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Full Length Research Paper

Effects of *Plantago major* L. seeds extract on endurance exercise capacity in mice

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The present study was designed to investigate the effects of *Plantago major* L. seeds extract (PE) on endurance exercise capacity in mice. Forty eight male mice were randomly divided into four groups, such as control, low-dose PE treated, middle-dose PE treated and high-dose PE treated. The mice in the treated group were received PE (30, 60 and 120 mg/kg, i.g), and the mice in the control group received drinking water i.g for 4 weeks. The results showed that PE prolonged the swimming time as well as glycogen amount in mice, but decrease serum urea nitrogen and blood lactate. These data suggest that PE improved endurance exercise capacity and possessed anti-fatigue effect. PE may be useful for the development of physical strength.

Key words: *Plantago major* L., seeds extract, endurance exercise capacity, mice, anti-fatigue effect.

INTRODUCTION

Plantago major L. (Da-Cheqian in China) is a perennial plant that belongs to the Plantaginaceae family (Samuelsen, 2000; Stanisavljević et al., 2008; Ozaslan et al., 2009). It grows up to 15 cm height, though size varies depending on the growth habitats. Leaves (observed as denate margin) grow in rosettes, and ovate to elliptical with parallel venation. Flowers are small, brownish-green on long non-ramified spikes. Seeds are small with an ovate shape and a slightly bitter taste (Chen et al., 1999; Sharifa et al., 2008). In China, *P. major* L. seeds have been used in traditional Chinese medicine (TCM) as a diuretic, tonic and cough mixture (Sun, 2003; Li et al., 2007; Reng et al., 2009). It is also a folk remedy for the blacks in South Africa, Spanish and Mexican and the natives in Brazil (Veale et al., 1992; França et al., 1996; Noor et al., 2000). Different pharmacological effects have been reported for the *P. major* L. seeds including anti-metastasis, anti-oxidant, anti-inflammatory, anti-diabetic, antimicrobial, analgesic, antiviral, hepatoprotective effect, as well as therapeutic effect on urolithiasis (Nunez Guillen et al., 1997; Chen et al., 1999; Gomez-Flores et al., 2000; Noor et al., 2000; Chiang et al., 2002; Aziz et al., 2005; Velasco-Lezama et al., 2006 Stanisavljević et al., 2008). But studies on whether *P. major* L. seeds have an effect on fatigue are very limited. Fatigue can generally be defined as a difficulty in initiating or sustaining a voluntary activity, and when it is used in exercise physiology, we usually think of fatigue as an exercise-induced decrease of the maximal muscular force (Edwards et al., 1977; Bigland-Ritchie and Woods, 1984; Gandevia, 2001). Many studies demonstrated that some traditional Chinese medicine (TCM) can stimulate blood circulation, improving the transport of ability to transport highly nutritional minerals and assisting excretion and the elimination of the by-products of metabolism, as well as accelerating recovery from fatigue (Bahrke and Morgan, 1994; Li et al., 1999; Kim et al., 2001; Zhang et al., 2006; Zhao et al., 2009). Therefore, the purpose of the study was to determine effect of *P. major L.* seeds extract (PE) on endurance exercise capacity in mice. Based on the results, it will be discussed if *P. major* L. have anti-fatigue effects and how these effects are produced.

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MATERIALS AND METHODS

Plant and extract

P. major L. seeds was generously provided by Shandong Province Medicine Co., Ltd (Jinan, China). Identity of the plant was confirmed and preserved in the Herbarium of Shandong Normal University (Jinan, China). The dried seeds were then pulverized using an electric blender (JFSD-70, Mazhe Co., Ltd. Hangzhou, China). 50 g powdered seed was extracted with 500 ml 96% ethanol by STSXT-06 Soxhlet extraction apparatus (Taian Technical Services Co., Ltd, Beijing, China). The obtained of *P. major L.* seeds extract (PE) were then concentrated and later lyophilized at -70°C to obtain the powder form.

