

Full Length Research Paper

Polysaccharides from *Portulaca oleracea* (purslane) supplementation lowers acute exercise induced oxidative stress in young rats

Lou Xiaojuan^{1*}, Huang Yong², Guo Xueqi¹, Zheng Fanhui³, Jiang Zhihong⁴
and Yang Yong ping¹

¹Donghua University, Shanghai 201620, PR, China.

²Tongji University, Shanghai 200092, PR. China.

³Shanghai Research Institute of Sports Science, Shanghai 200030, PR, China.

⁴Zhejiang Sci-Tech University, Hangzhou 310018, PR, China.

Accepted 2 March, 2011

The present study was designed to determine the effects of polysaccharides from purslane (PFP) supplementation on acute exercise induced oxidative stress in young male Sprague-Dawley rats. Animals were divided randomly into four groups, that is control group (C), low-dose PFP supplemented group (LP), middle-dose PFP supplemented group (MP) and high-dose PFP supplemented group (HP). Each group contains eight animals. The mice in the control group were orally administered physiological saline of 50 ml/kg bodyweight per day for 30 days, while the PFP supplemented group received the same volume of PFP of 100, 200 and 400 mg/kg bodyweight. On the final day of the study, rats were exercised to exhaustion (22 m/min at 10% inclination on the treadmill) and then all the rats were sacrificed. Body weight, running time, blood lactate, malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPX) of rats were measured. Results of the above study showed that PFP supplementation could elevate the exercise tolerance and inhibited the production of blood lactate during acute exercise. PFP supplementation lowers exercise induced oxidative stress by means of decreasing SOD, GPX activities and MDA level in skeletal muscle of young rats.

Key words: Polysaccharides from purslane, acute exercise, oxidative stress, rats.

INTRODUCTION

Purslane (*Portulaca oleracea* L.) is listed in the World Health Organization as one of the most used medicinal plants and it has been given the term "Global Panacea" (Lim and Quah, 2007). It is a warm climate, annual, green herb, with branched and succulent stems which are decumbent near the base and ascending near the top to a height of 15 to 30 cm (Boroushaki et al., 2004). It has a cosmopolitan distribution in China, Africa, India, Australia, Middle East, Europe, and United States (Chan et al., 2000; Oran and Al-Eisawi, 1998; Mitich, 1997) and has a

long history of use as a medicinal and edible plant (Chan et al., 2000; Rasheed et al., 2004).

As a traditional Chinese medicine, it has been used for treating dysentery with bloody stools, eczema, erysipelas, and used as febrifuge and antiseptic (Hongxing et al., 2007; Dong et al., 2010). Recent pharmacological studies show that it exhibits a wide range of biological effects, including skeletal muscle relaxant effect, analgesic, anti-inflammatory, antifungal, antidiabetic, antiulcerogenic, anti-hypoxic, and antifertility effects (Karimi et al., 2004; Costa et al., 2007; Chen et al., 2009; Li et al., 2009). Purslane contains several biologically active compounds include organic acids, alkaloids, coumarins, flavonoids, cardiac glycosides and polysaccharides etc. (Rasheed et al., 2004; Yang et al., 2007). Polysaccharides from purslane (PFP) has been recently studied for their

*Corresponding author. E-mail: louxiaojuandh@yahoo.com.cn or louxiaojuandh@sina.com. Tel: +086-021-52705229 or +086-13817627178.

Table 1. The composition of the basal diet.

Ingredient	Content (%)	Ingredient	Content (%)
Corn starch	20	Bone meal	3
Bran	19	Yeast powder	2.3
Rice	16	Salt	0.5
Soybean oil meal	20	Vitamin mix	0.1
Calcium flour	3	Microelement	0.1
Fish flour	16	—	—

physiological and pharmaceutical activities (Gong et al., 2009; Li et al., 2010).

