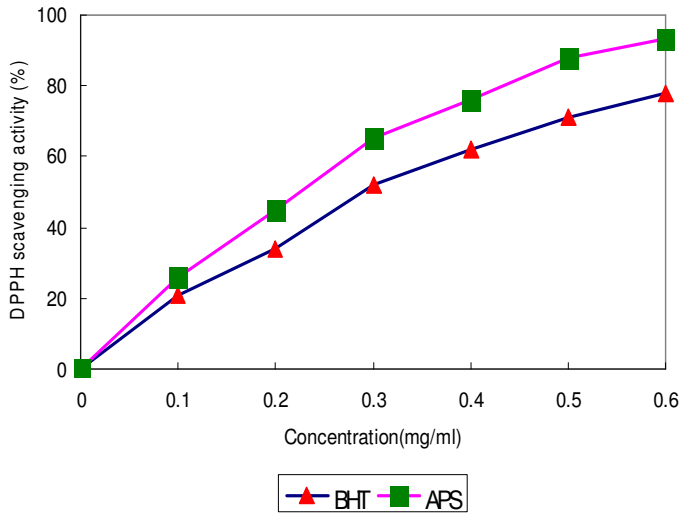

Figure 3. DPPH radical scavenging activity of APS.

performance capacity.

Conclusion

In summary, we have shown that intake of Astragalus membranaceus polysaccharides (APS) is beneficial in improving endurance capacity. It possess the ability to retard and lower the blood lactate produced, and prevent the increase of serum BUN after exercise. In addition, it is clear that APS possess effective antioxidative potential, and as antioxidants could play a role in enhancing performance capacity. Further research is required to elucidate the mechanism of action relevant to its antioxidative activity.

ACKNOWLEDGEMENT

We thank the laboratory staff of the Biology Department of Dezhou University for their advice and support.

REFERENCES

Bonen A (2000). Lactate transporters (MCT proteins) in heart and skeletal muscles. Med. Sci. Sports Exercise 32: 778-789.
Chen YG, Shen ZJ, Chen XP (2009). Evaluation of free radicals scavenging and immunity-modulatory activities of Purslane polysaccharides. Inte. J. Biol. Macromol. 45: 448-452.
Dang SS, Jia XL, Cheng YA, Chen YR, Liu EQ, Li ZF (2004). Inhibitory effect of Huangqi Zhechong decoction on liver fibrosis in rat. World J. Gastroenterol. 10: 2295-2298.
Ding LF, Wu QP (2003). Extraction of polysaccharide from astragalus membranaceus and effects of scavenging free radicals. Food Machin. 4: 7-8.
Duan P, Wang ZM (2002). Clinical study on effect of Astragalus in efficacy enhancing and toxicity reducing of chemotherapy in patients of malignant tumor. Zhongguo Zhongxiyi Jiehe Zazhi 22: 515-517.
Duan YL, Xie MD (2005). Studies on the chemical components of Astragalus membranaceus and the method of determinating effectual constituent-astragaloside IV. Chinese J. Vet. Drug 39: 35-38.
Fushiki T, Matsumoto K, Inoue K, Kawada T, Sugimoto E (1995). Swimming endurance capacity of mice is increased by chronic consumption of medium-chain triglycerides. J. Nut. 125: 531-539.
Ganzera M, Bedir E, Calis I, Khan IA (2001). Separation of Astragalus saponins by reversed phase high performance liquid chromatography and evaporative light scattering detection. Chromatographia 53: 131-134.
Gu JW, Zhang LX, Guo TT, He PM (2007). Study on Anti-fatigue Bioactivity of Polysaccharide from Porphyra yezoensis. Biotechnol. Bull. 4: 32-35.
Han LJ, Yan QJ, Jiang ZQ, Liu XY (2000). Study on Extracting Technology of Astragalan Polysaccharide (ASP) and Astragaloside (AS). Transactions Chin. Soc. Agric. Eng. 16: 118-121.
Han SP, Zhang CY (2008). Effects of Astragalus Hoangtchy on Enzyme Related to Glycogen Metabolism in Swimming Rats. Sport Sci. Technol. 44: 129-130.
He FL, Wang L, Zhang XK, Zeng JZ (2008). Emodin induces apoptosis of cancer cells and inhibits retinoid X receptor transcriptional activity. Yaoxue Xuebao 43: 350-355.
He ZQ, Findlay JA (1991). Constituents of Astragalus membranaceus. J. Nat. Prod. 54: 810-815.
Huo GH (2007). Investigating Progression of Astragalus Mongholicus in Angiomyocardiac Pharmacologic Action. J. Henan Univers. Chinese Med. 22: 86-88.
Lv H, Guo WD, Yang J, Bao HGJLT, Yu B (2004). Experimental Study Against Exercise Fatigue with Compound Huangqi Extract. J. Shandong Phys. Edu. Inst. 20: 48-50.
Ma XQ, Shi Q, Duan JA, Dong TT, Tsim KW (2002). Chemical analysis of Radix Astragali (Huangqi) in China: a comparison with its adulterants and seasonal variations. J. Agric. Food Chem. 50: 4861-4866.
Mao XQ, Wu Y, Wu K, Liu M, Zhang JF, Zou F, OuYang JP (2007). Astragalus polysaccharide reduces hepatic endoplasmic reticulum stress and restores glucose homeostasis in a diabetic KKAy mouse model. Acta Pharmacolo. Sinica 28: 1947-1956.
Mao XQ, Yu F, Wang N, Wu Y, Zou F, Wu K, Liu M, Ouyang JP (2009). Hypoglycemic effect of polysaccharide enriched extract of Astragalus membranaceus in diet induced insulin resistant C57BL/6J mice and its potential mechanism. Phytomed. 16: 416-425.
Matkowski A, Woźniak D, Lamer-Zarawska E, Oszmiański J, Leszczyńska A (2003). Flavonoids and phenol carboxylic acids in the oriental medicinal plant Astragalus membranaceus acclimated in Poland. Zeitschrift für Naturforschung C, 58: 602-604.
Matsumoto K, Ishihara K, Tanaka K, Inoue K, Fushiki T (1996). An adjustable-current swimming pool for the evaluation of endurance capacity of mice, J. Appl. Physiol. 81: 1843-1849.
Na D, Liu FN, Miao ZF, Du ZM, Xu HM (2009). Astragalus extract inhibits destruction of gastric cancer cells to mesothelial cells by anti-apoptosis. World J. Gastroenterol. 15: 570-577.
Nie YT, Yang JF, Chen J (2009). Effects of Longan Polysaccharide on Swimming Ability of Rat. J. Anhui Agric. Sci. 37: 287-289.
Nieman DC, Henson DA, McAnulty SR, McAnulty L, Swick NS, Utter AC, Vinci DM, Opiela SJ, Morrow JD (2002). Influence of vitamin C supplementation on oxidative and immune changes after an ultramarathon. J. Appl. Physiol. 92: 1970-1977.
Shao BM, Xu W, Dai H, Tu P, Li Z, Gao XM (2004). A study on the immune receptors for polysaccharides from the roots of Astragalus membranaceus, a Chinese medicinal herb. Biochem. Biophysic. Res. Commun. 320: 1103-1111.
Sun Y, Jin L, Wang T, Xue J, Liu G, Li X, You J, Li S, Xu Y (2008). Polysaccharides from Astragalus membranaceus promote phagocytosis and superoxide anion (O2-) production by coelomocytes from sea cucumber Apostichopus japonicus *in vitro*. Comparative Biochemistry and Physiology - Part C: Toxicol. Pharmacol. 147: 293-298.
Tang WY, Zhang Y, Gao J, Ding XY, Gao S (2008). The Anti-fatigue Effect of 20(R)-Ginsenoside Rg3 in Mice by Intranasally Administration, Biolo. Pharmaceut. Bull. 31: 2024-2027.
Tsopanakis C, Tsopanakis A (1998). Stress hormonal factors, fatigue, and antioxidant responses to prolonged speed driving, Pharmacol.