Animals and grouping

All experiments were carried out in accordance with the recommendations of the Institutional Animal Care and Use Committee of Shandong University. Male Kunming mice weighing 18~22 g (Laboratory Animal Center of Shandong Normal University, Jinan, China) were used in the present study. The animals were housed at a temperature of 23 ± 1°C with a 12□12 h light dark cycle. Food and water were available *ad libitum*. The mice were allowed to adapt to the laboratory housing for at least 1 week. A total of 48 male mice were randomly divided into four groups, such as control (Con group), low-dose PE treated (LPT group), middle-dose PE treated (MPT group) and high-dose PE treated (HPT group). The mice in the treated group were received PE (30, 60 and 120 mg/kg, ig), and the mice in the control group received drinking water ig for 4 weeks. The doses of PE and 4-week treatment time used in this study were confirmed to be suitable and effective in tested mice, according to preliminary experiments.

Forced swimming test (FST)

To determine anti-fatigue activity, the endurance exercise capacity of male Kunming mice was assessed through a FST. The mice were submitted to weekly swimming exercise supporting constant loads (lead fish sinkers, attached to the tail) corresponding to 10% of their body weight (Ikeuchi et al., 2006). The mice were assessed to be fatigued when they failed to rise to the surface of the water to breathe within 5 s and the time was immediately recorded (Lu et al., 2009). The swimming exercise was carried out in a swimming tank of 30 x 35 cm with a depth of 35 cm, filled with tap water. The water temperature was kept at 30 ± 2°C.

Biochemical assays

After a period of 4 weeks, the mice were sacrificed by dislocation of the neck after swimming exercise. Blood samples were collected from the caudal vena cava with a heparinized syringe and put into ice-cold tubes for determination of serum urea nitrogen (SUN) and lactate. Samples of liver and muscle tissue from the hind limbs were then removed and stored at -40°C for determination of glycogen content.

Serum was prepared by centrifugation at 3000 rpm at 4°C for 10 min. SUN was determined according to the procedures provided by the kits (Biosino Biotechnology and Science Inc., Beijing, China). The SUN content expressed as mol nitrogen per litre blood serum. Blood lactate and glycogen were determined according to the procedures provided by the kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China). The Blood lactate level expressed as milimol blood lactate per litre bood. The liver/ muscle glycogen content expressed as milligram liver/ muscle glycogen per gram tissue.

Statistical methods

The values are presented as mean ± SD. Two-way ANOVA was performed for the effect of *P. major* L. seeds extract treatments and the paired Student's t-test was used to determine significant differences among groups. All analyses were performed using SPSS11.0 software and P values ＜0.05 were accepted as statistically significant.

RESULTS AND DISCUSSION

Effect of *Plantago major* L. seeds extract on forced swimming capacity in mice

Recently, FST is used to examine whether certain agents have an anti-fatigue effect and is also used as an endurance test (Oztürk et al., 2002; Kim et al., 2002; Koo et al., 2004). Swimming is frequently preferred as an exercise model for small laboratory animals, and it has several advantages over other types of exercise. The intensity of labor during exercise is greater than running for equal periods, and aversive stimulation used to promote running is not used in swimming (Kramer et al., 1993; Yalcin et al., 2000). In this study, the mice loaded with 10% of their body weight were placed in the water at room temperature (30 ± 2°C) to swim and the mice were assessed to be fatigued when they failed to rise to the surface of the water to breathe within 5 s. As shown in Figure 1, MPT and HPT groups showed a significant increase in swimming time to exhaustion as compared to the control group from the first week ($P < 0.05$). In the LPT group, a significant increase in swimming time to exhaustion as compared to the control group was evident after 4 weeks ($P < 0.05$). Data presented herein indicate that PE prolongs the swimming time of mice and improved endurance exercise capacity.

Effect of *P. major* L. seeds extract on blood lactate in mice

Lactate is commonly produced in the body during physiological (e.g. severe exercise) or pathological (e.g. tissue hypoxia) anaerobic metabolism (Hong et al., 1997; Akova et al., 2001). In both humans and a large number of animals, strenuous exercise is associated with accumulation of lactate. There is accumulating evidence that fatigue occurs when lactate builds up within the myocyte, this causes intracellular pH (pHi) to drop, inhibiting both glycolysis and contractile activity (McCullagh et al., 1996; Nielsen et al., 2001; Allen et al., 2008). Therefore, blood lactate is a key indicator of fatigue. As shown in Figure 2, after swimming, blood lactate level of PE treated groups were significantly lower than that of control group ($P < 0.05$) suggesting PE inhibited the production of blood

