Journal of Medicinal Plants Research Vol. 6(9), pp. 1605-1611, 9 March, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1254
ISSN 1996-0875 ©2012 Academic Journals

*Full Length Research Paper*

# Protective effect of *laminarin* polysaccharides on oxidative stress caused by exhaustive exercise

**Daye Cheng[1]* Bin Liang[2] and Yunhui Li[3]**

[1]Department of Transfusion, First Hospital of China Medical University, Shenyang 110001, P.R. China.
[2]High Vocational Technological College, China Medical University, Shenyang 110001, P.R. China.
[3]Department of Clinical Laboratory, No 202 Hospital of Shenyang, Shenyang 110003, P.R. China.

Accepted 27 October, 2011

**The aim of this study was to investigate the effect of *laminarin* polysaccharides (LP) on exhaustive exercise-induced oxidative stress in rats. A total of thirty-two rats were randomly divided into the following four groups: Sedentary control group (C), exhaustive exercise group (E), LP treatment group (L) and exercise group subjected to exhaustive exercise and LP treatment (EL). Rats in the L and EL group received supplemental LP diet (300 mg/Kg body weight) for 30 consecutive days, and rats in C and E groups received the same volume of 0.9% NaCl for the same days as rats in the L and EL group. Rats in the E and EL group underwent 30 day exhaustive exercise experiments on a treadmill. Blood and skeletal muscle were collected. The biochemical markers in serum were determined and the level of lipid peroxidation and antioxidants in muscle were measured. Exhaustive exercise caused elevations of biochemical markers and lipid peroxidation (*P*<0.05) and decreases of antioxidants (*P*<0.05). The presence of LP with exhaustive exercise significantly decreases the blood biochemical markers and lipid peroxidation (*P<0.05*) and significantly increased the levels of the antioxidants in muscle (*P*<0.05). Our results indicate that LP could be a potent protective agent against exhaustive exercise-induced biochemical alteration and oxidative stress in rats.**

**Key words:** Antioxidant activity, polysaccharides, animal models, exercise.

## INTRODUCTION

Moderate physical activity is widely considered an essential component of a healthy lifestyle. Human cells are known to be either positively or negatively responding to exercise, depending on intensity and/or volume (Aoi et al., 2003; Ji et al., 2004; Nikolaidis et al., 2008). In contrast to moderate exercise, exhaustive exercise is believed to be linked to increased levels of reactive oxygen species (ROS), exceeding the capacity of antioxidant defenses, evidence from impairment of membrane lipids, proteins and deoxyribonucleic acid (DNA) molecular and function damage (Aoi et al., 2004; Koyama et al., 1999; Tappel, 1973). Oxidative stress represents an imbalance between the production and manifestation of ROS and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. While it is well accepted that a low level of ROS production is absolutely necessary to maintain normal physiological function (Droge, 2002), as well as to allow exercise-induced adaptations to the endogenous antioxidant defense system (Ji et al., 2006; Radak et al., 2008), excessive ROS production may lead to the oxidation of lipids, proteins and nucleic acids, which may ultimately impair normal cellular function (Dalle-Donne et al., 2006). Oxidative stress plays an important role in pathophysiology of many diseases such as cancer, diabetes and cardiovascular disease. Therefore, many investigators and clinicians attempt to

*Corresponding author. E-mail: dayecheng_cmu@yahoo.cn. Tel: +862483283040.

**Abbreviations: ROS,** Reactive oxygen species; **DNA,** deoxyribonucleic acid; **DEAE,** diethylaminoethyl; **DPPH,** 1,1 - diphenyl-2-picrylhydrazyl; **SOD,** superoxide dismutase; **LDH,** lactate dehydrogenase; **CK,** creatine kinase; **GPx,** glutathione-dependent peroxidase; **CAT,** catalase; **GSH,** glutathione; **MDA,** malondialdehyde.