Oxidative stress occurs when the production of reactive oxygen species (ROS), often referred to as “free radicals”, exceeds antioxidant defense (Sies, 1997). The antioxidant system is comprised of both endogenous (within the body) and exogenous (outside of the body) defense mechanisms. Oxidative stress may progress to oxidative damage involving cellular proteins (contractile, structural, and enzymatic), lipids, DNA, and other molecules in ways that might lead to abnormal cellular function (Therond, 2006; Kulbacka et al., 2009). With excessive oxidative damage, the onset of poor health and a variety of diseases exists (Dhalla et al., 2000). Prior studies have indicated that acute exercise increases oxidative stress, especially when the exercise intensity is high (Urso and Clarkson, 2003; Vincent et al., 2006; Youssef et al., 2009). Two mechanisms linking acute exercise and oxidative stress are increased pro-oxidant activity through a mass action effect when VO_2 is elevated 10 to 15 fold above rest, and inadequate antioxidant activity relative to pro-oxidants (Belvirani and Gökbel, 2006). Therefore to avoid or minimize deleterious affects of exercise induced oxidative stress the antioxidant capacity of the cell must be increased. This increased capacity may be achieved through appropriate training, diet and antioxidant supplementation.

In recent years, many studies have shown that PFP exhibit a strong antioxidant activity (Wang and Yang, 2008; YouGuo et al., 2009; Li et al., 2010). However, the role of PFP in exhaustive exercise-induced oxidative stress in human subjects or animal studies has not been investigated so far. Therefore, the present study has been undertaken with an animal model to investigate the effects of PFP supplementation on exercise-induced oxidative stress in young rats.

MATERIALS AND METHODS

Plant materials

Aerial parts of purslane (*P. oleracea* L. cv. “Kuanye”) were collected in its fresh state at a local farm in August in Shanghai city and identified by Botany Institute. A voucher specimen of the plant materials (voucher No. KPT-762) was deposited in herbarium of onghua University. Fresh purslane was washed with distilled water, and dried with air. The dried purslane was ground to a fine

powder using a grinder (IKAM 20, IKA, Staufen, Germany).

Preparation of polysaccharides from purslane

As described previously (Li et al., 2009; Gong et al., 2009; YouGuo et al., 2009), purslane powder was extracted in a soxhlet apparatus with a mixture of chloroform-methanol (2:1, 75°C), and pretreated with 80% ether twice to remove some coloured materials, oligosaccharides, and some small molecule materials. The organic solvent was volatilized and pretreated dry powder was obtained. The pretreated dry powder (500 g) was extracted with boiling water (4000 ml) for 3 h twice. The aqueous extracts combined and centrifuged (2000 g, 20 min), then the supernatant was separated from insoluble residue with nylon cloth (pore diameter: 38 µm). The aqueous extracts were then defatted by the method of Sevag et al. (1938), precipitated by the addition of ethanol to a final concentration of 80% (v/v) and the precipitates were collected by centrifugation (2000 g, 20 min). It was then solubilized in deionized water and lyophilized to get PFP.

Animals and exercise

All experimental procedures involving animals received the approval from the Animal Care and Use Committee of the Donghua University (Shanghai, China). Guidelines and Policy on using and caring of the laboratory animals were followed at all time. Male Sprague-Dawley rats (8 week-old, 260~280 g) were purchased from the Shanghai Slack Laboratory Animal Co., Ltd. (Shanghai, China). Animals were housed in a ventilated room under a 12/12 h light/dark cycle at 24±2°C and had free access to water and basal diet. The composition of the basal diet was showed in Table 1.

All animals, at the beginning of the experiments, were divided randomly into four groups, such as control group (C), low-dose PFP supplemented group (LP), middle-dose PFP supplemented group (MP) and high-dose PFP supplemented group (HP). Each group contains eight animals. The mice in the control group were orally administered physiological saline of 50 ml/kg bodyweight per day for 30 days, while the PFP supplemented group received the same volume of PFP of 100, 200 and 400 mg/kg bodyweight. The dose of 100, 200 and 400 mg/kg was chosen based on estimates from prior studies.