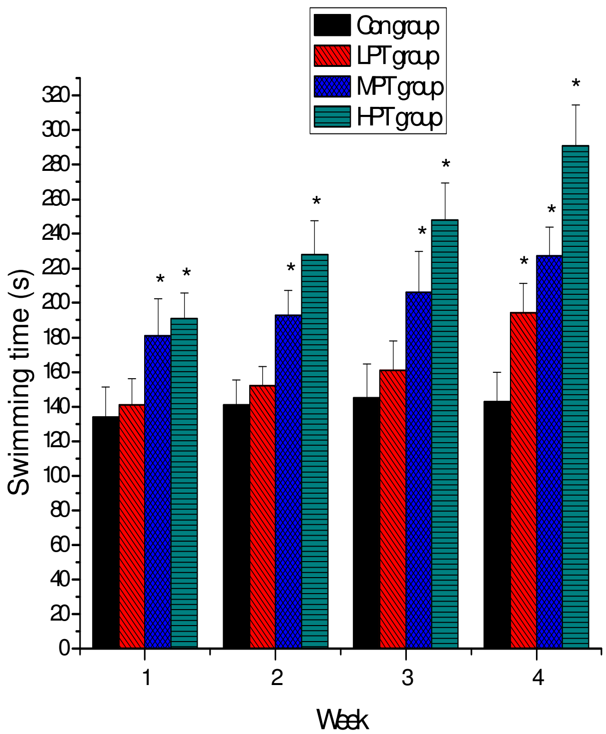

Figure 1. Effect of *P. major L.* seeds extract on forced swimming capacity in mice. Con group was received drinking water; LPT, MPT and HPT groups were received PE of 30, 60 and 120 mg/kg body weight (n= 12 mice per group). *P＜0.05 vs. Con group.

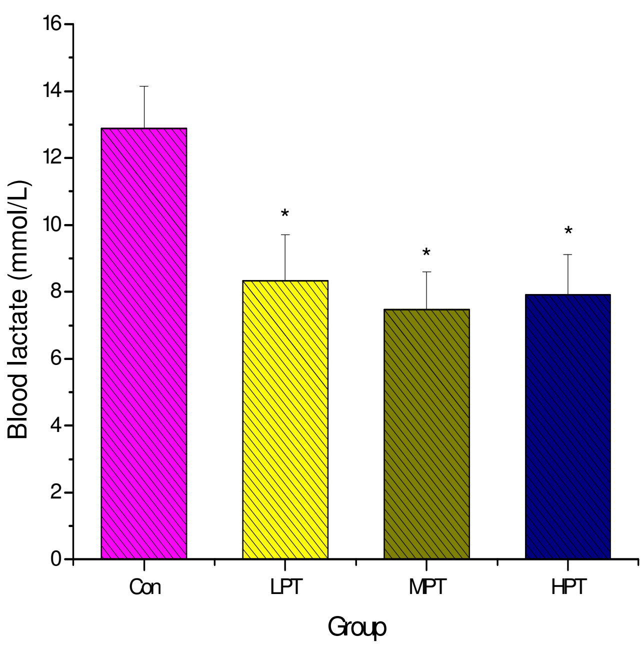

Figure 2. Effect of *P. major L.* seeds extract on blood lactate in mice. Con group was received drinking water; LPT, MPT and HPT groups were received PE of 30, 60 and 120 mg/kg body weight (n= 12 mice per group). *P < 0.05 vs. con group.