minimize oxidative stress levels, often done with the use of supplemental antioxidant nutrient intake. Nowadays, supplemental antioxidant use is a popular practice, consuming some sorts of antioxidant supplement. While many popular antioxidants have been studied primarily within animal models or *in vitro* systems, it is unknown what the optimal antioxidant(s) is for human supplementation. *Laminarin* is common seafood in China and many other countries and is documented as a drug in traditional Chinese medicine for over a thousand years. In recent years, polysaccharides isolated from seaweeds have been demonstrated to be potential ROS scavengers by protecting polyunsaturated fatty acid in cell membranes against lipid peroxidation. There is evidence that sulfated polysaccharide fractions extracted from *Laminaria japonica* possessed considerable antioxidant activity (Wang et al., 2008). Our preliminary study had shown that *Laminaria* polysaccharides (LP) appeared to be more effective in reducing sepsis-induced oxidative stress and lipid peroxidation in rats (Cheng et al., 2011). Based on the aforementioned considerations, the purpose of the present study was to investigate the effect of *l. polysaccharides* on exhaustive exercise-induced anti-oxidant capacity and oxidative biomarkers.

## MATERIALS AND METHODS

*Laminarin* was purchased from a seafood shop (Shenyang, China). All reagents used in this research were of analytical grade and obtained from Shenyang Biotechnology Company Limited.

### Extraction of polysaccharides

A total of 150.00 g *Laminarin* powder was weighted. The powder was extracted with 200 ml of 80% ethanol solution at 50°C for 2 h to remove ethanol-soluble substances. After vacuum filtration, the residues were then extracted with 200 ml of distilled water at 50°C for 2 h. After centrifuged at 8000 × g for 15 min, the residues were discarded and the supernatant was collected. The supernatant was concentrated by rotary vacuum evaporator (BC-R203, Shanghai Biochemical Equipment Company China) at 65°C and a four-fold volume of ethanol was added to precipitate polysaccharides, which was finally obtained by centrifugation. The crude polysaccharide was further purified by column chromatography. Fifty milligrams of crude polysaccharide dissolved in 10 ml of $dH_2O$ was applied to a Diethylaminoethyl (DEAE)–cellulose column (3 × 45 cm) pre-equilibrated with water and eluted in NaCl gradient (0 to 3 M) until no carbohydrate is detected. The content of polysaccharides was determined by the phenol-sulphuric acid method.

### 1,1 -Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable DPPH free radical was determined by the method described by Braca et al. (2001). LP (0.1 ml) was added to 3 ml of a 0.004% MeOH solution of DPPH. Absorbance at 517 nm was determined after 30 min and the percentage inhibition activity was calculated from $[(A_0 - A_1)/A_0] \times 100$, where $A_0$ is the absorbance of the control and $A_1$ is the absorbance of the extract/standard.

### Animal experiment

A total of 32 male Wistar rats averaging 12 weeks old were housed in a controlled environment with a 12 h light-dark cycle at 25°C and provided with rat chow and water. All experiments were done under Health Guidelines for Care and Use of Laboratory Animals and approved by the Ethics Committee of China Medical University. All rats were randomly divided into the following four groups ($n$=8 in each group): Sedentary control group (C), exhaustive exercise group (E), LP treatment group (L) and exercise group subjected to exhaustive exercise and LP treatment (EL). Rats in the L and EL group received supplemental *l. polysaccharides* diet (300 mg/kg body weight) for 30 consecutive days and rats in C and E groups received the same volume of 0.9% NaCl for the same days. Rats in the E and EL group underwent 30 day exhaustive exercise experiments on a treadmill. One week before the experiment, the rats were accustomed to treadmill running for 15 min a day, during the course of which the speed was gradually increased from 10 to 20 min. At the time of experiment, rats in the E and EL groups were placed on the treadmill at 9% slope and the speed was rapidly increased up to 24 min. Exhaustion was defined as the point at which a rat refused to run despite being given mild touches. In this study, the mean treadmill running time was 100 ± 8 min/day. Rats in the C and L group were maintained in a sedentary condition for 100 min without access to food and water.

### Blood and muscle analysis

After the rats had finished the last exercise, they were weighted and killed by decapitation. Blood samples were collected and centrifuged at 1500×g at 4°C for 10 min to obtain serum, which was stored at 80°C for further analyses. Vastus lateralis muscle of rats were removed and placed in 10% KCl (10 ml/g tissue) and homogenized on ice for 120 s with a DY89-II homogenizer (Ningbo Scientz Biotechnology Company Limited) at 600 rpm. Tissue homogenates were centrifuged at 1000×g at 4°C for 10 min to remove tissue debris and clear supernants was used for further analyses.