On the final day of the study, all the rats were exercised vigorously in one session until they were exhausted. The exercise protocol was 22 m/min at 10% inclination on a treadmill (FHN-361 Treadmill, Huian Instrument, Chengdu, China) (Chen et al., 2002; Myers, 2003). A rat was considered exhausted when it would not be prompted to run any more. The rats would stop running and when overturned would continue to lie on their backs disregarding gentle prods and electric shock (Chen et al., 2002; Huang et al., 2009). The running time of each rat was recorded. To eliminate diurnal effects, the experiments were performed at the same time of day (08:00~11:00).

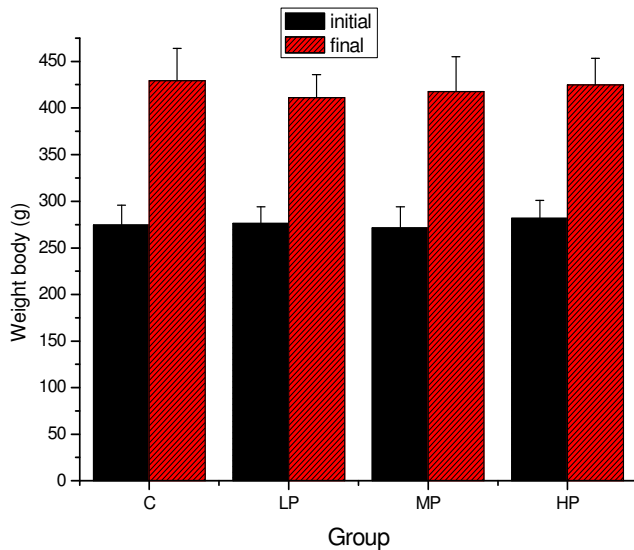


Figure 1. Effect of PFP supplementation on body weight of rats. Values are means \pm SD, Each group contains eight rats.

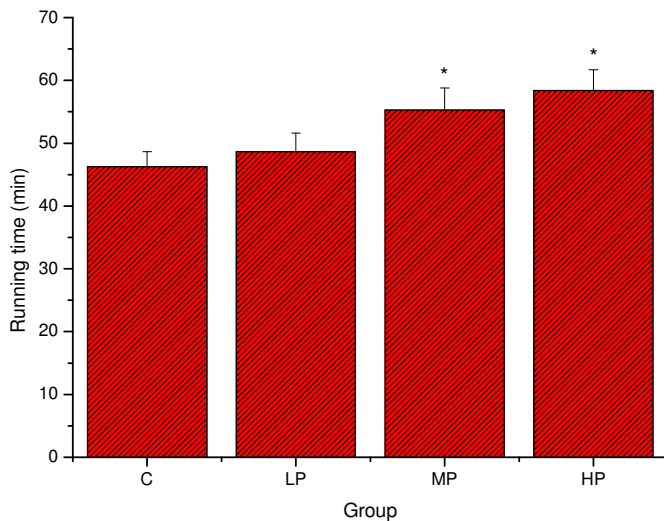


Figure 2. Effect of PFP supplementation on running ability of rats. Values are means \pm SD, Each group contains eight rats. * $P > 0.05$ when compared to the control group.

Analysis of biochemical parameters

Immediately after acute exhaustive exercise, all the rats were sacrificed by decapitation. Heparinised blood samples were collected from the abdominal aorta for the determination of lactate, and skeletal muscle tissue was carefully removed, rinsed in ice-cold normal saline, blotted dry and stored at -80°C for MDA and anti-oxidant enzymes analysis.

Blood lactate, MDA and anti-oxidant enzymes (SOD and GPx) was tested according to the recommended procedures provided by the kits purchased from Nanjing Jiancheng Bioengineering Institute (Jiangsu, China).

Statistical analysis

All data were expressed as means \pm SD of three replications and subjected to the analysis of variance and tested for significant differences by Duncan's multiple range tests (SAS Institute, Cary, NC). A p value < 0.05 was considered to be significant.