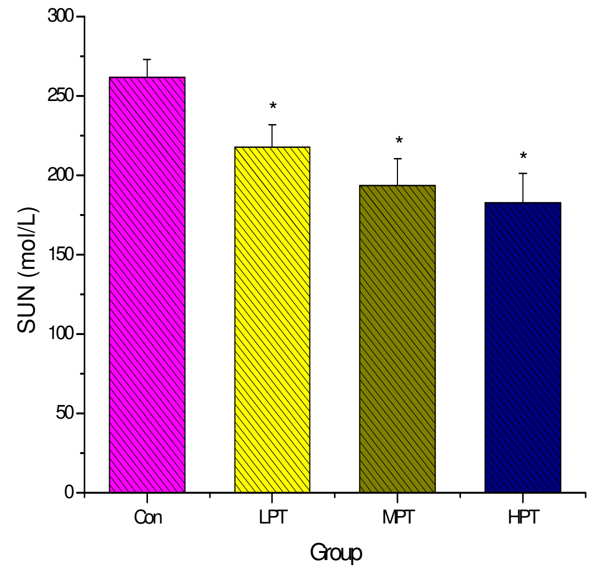

Figure 3. Effect of *P. major L.* seeds extract on serum urea nitrogen in mice.Con group was received drinking water; LPT, MPT and HPT groups were received PE of 30, 60 and 120 mg/kg body weight (n= 12 mice per group). *P < 0.05 vs. Con group.

lactate during exercise and postpone the appearance of fatigue.

Effect of *P. major* L. seeds extract on serum urea nitrogen in mice

Urea is formed in the liver as the end product of protein-metabolism. During digestion, protein is broken down into amino acids. Amino acids contain nitrogen, which is removed as NH_4^+ (an ammonium ion), while the rest of the molecule is used to produce energy or other substances needed by the cell (Ozcelik et al., 2003; An et al., 2006). There is a positive correlation between the urea nitrogen *in vivo* and the exercise tolerance (Komiyama et al., 1997; Zhang et al., 2006; Koo et al., 2008). Therefore SUN is an important blood biochemical parameter related to fatigue. As shown in Figure 3, content of SUN of PE treatment groups were significantly lower than that of control group (P < 0.05). These results indicate that PE reduced the decomposition of nitrogenous substances in the body and improved endurance capacity during exercise.

Effect of *P. major* L. seeds extract on tissue glycogen in mice

It is well known that energy for exercise is derived initially from the breakdown of glycogen. Glycogen-sparing effect provides an important survival advantage for improving

Table 1. Effect of P. *major L*. seeds extract on tissue glycogen in mice.

Group	Glycogen (mg/g)	
	Liver	**Muscle**
Con	7.34 ± 1.28	1.26 ± 0.65
LPT	12.21 ± 1.74*	1.91 ± 0.52*
MPT	14.89 ± 1.37*	2.36 ± 0.87*
HPT	15.21 ± 1.49*	2.49 ± 0.77*

Con group was received drinking water; LPT, MPT and HPT groups were received PE of 30, 60 and 120 mg/kg body weight (n= 12 mice per group). *P < 0.05 vs. Con group.

endurance performance time, because glycogen depletion is associated with physical exhaustion and slower utilization of glycogen results in improved endurance capacity (Holloszy et al., 1998; Oh and Ohta, 2003). As shown in Table 1, content of liver glycogen and muscle glycogen of PE treatment groups were significantly higher than that of control group ($P < 0.05$).

These results showed that PE may increase the glycogen content of mice post exercise by improving glycogen reserve and glycogen-sparing effect.

Conclusion

In summary, the data from this study showed that PE prolongs the swimming time in mice as well as increase the tissue glycogen, but decrease the serum urea nitrogen and blood lactate. Therefore, the results suggest that PE improved endurance exercise capacity and possessed anti-fatigue effect. PE may be useful for the development of physical strength. However, this suggestion, based on our preliminary results, should be confirmed by further experimental studies.

REFERENCES

Akova B, Sürmen-Gür E, Gür H, Dirican M, Sarandöl E, Küçükoglu S (2001). Exercise-induced oxidative stress and muscle performance in healthy women: role of vitamin E supplementation and endogenous oestradiol, Eur. J. Appl. Physiol., 84(1-2): 141-147.

Allen DG, Lamb GD, Westerblad H (2008). Skeletal muscle fatigue: cellular mechanisms, Physiol. Rev., 88(1): 287-332.

An HJ, Choi HM, Park HS, Han JG, Lee EH, Park YS, Um JY, Hong SH, Kim HM (2006). Oral administration of hot water extracts of Chlorella vulgaris increases physical stamina in mice. Ann. Nutr. Metab., 50(4): 380-386.