### Determination

The degree of muscle damage was evaluated by lactate dehydrogenase (LDH) and creatine kinase (CK) activities in serum using a commercially available kit (Abbott Company, USA) by HITACHI 7170 auto analyzer according to the manual. Determination of different oxidative stress related biochemical parameters in muscle was carried out. Superoxide dismutase (SOD) activity was determined using SOD assay kit (Cayman Chemical, USA) in accordance with the manufacture's protocol. Glutathione-dependent peroxidase (GPx) activity was determined by GPx assay kit (Nanjing Jiancheng Biology Research Institute, Nanjing). Catalase (CAT) activity was assayed by the method described by Ferro Cde et al. (2010). The non-enzymic antioxidant reduced glutathione (GSH) was analyzed by the method of Moron, Dipierre and Mannervik (Moron et al., 1979). Lipid peroxidation was evaluated in terms of malondialdehyde (MDA) production described by Shafiq-ur-Rehman et al. (1995).

### Statistical analyses

All data were expressed as mean±S.D. Statistical significance was analyzed by two-way ANOVA followed by Bonferroni's test. A value

**Table 1.** Effect of LP on body weight (BW) of rats.

| Parameter | Initial BW | Final BW |
|---|---|---|
| C group | 191.81 ± 14.27 | 275.31 ± 32.46 |
| E group | 189.02 ± 19.01 | 250.66 ± 37.03 |
| L group | 190.11 ± 20.22 | 264.11 ± 18.75 |
| EL group | 186.33 ± 15.77 | 257.48 ± 28.74 |

Values are means ± SD, C group (sedentary control group), E group (exhaustive exercise group), L group (LP) treatment group) and EL group (exercise group subjected to exhaustive exercise and LP treatment).

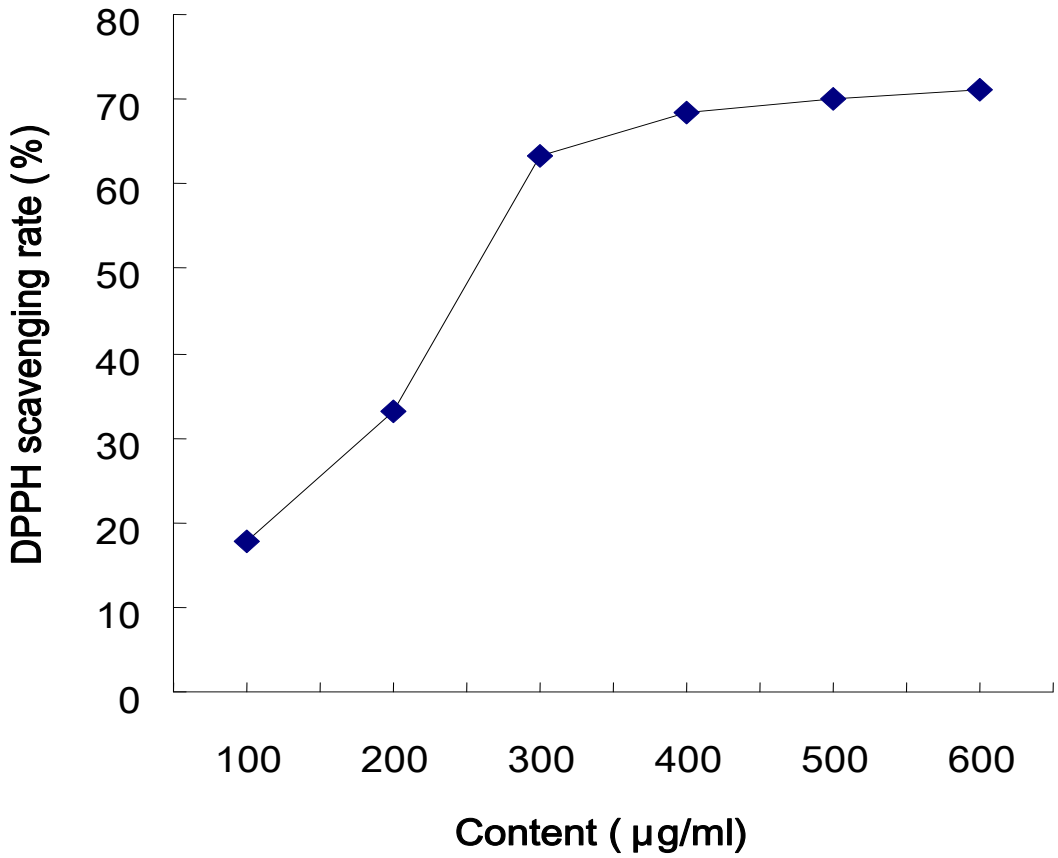


**Figure 1.** Scavenging activity of LP against 1, 1-diphenyl-2-picrylhydrazyl radical.

of $P<0.05$ was considered to be statistically significant. All statistical analyses were carried out using SPSS 15.0 software (SPSS, Chicago, IL).