RESULTS AND DISCUSSION

Effect of PFP supplementation on body weight of rats

As shown in Figure 1, significant difference were not observed in initial and final body weights between different groups ($P > 0.05$), which means the PFP supplementation did not affect body weight.

Effect of PFP supplementation on running ability of rats

As shown in Figure 2, the average running times of rats in HP and MP groups were significantly prolonged compared to the control group ($P < 0.05$), and increased by 26.1 and 19.4%, respectively. However, average running time of rats in LP group showed no significant changes compared to the control group. The results showed that PFP supplementation could elevate the exercise tolerance.

Effect of PFP supplementation on blood lactate of rats

Prior research has indicated that lactate is the metabolic byproduct of excess pyruvate without available oxygen to break it down. When pyruvate is not utilized in the mitochondria as a source of energy production through the krebs cycle, which requires an aerobic environment, lactate will form from the excess pyruvate. In both humans and a large number of animals, strenuous exercise is associated with accumulation of lactate. Blood lactate represents the degree of fatigue after exercise and the condition of recovery (Korzeniewski and Liguzinski, 2004; Wang et al., 2006). As shown in Figure 3, after acute exhaustive exercise, blood lactate concentration of rats in LP, MP and HP groups were significantly decreased compared to the control group ($P < 0.05$), and decreased by 28.2, 33.6 and 38.2%, respectively. The results showed that PFP supplementation inhibited the production of blood lactate during acute exercise.

Effect of PFP supplementation on MDA of rats

Evidence has shown that pathological acute exercise lead to an excessive production of ROS which in turn, promotes severe oxidative damages (Powers and

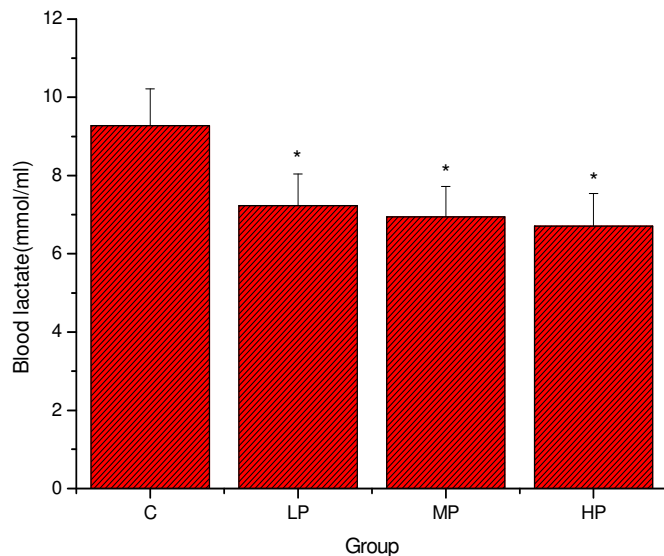


Figure 3. Effect of PFP supplementation on blood lactate of rats. Values are means \pm SD, Each group contains eight rats. * $P < 0.05$ when compared to the control group.

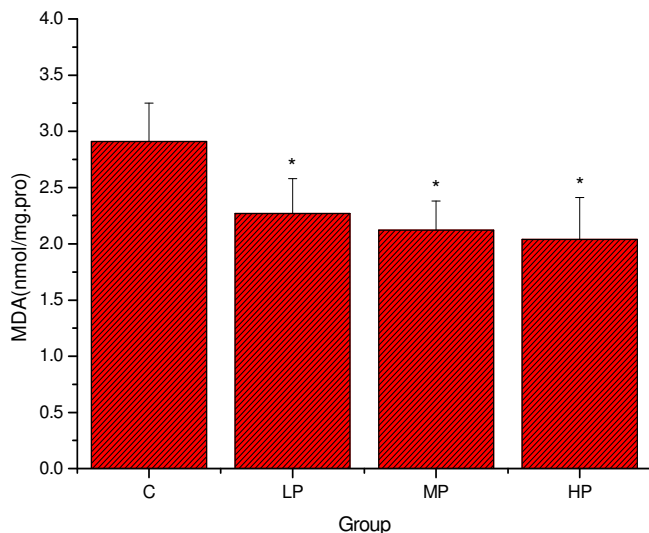


Figure 4. Effect of PFP supplementation on MDA of rats skeletal muscle. Values are means \pm SD, Each group contains eight rats. * $P < 0.05$ when compared to the control group.