Aziz SA, See TL, Khuay LY, Osman K, Bakar MAA (2005). *In Vitro* Effects of *Plantago major.* Extract on Urolithiasis, Malaysian J. Med. Sci., 12 (2): 22-26.

Bahrke MS, Morgan WP (1994). Evalution of the ergogenic properties of Ginseng. Sports Med., 18: 229-248.

Bigland-Ritchie B, Woods JJ (1984). Changes in muscle contractile properties and neural control during human muscular fatigue, Muscle Nerve, 7: 691-699.

Chen X, Wang Z, Ling R, Hong X (1999). Effects of Altitude on Antioxidative System in Leaves and Roots of *Plantago major*, Acta Bot. Sin., (8): 87 - 89.

Chiang LC, Chiang W, Chang MY, Ng LT, Lin CC (2002). Antiviral activity of *Plantago major* extracts and related compounds in vitro, Antiviral Res., 55(1): 53-62.

Edwards RH, Hill DK, Jones DA, Merton PA (1977). Fatigue of long duration in human skeletal muscle after exercise. J. Physiol. 272: 769-778.

França F, Lago EL, Marsden PD (1996). Plants used in the treatment of leishmanial ulcers due to Leishmania (Viannia) braziliensis in an endemic area of Bahia, Brazil. Rev. Soc. Bras. Med. Trop., 29(3): 229-232.

Gandevia SC (2001). Spinal and supraspinal factors in human muscle fatigue. Physiol. Rev., 81: 1725-1789.

Gomez-Flores R, Calderon CL, Scheibel LW, Tamez-Guerra P, Rodriguez-Padilla C, Tamez-Guerra R, Weber RJ (2000). Immunoenhancing properties of *Plantago major* leaf extract, Phytother. Res., 14(8): 617-622.

Holloszy JO, Kohrt WM, Hansen PA (1998). The regulation of carbohydrate and fat metabolism during and after exercise. Front. Biosci., 3: D1011-27.

Hong JL, Kwong K, Lee LY (1997). Stimulation of pulmonary C fibres by lactic acid in rats: contributions of H^+ and lactate ions. J. Physiol., 500: 319-329.

Ikeuchi M, Koyama T, Takahashi J, Yazawa K (2006). Effects of astaxanthin supplementation on exercise-induced fatigue in mice, Biol. Pharm. Bull., 29(10): 2106-2110.

Kim KM, Yu KW, Kang DH, Koh JH, Hong BS, Suh HJ (2001). Anti-stress and anti-fatigue effects of fermented rice bran, Biosci. Biotechnol. Biochem., 65(10): 2294-2296.

Kim KM, Yu KW, Kang DH, Suh HJ (2002). Anti-stress and anti-fatigue effect of fermented rice bran, Phytother. Res., 6(7): 700-702.

Komiyama Y, Kimura Y, Nishimura N, Hara K, Mori T, Okuda K, Munakata M, Masuda M, Murakami T, Takahashi H (1997). Vasodepressor effects of exercise are accompanied by reduced circulating ouabainlike immunoreactivity and normalization of nitric oxide synthesi. Clin. Exp. Hypertens., 19(3): 363-372.

Koo HN, Lee JK, Hong SH, Kim HM (2004). Herbkines increases physical stamina in mice, Biol. Pharm. Bull., 27(1):117-119.

Koo HN, Um JY, Kim HM, Lee EH, Sung HJ, Kim IK, Jeong HJ, Hong SH (2008). Effect of pilopool on forced swimming test in mice. Int. J. Neurosci., 118(3):365-374.

Kramer K, Dijkstra H, Bast A (1993). Control of physical exercise of rats in a swimming basin. Physiol. Behav., 53: 271-276.

Li B, Li L, Zhang L, Cheng T, Li Z, Liao F, Li W (1999). Effect of bushenjiannao 1 decoction on learning and memory functions and anti-fatigue ability in mice, Zhongguo Zhong Yao Za Zhi, 24(2):104-106.

Li H, Zhang ML, Zhang ZF, Ou XM, Zhou Y (2007). Experiment on acute toxicity reaction of Daye Cheqianzi Capsules in mice, J. Gansu Coll. Tradit. Chinese Med., 24(3): 18- 19.