## RESULTS

### Effect of LP treatment on body weight of rats

Table 1 showed the effect of *LP* treatment on body weight of rats. At the initial day of experiment, there was no significant difference in four groups ($P>0.05$). After 30 day exhaustive exercise, no significant difference in body weight was found between the groups ($P>0.05$).

### DPPH radical scavenging activity

The scavenging ability of LP on DPPH free radical was examined in the concentration range from 100 to 600 μg/ml. As shown in Figure 1, a dose-response relationship was found in the DPPH radical scavenging activity. The activity increased as the concentration of LP increases.

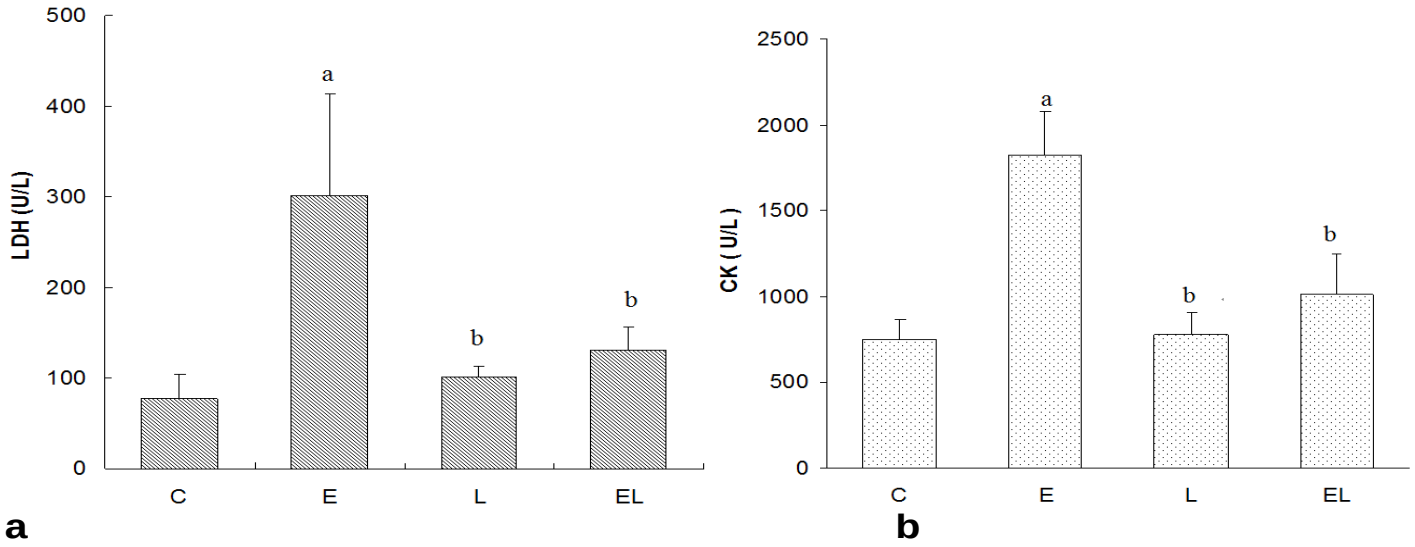


**Figure 2. a.** LDH activities; **b.** CK activities. Each bar represents mean ± SD of eight rats. Rats were randomly divided into the following four groups: Sedentary control group (C), exhaustive exercise group (E), *LP* treatment group (L) and exercise group subjected to exhaustive exercise and *l. polysaccharides* treatment (EL). [a], $P<0.01$ *vs*. C group, [b], $P<0.01$, E group.

### Effect of *LP* treatment on serum biochemical markers

Figure 2a and b showed serum LDH and CK activities of rats subjected to exhaustive exercise in the absence or presence of *LP* treatment. LDH and CK activities in serum were significantly higher in the E group than in the C group ($P<0.05$). Obviously, serum LDH and CK activities of the EL group were comparable with those of the C group and lower than those of the E group ($P<0.05$). There was no difference between C group and L group ($P>0.05$).