Lennon, 1999; Cepinskas et al., 2002). The extent of oxidative damage is commonly measured through quantitative assessment of lipid peroxidation products such as malondialdehyde (MDA) (Bloomer and Goldfarb, 2004; Fisher-Wellman and Bloomer, 2009). As shown in Figure 4, MDA levels in skeletal muscle of rats in LP, MP and HP groups were significantly decreased compared to the control group ($P < 0.05$), and decreased by 28.1, 37.2 and 42.6%, respectively. The results showed that PFP supplementation protected skeletal muscles from ROS-mediated oxidative damage during acute exercise.

Table 2. Effect of PFP supplementation on anti-oxidant enzymes of rats skeletal muscle.

Group	SOD (U/mg.pro)	GPX(U/mg.pro)
C	15.31 \pm 2.34	0.039 \pm 0.005
LP	11.84 \pm 2.89*	0.021 \pm 0.011*
MP	11.12 \pm 2.17*	0.017 \pm 0.008*
HP	10.64 \pm 1.84*	0.018 \pm 0.009*

Values are means \pm SD, Each group contains eight rats. * $P > 0.05$ when compared to the control group.

Effect of PFP supplementation on anti-oxidant enzymes of rats

It is well known that SOD and GPX are regarded as the first line of defense by the antioxidant enzyme system against ROS generated during exhaustive exercise (Gago-Dominguez et al., 2007; Huang et al., 2009). As shown in Table 2, SOD and GPX levels in skeletal muscle of rats in LP, MP and HP groups were significantly decreased compared to the control group ($P > 0.05$).

It has been indicated that oxidative stress induced by acute exercise can elevate tissue antioxidant enzyme activities because physical exercise promotes the production of ROS due to a substantial increase in oxygen consumption. And increasing the circulating levels of certain antioxidants will help to down-regulate antioxidant enzymes to protect against oxidative stress-induced injury during acute exercise. (Ji, 1995; Somani et al., 1995; Ayres et al., 1998; Childs et al., 2001; Kirschvink et al., 2008). Prior studies have indicated that PFP exhibits a strong antioxidant activity, and our results showed that supplementation with PFP decreased SOD and GPX levels during acute exercise. This is probably due to the per se antioxidant activity of PFP. Thus, the current results indicated that PFP supplementation had beneficial effects on attenuating the oxidative stress induced by acute exercise.

Conclusions

In conclusion, the present results suggest that PFP supplementation could elevate the exercise tolerance and inhibited the production of blood lactate during acute exercise. PFP supplementation lowers exercise induced oxidative stress by means of decreasing SOD, GPX activities and MDA level in skeletal muscle of young rats.

REFERENCES

- Ayres S, Abplanalp W, Liu JH, Subbiah MT (1998). Mechanisms involved in the protective effect of estradiol-17beta on lipid peroxidation and DNA damage. *Am. J. Physiol.*, 274(6 Pt 1): E1002-1008.
- Belvirani M, Gökbel H (2006). Acute exercise induced oxidative stress and antioxidant change. *Eur. J. General Med.*, 3(3): 126-131.