Biochem. Behav. 60: 747-751.
Van Hall G, Calbet JA, Sondergaard H, Saltin B (2001). The re-establishment of the normal blood lactate response to exercise in humans after prolonged acclimatization to altitude. J. Physiol. 536: 963-975.
Wang PC, Zhang ZY, Zhang J, Tong TJ (2008). Two isomers of HDTIC isolated from Astragali Radix decrease the expression of p16 in 2BS cells. Chinese Med. J. (Engl) 121: 231-235.
Wang RT, Shan BE, Li QX, Tang JF, Qiao F, Du XN, Li H, Ye Q (2002). Extracorporeal Experimental Study on Immuno-Modulatory Activity of Astragalus memhranaceus Extract. Chinese J. Integrated Trad. Western Med. 22: 453-455.
Wang SH, Wang WJ, Wang XF, Chen W (2004). Effect of Astragalus polysaccharides and berberine on carbohydrate metabolism and cell differentiation in 3T3-L1 adipocytes. Zhongguo Zhong Xi Yi Jie He Za Zhi 24: 926-928.
Wang SH, Wang WJ, Wang XF, Chen W (2008). Effects of astragalus polysaccharides on antioxidant status of chicks. J. Herbal Med. Toxicol. 2: 45-49.
Wang YG, Zhu FR, Han FS, Wang HY (2007). The anti-fatigue and anti-oxidant effects of protamine hydrolysate. Food Sci. Technol. 7: 48-83.
You Y, Kim K, Heo H, Lee K, Lee J, Shim S, Jun W (2006). Stimulatory effects of *Pseudosasa japonica* leaves on exercise performance. Biosci. Biotechnol. Biochem. 70: 2532-2535.
Yu RM, Yang W, Song LY, Yan CY, Zhang Z, Zhao Y (2007). Structural characterization and antioxidant activity of a polysaccharide from the fruiting bodies of cultured Cordyceps militaris. Carbohydrate Polymers 70: 430-436.
Yuan C, Pan X, Gong Y, Xia A, Wu G, Tang J, Han X (2008). Effects of Astragalus polysaccharides (APS) on the expression of immune response genes in head kidney, gill and spleen of the common carp, Cyprinus carpio L. Inter. Immunopharmacol. 8: 51-58.
Yuan XL, Zhang XW, Zheng DW, Yu YP, Wu HL, Liu GH, Lin HC (2005). Effects of treatment with milkvetch root and red sage root injections on changes in blood viscosity and renal function in patients with early diabetic nephropathy. Chinese J. Pract. Inter. Med. 25: 138-143.
Zee-Cheng RK (1992). Shi-quan-da-bu-tang (ten significant tonic decoction), SQT. A potent Chinese biological response modifier in cancer immunotherapy, potentiation and detoxification of anticancer drugs. Methods Findings Experim. Clin. Pharmacol. 14: 725-736.
Zhang CM (2009). On the Influence of Astragalus Hoangtchy on Trained Rats' Skeletal Muscle Damage Caused by Free Radicals. J. Beijing Sport Univers. (6): 54-57.
Zhang ZL, Wen QZ, Liu CX (1990). Hepato protective effects of astragalus root. J. Thnopharmacol. 30: 145-149.