### Effect of LP treatment on oxidative stress parameters in rat muscle

The end products of lipid peroxidation were assessed through detections of the level of MDA, a biomarker of lipid peroxidation and oxidative stress. Figure 3a to e showed that the activities of SOD, CAT, GPx, and GSH level in rat muscle of the E group significantly decreased, whereas MDA level was enhanced compared with those of the C group ($P<0.05$). SOD, GPx, CAT activities and GSH level significantly increased, whereas MDA level decreased in rat muscle in the EL group compared with those of the E group ($P<0.05$). The results showed there was no difference between C and L groups ($P>0.05$).

## DISCUSSION

The regular, moderate physical exercise improves health conditions, maintains skeletal muscle function and stimulates cellular antioxidant defenses (Gomez-Cabrera et al., 2008; Ji, 2002). There are many evidences that moderate exercise to effectively prevent several chronic diseases such as cardiovascular disease, diabetes, hypertension, osteoporosis and obesity (Warburton et al., 2006). Although regular exercise is beneficial to the body, it is well known that intense and exhaustive exercise causes muscle damage through increased ROS production in the skeletal muscle and can be a harmful source of oxidative stress. Davies et al. (1999) reported that free radical increased in rat skeletal muscle after running until exhaustion. In Niu's experiment, antioxidant levels in muscle were reduced in rats with exhaustive exercise (Niu et al., 2008). To date, many researchers provided the evidence that oxidative stress induced by exhaustive exercise can be counteracted by optimizing nutrition, especially by increasing the dietary content of nutrition antioxidant. Castell (2002) presented that provision of glutamine or a glutamine precursor had been found to decrease the incidence of illness in endurance athletes. Tauler et al., 2003; showed that dietary vitamin C supplementation play an important role in the defense against oxidative stress induced by exercise and in avoiding negative effects on erythrocyte integrity. Jackson (1987) observed that muscle damage associated with exercise could be prevented, at least in part, by vitamin E administration. Moreover, xanthine oxidase is a signicant source of ROS and allopurinol affords protection against free radical production and against tissue damage associated with exhaustive exercise by inhibiting xanthine oxidase (Vina et al., 2000). In these reports, glutamine, vitamin C, E and allopurinol play a part in preventing oxidative stress by acting as