- Bloomer RJ, Goldfarb AH (2004). Anaerobic exercise and oxidative stress: a review. *Can. J. Appl. Physiol.*, 29(3): 245-263.
- Borouhaki MT, Boskabady MH, Malek F (2004). Antitussive effect of *Portulaca oleracea* L. in guinea pigs. *Iranian J. Pharm. Res.*, 3: 187-190.
- Cepinskas G, Rui T, Kvietys PR (2002). Interaction between reactive oxygen metabolites and nitric oxide in oxidant tolerance. *Free Radic. Biol. Med.*, 33(4): 433-440.
- Chan K, Islam MW, Kamil M, Radhakrishnan R, Zakaria MNM, Habibullah M, Attas A (2000). The analgesic and anti-inflammatory effects of *Portulaca oleracea* L. subsp. *sativa* (Haw.) Celak. *J. Ethnopharm.*, 73(3): 445-451.
- Chen CJ, Wang WY, Wang XL, Dong LW, Yue YT, Xin HL, Ling CQ, Li M (2009). Anti-hypoxic activity of the ethanol extract from *Portulaca oleracea* in mice. *J. Ethnopharm.*, 124(2): 246-250.
- Chen CY, Holtzman GI, Bakhit RM (2002). High-Genistin Isoflavone Supplementation Modulated Erythrocyte Antioxidant Enzymes and Increased Running Endurance in Rats Undergoing One Session of Exhausting Exercise – A pilot study. *Pakistan J. Nutr.*, 1(1): 1-7.
- Childs A, Jacobs C, Kaminski T, Halliwell B, Leeuwenburgh C (2001). Supplementation with vitamin C and N-acetyl-cysteine increases oxidative stress in humans after an acute muscle injury induced by eccentric exercise. *Free Radic. Biol. Med.*, 31(6): 745-753.
- Costa JF, Kiperstok AC, David JP, David JM, Giulietti AM, de Queiroz LP, dos Santos RR, Soares MB (2007). Anti-leishmanial and immunomodulatory activities of extracts from *Portulaca hirsutissima* and *Portulaca werdermannii*. *Fitoterapia*, 78(7-8): 510-514.
- Dhalla NS, Temsha RM, Netticadan T (2000). Role of oxidative stress in cardiovascular diseases. *J. Hypertens*, 18: 655-673.
- Dong CX, Hayashi K, Lee JB, Hayashi T. (2010). Characterization of structures and antiviral effects of polysaccharides from *Portulaca oleracea* L. *Chem. Pharm. Bull. (Tokyo)*, 58(4): 507-510.
- Fisher-Wellman K, Bloomer RJ (2009). Acute exercise and oxidative stress: a 30 year history. *Dyn. Med.*, 8: 1.
- Gago-Dominguez M, Jiang X, Esteban Castela J (2007). Lipid peroxidation and the protective effect of physical exercise on breast cancer. *Med. Hypotheses*, 68(5): 1138-1143.
- Gong F, Li F, Zhang L, Li J, Zhang Z, Wang G (2009). Hypoglycemic effects of crude polysaccharide from purslane. *Int. J. Mol. Sci.*, 10(3): 880-888.
- Hongxing Z, Nancai Y, Guofu H, Jianbo S, Yanxia W, Hanju H, Qian L, Wei M, Yandong Y, Hao H (2007). Neuroprotective effects of purslane herb aqueous extracts against D-galactose induced neurotoxicity. *Chem. Biol. Interact*, 170(3): 145-152.
- Huang CC, Lin TJ, Lu YF, Chen CC, Huang CY, Lin WT (2009). Protective effects of L-arginine supplementation against exhaustive exercise-induced oxidative stress in young rat tissues. *Chin. J. Physiol.*, 52(5): 306-315.
- Ji LL (1995). Exercise and oxidative stress: role of the cellular antioxidant systems. *Exerc. Sport Sci. Rev.*, 23: 135-166.
- Karimi G, Hosseinzadeh H, Ettehad N (2004). Evaluation of the gastric antiulcerogenic effects of *Portulaca oleracea* L. extracts in mice. *Phytother. Res.*, 18(6): 484-487.
- Kirschvink N, de Moffarts B, Lekeux P. (2002). The oxidant/antioxidant equilibrium in horses. *Vet. J.*, 177(2): 178-191.