Lu JR, He TR, Putheti R (2009). Compounds of Purslane Extracts and Effects of Anti-kinetic Fatigue, J. Med. Plant Res., 3(7): 506-510.

McCullagh KJA, Poole RC, Halestrap AP, O'Brien M, Bonen A (1996). Role of lactate transporter (MCT) in skeletal muscles, Am. J. Physiol., 271:E143-E150.

Nielsen OB, de Paoli F, Overgaard K (2001). Protective effects of lactic acid on force production in rat skeletal muscle. J. Physiol., 536(1): 161-166.

Noor H, Juing M, Chee B, Kueh BL, Othman Z (2000). Medicinal Properties of *Plantago major*: Hypoglycaemic and Male Fertility Studies, Trap Agric. Sci. 23(1): 29-35.

Nunez Guillen ME, Da Silva Emim JA, Souccar C, Lapa AJ (1997). Analgesic and antiinflammatory activities of the aqueous extract of *Plantago major L*, Int. J .Pharmacog., 35 (2): 99-104.

Oh TW, Ohta F (2003). Dose-dependent effect of capsaicin on endurance capacity in rats, Br. J. Nutr., 90(3):515-520.

Ozaslan M, Karagoz DI, Kili IH, Cengiz B, Kalender ME, Güldür ME, Karagoz A, Zümrütdal ME (2009). Effect of *Plantago major* sap on Ehrlich ascites tumours in mice, Afr. J. Biotechnol., 8 (6): 955–959.

Ozcelik D, Ozaras R, Gurel Z, Uzun H, Aydin S (2003). Copper-mediated oxidative stress in rat liver. Biol Trace Elem Res., 96(1-3): 209-215.

Oztürk N, Başer KH, Aydin S, Oztürk Y, Caliş I (2002). Effects of Gentiana lutea ssp. symphyandra on the central nervous system in mice, Phytother. Res. 16(7): 627-631.

Reng YJ, Zhou H, Yang YR, Deng XH (2009). Research Situation of Plantain. J. Anhui Agric. Sci., (18): 8467 - 8469.

Samuelsen AB (2000). The traditional uses, chemical constituents and biological activities of *Plantago major L.* A review. J. Ethnopharmacol., 71(1-2): 1-21.

Sharifa AA, Neoh YL, Iswadi MI, Khairul O, Abdul Halim M, Jamaludin M, Mohamed Azman AB, Hing H (2008). Effects of methanol, ethanol and aqueous extract of *Plantago major* on gram positive bacteria, gram negative bacteria and yeas. Ann. Microsc., (8): 42-44.

StanisavljeviĆ IT, StojiČeviĆ SS, VeliČkovi DT, LaziĆ ML, VeljkoviĆ VB (2008). Screening the Antioxidant and Antimicrobial Properties of the Extracts from Plantain (*Plantago major L.*) Leaves, Separation Sci. Technol., 43 (14): 3652 - 3662.

Sun ZP (2003). Microwave-assisted Extraction and Determination of Total Flavonoid in Leaves of *Plantago major L.* Primary J. Chinese Mater. Med., 17(1): 48-49.

Veale DJ, Furman KI, Oliver DW (1992). South African traditional herbal medicines used during pregnancy and childbirth. J. Ethnopharmacol., 36(3): 185-191.

Velasco-Lezama R, Tapia-Aguilar R, Román-Ramos R, Vega-Avila E, Pérez-Gutiérrez MS (2006). Effect of *Plantago major* on cell proliferation *in vitro*. J. Ethnopharmacol., 103(1): 36-42.

Yalcin O, Bor-Kucukatay M, Senturk UK, Baskurt OK (2000). Effects of swimming exercise on red blood cell rheology in trained and untrained rats. J. Appl. Physiol., 88(6): 2074-2080.

Zhang Y, Yao X, Bao B, Zhang Y (2006). Anti-fatigue activity of a triterpenoid-rich extract from Chinese bamboo shavings (Caulis bamfusae in taeniam), Phytother. Res., 20(10): 872-876.

Zhao W, Zhang X, Wang W, Zhang L (2009). Experimental study for the anti-fatigue effect of ginseng general ginsenosides P.E. *in vivo*, Wei Sheng Yan Jiu, 38(2): 184-187.