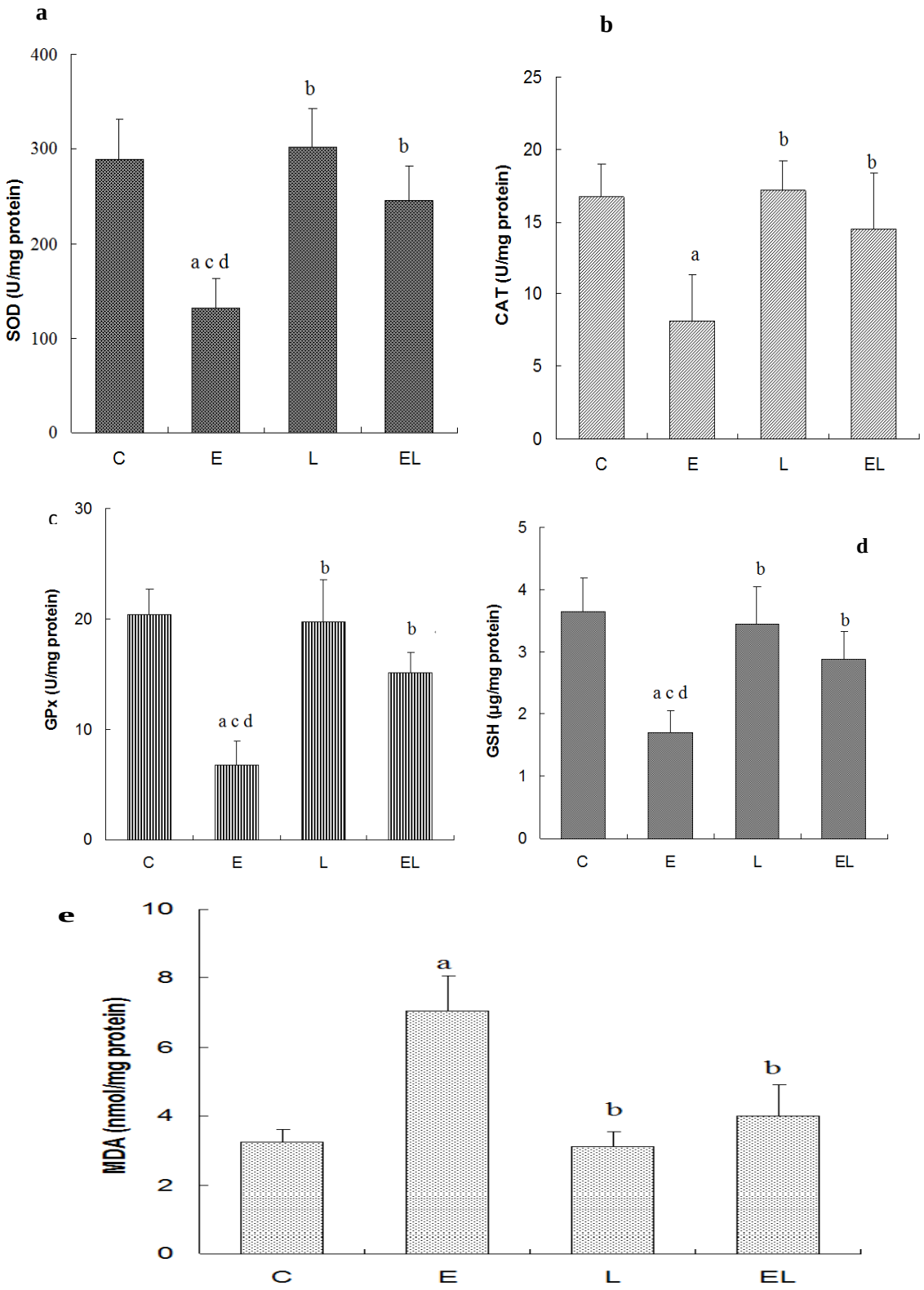


**Figure 3.** Effects of *LP* administration and exhaustive exercise on oxidative stress parameters in vastus lateralis muscle of rats; **a,** SOD activities; **b,** CAT activities; **c,** GPx activities; **d,** GSH levels and; **e,** MDA levels of rats randomly divided into the following four groups. Each bar represents mean ± SD of eight rats. Rats were randomly divided into the following four groups: Sedentary control group (C), exhaustive exercise group (E), LP treatment group (L) and exercise group subjected to exhaustive exercise and LP treatment (EL).[a], *P*<0.05, *vs*. C group, [b],*P*<0.05, *vs*. E group, [c], *P*<0.05, *vs*. L group,[d], *P*<0.05, *vs*. EL group.

ROS scavengers. This opened up the possibility of minimizing damage caused by exercise by antioxidant administration. In recent years, LP a heteropolysaccharide extracted from *Laminaria*, has gained more attention mainly for its diversity function. In addition, (Neyrinck et al., 2007), suggested that *Laminaria* merits consideration as a potential therapeutic agent in the oral treatment of systemic inflammatory syndrome and associated hepatic damages (Neyrinck et al., 2007). The preliminary study had shown that the fucoidan, a type of polysacchrides extracted from *Laminaria*, was active as an antioxidant, being strong in scavenging superoxide radical and also hydroxyl radical, and possessed antioxidant properties against $CCl_4$-induced oxidative stress (Kang et al., 2008). Although it has been demonstrated that LP is an inducer of endogenous antioxidant in liver cells, the inducibility of the cellular defenses in skeletal muscle cells has not been studied. In our study, LDH and CK in serum, two well-known biomarkers of muscle damage that significantly increased after an exhaustive exercise (Ebbeling and Clarkson, 1989), were significantly reduced by LP treatment. The higher levels of LDH and CK observed in the E group may have resulted from the disruption of the muscle cell membrane, with the consequent leakage of proteins. Due to LP treatment, both enzymes activities in serum decreased, evidencing a protection of muscle tissue against exhaustive exercise-induced damage. ROS formation is related to respiration chain. Investigators assumed that exercise can cause an increase in mitochondrial respiration; an increase in ROS formation should be expected. Exhaustive exercise is associated with accelerated generation of ROS that results in oxidative stress, which can induce adverse effects on health.