- Korzeniewski B, Liguzinski P (2004). Theoretical studies on the regulation of anaerobic glycolysis and its influence on oxidative phosphorylation in skeletal muscle. *Biophys. Chem.*, 110(1-2): 147-169.
- Kulbacka J, Saczko J, Chwilkowska A (2009). Oxidative stress in cells damage processes. *Pol. Merkur. Lekarski*, 27(157): 44-47.
- Li FL, Li QW, Gao DW, Peng Y, Feng CN (2009). Preparation and antidiabetic activity of polysaccharide from *Portulaca oleracea* L. *Afr. J. Biotechnol.*, 8 (4): 569-573.
- Li X, Feng Lei, Hu ZL, Xu CS, Zhao YF (2010). Antioxidant activities of polysaccharide from *Portulaca Oleracea* L. *Chin. J. Biochem. Pharm.*, 31(4): 123-125.
- Lim YY, Quah EPL (2007). Antioxidant properties of different cultivars of *Portulaca oleracea*. *Food Chem.*, 103(3): 734-740.
- Powers SK, Lennon SL (1999). Analysis of cellular responses to free radicals: focus on exercise and skeletal muscle. *Proc. Nutr. Soc.*, 58(4): 1025-1033.
- Rasheed AN, Afifi FU, Shaedah M, Taha MO (2004). Investigation of the active constituents of *Portulaca oleracea* L. (Portulacaceae) growing in Jordan. *Pak. J. Pharm. Sci.*, 17(1): 37-45.
- Sevag MG, Lackman DB, Smolens J (1938). The isolation of components of streptococcal nucleoproteins in serologically active form. *J. Biol. Chem.*, 124: 425.
- Sies H (1997). Oxidative stress: oxidants and antioxidants. *Exp. Physiol.*, 82: 291-295.
- Somani SM, Frank S, Rybak LP (1995). Responses of antioxidant system to acute and trained exercise in rat heart subcellular fractions. *Pharmacol. Biochem. Behav.*, 51: 627-634.
- Therond P (2006). Oxidative stress and damages to biomolecules (lipids, proteins, DNA). *Ann. Pharm. Fr.*, 64(6): 383-389.
- Urso ML, Clarkson PM (2003). Oxidative stress, exercise, and antioxidant supplementation. *Toxicol.*, 14(12): 189(1-2): 41-54.
- Vincent HK, Bourguignon CM, Vincent KR, Weltman AL, Bryant M, Taylor AG (2006). Antioxidant supplementation lowers exercise-induced oxidative stress in young overweight adults. *Obesity (Silver Spring)*, 14(12): 2224-2235.
- Wang JJ, Shieh MJ, Kuo SL, Lee CL, Pan TM (2006). Effect of red mold rice on antifatigue and exercise-related changes in lipid peroxidation in endurance exercise. *Appl. Microbiol. Biotechnol.*, 70(2): 247-253.
- Wang JT, Yang MY. (2008). Research on Chemical Purification and Physiological Activity of Polysaccharide from *Portulaca oleracea* L. *J. Chongqing Inst. Technol.*, (1): 43-47.
- Yang ZJ, Zheng YN, Xiang L (2007). Study on chemical constituents of *Portulaca oleracea*. *Zhong Yao Cai*, 30(10): 1248-1250.
- YouGuo C, Zongji S, XiaoPing C (2009). Evaluation of free radicals scavenging and immunity-modulatory activities of Purslane polysaccharides. *Int. J. Biol. Macromol.*, 45(5): 448-452.
- Youssef H, Groussard C, Pincemail J, Moussa E, Jacob C, Lemoine S, Zind M, Defraigne JO, Cillard J, Delamarche P, Gratas-Delamarche A (2009). Exercise-induced oxidative stress in overweight adolescent girls: roles of basal insulin resistance and inflammation and oxygen overconsumption. *Int. J. Obes. (Lond)*, 33(4): 447-455.
- Myers J (2003). A treadmill ramp protocol using simultaneous changes in speed and grade - a (ramp) step forward in exercise testing. *Med. Sci. Sports Exerc.*, 35(9): 1604-1605.