Aerobic organisms synthesize numerous antioxidant enzymes and some proteins in attempt to minimize oxidative stress. SOD, the best known of these enzymes, is a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. CAT is exclusively found within peroxisomes where it has a clear function of removing the $H_2O_2$ generated by β-oxidation of long-chain fatty acids. GPx utilize the reducing power of GSH to detoxify hydrogen peroxide. GSH are oxidized to form the disulfide-bonded compound, GS-SG in the reduction of hydrogen peroxide, and also is the most abundant intracellular antioxidant serving as an important defense against the oxidative stress and playing a key role in maintaining cellular integrity (Mallikarjuna et al., 2007). These antioxidants act cooperatively at different sites in the metabolic pathway of free radicals. MDA is usually used as one of the biomarkers to measure the level of oxidative stress. MDA is formed by degrading polyunsaturated lipids and reacts with deoxyadenosine and deoxyguanosine in DNA, forming DNA adducts, primarily $M_1G$, which is mutagenic (Del Rio et al., 2005; Lykkesfeldt, 2007; Marnett, 1999; Valenzuela, 1991). In present study, rats that received LP along with exhaustive exercise showed increases in antioxidant enzymes and protein and decrease in MDA level as compared to exhaustive exercise alone treated rats. These results also showed that LP treatment prevents the elevation of lipid peroxidation and also decrease of antioxidants. This finding is in agreement with Wang's observation that LP exerts its protective effect by modulating the biochemical marker enzymes, lipid peroxidation and augmenting antioxidant defense system (Wang et al., 2008). Therefore, LP has been shown to restore the activities of SOD, CAT, GPx and GSH level during exhaustive exercise-induced toxicity.

## Conclusion

In conclusion, our study indicates that antioxidant enzymes and treatment has the protective effect against exhaustive exercise-induced biochemical alterations and oxidative damage in the skeletal muscle of rats. The mechanism for this protective effect may be its free radical scavenging activity and increased antioxidant enzymes in rats.

## ACKNOWLEDGMENTS

This study was supported by the grant of 2008 fund project of the Department of Education, Liaoning Province, China (Topic No: 2008752).

### REFERENCES

Aoi W, Naito Y, Sakuma K, Kuchide M, Tokuda H, Maoka T, Toyokuni S, Oka S, Yasuhara M, Yoshikawa T (2003). Astaxanthin limits exercise-induced skeletal and cardiac muscle damage in mice. Antioxid. Redox. Sign., 5(1): 139-144.

Aoi W, Naito Y, Takanami Y, Kawai Y, Sakuma K, Ichikawa H, Yoshida N, Yoshikawa T (2004). Oxidative stress and delayed-onset muscle damage after exercise. Free Radic. Biol. Med., 37(4): 480-487.

Braca A, De Tommasi N, Di Bari L, Pizza C, Politi M, Morelli I (2001). Antioxidant principles from Bauhinia tarapotensis. J. Nat. Prod., 64(7): 892-895.

Castell LM (2002). Can glutamine modify the apparent immunodepression observed after prolonged, exhaustive exercise? Nutrition, 18(5): 371-375.

Cheng D, Liang B, Li M, Jin M (2011). Influence of Laminarin polysaccahrides on oxidative damage. Int. J. Biol. Macromol., 48(1): 63-66.

Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A (2006). Biomarkers of oxidative damage in human disease. Clin. Chem., 52(4): 601-623.

Davies MJ, Fu S, Wang H, Dean RT (1999). Stable markers of oxidant damage to proteins and their application in the study of human disease. Free Radic. Biol. Med., 27(11-12): 1151-1163.

Del Rio D, Stewart AJ, Pellegrini N (2005). A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr. Metab. Cardiovasc. Dis., 15(4): 316-328.

Droge W (2002). Free radicals in the physiological control of cell function. Physiol. Rev., 82(1): 47-95.

Ebbeling CB, Clarkson PM (1989). Exercise-induced muscle damage and adaptation. Sports Med., 7(4): 207-234.

Ferro CO, Chagas VL, de Oliveira MF, de Oliveira PL, Schanaider A (2010). Catalase activity in lung, kidney and small bowel non-.,

ischemic in rats after intestinal reperfusion. Rev. Col. Bras. Circ., 37(1): 31-38.
Gomez-Cabrera MC, Domenech E, Vina J (2008). Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. Free Radic. Biol. Med., 44(2): 126-131.
Jackson MJ (1987). Muscle damage during exercise: possible role of free radicals and protective effect of vitamin E. Proc. Nutr. Soc., 46(1): 77-80.
Ji LL (2002). Exercise-induced modulation of antioxidant defense. Ann. N. Y. Acad. Sci., 959: 82-92.
Ji LL, Gomez-Cabrera MC, Steinhafel N, Vina J (2004). Acute exercise activates nuclear factor (NF)-kappaB signaling pathway in rat skeletal muscle. F.A.S.E.B. J., 18(13): 1499-1506.
Ji LL, Gomez-Cabrera MC, Vina J (2006). Exercise and hormesis: activation of cellular antioxidant signaling pathway. Ann. N. Y. Acad. Sci., 1067: 425-435.
Kang KS, Kim ID, Kwon RH, Lee JY, Kang JS, Ha BJ (2008). The effects of fucoidan extracts on CCl(4)-induced liver injury. Arch. Pharm. Res., 31(5): 622-627.
Koyama K, Kaya M, Ishigaki T, Tsujita J, Hori S, Seino T, Kasugai A (1999). Role of xanthine oxidase in delayed lipid peroxidation in rat liver induced by acute exhausting exercise. Eur. J. Appl. Physiol. Occup. Physiol., 80(1): 28-33.
Lykkesfeldt J (2007). Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking. Clin. Chim. Acta, 380(1-2): 50-58.
Mallikarjuna K, Nishanth K, Reddy KS (2007). Hepatic glutathione mediated antioxidant system in ethanol treated rats: Decline with age. Pathophysiology, 14(1): 17-21.
Marnett LJ (1999). Lipid peroxidation-DNA damage by malondialdehyde. Mutat. Res., 424(1-2): 83-95.
Moron MS, Depierre JW, Mannervik B (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim. Biophys. Acta., 582(1): 67-78.
Neyrinck AM, Mouson A, Delzenne NM (2007). Dietary supplementation with laminarin, a fermentable marine beta (1-3) glucan, protects against hepatotoxicity induced by LPS in rat by modulating immune response in the hepatic tissue. Int. Immunopharmacol., 7(12): 1497-1506.
Nikolaidis MG, Jamurtas AZ, Paschalis V, Fatouros IG, Koutedakis Y, Kouretas D (2008). The effect of muscle-damaging exercise on blood and skeletal muscle oxidative stress: magnitude and time-course considerations. Sports Med., 38(7): 579-606.
Niu AJ, Wu JM, Yu DH, Wang R (2008). Protective effect of Lycium barbarum polysaccharides on oxidative damage in skeletal muscle of exhaustive exercise rats. Int. J. Biol. Macromol., 42(5): 447-449.
Radak Z, Chung HY, Koltai E, Taylor AW, Goto S (2008). Exercise, oxidative stress and hormesis. Ageing. Res. Rev., 7(1): 34-42.
Shafiq ur R, Rehman S, Chandra O, Abdulla M (1995). Evaluation of malondialdehyde as an index of lead damage in rat brain homogenates. Biometals, 8(4): 275-279.
Tappel AL (1973). Lipid peroxidation damage to cell components. Fed. Proc., 32(8): 1870-1874.
Tauler P, Aguilo A, Gimeno I, Fuentespina E, Tur JA, Pons A (2003). Influence of vitamin C diet supplementation on endogenous antioxidant defences during exhaustive exercise. Pflugers. Arch., 446(6): 658-664.
Valenzuela A (1991). The biological significance of malondialdehyde determination in the assessment of tissue oxidative stress. Life Sci., 48(4): 301-309.
Vina J, Gimeno A, Sastre J, Desco C, Asensi M, Pallardo FV, Cuesta A, Ferrero JA, Terada LS, Repine JE (2000). Mechanism of free radical production in exhaustive exercise in humans and rats; role of xanthine oxidase and protection by allopurinol. I.U.B.M.B. Life, 49(6): 539-544.
Wang J, Zhang Q, Zhang Z, Li Z (2008). Antioxidant activity of sulfated polysaccharide fractions extracted from *Laminaria japonica*. Int. J. Biol. Macromol., 42(2): 127-132.
Warburton DE, Nicol CW, Bredin SS (2006). Health benefits of physical activity: the evidence. C.M.A.J., 174(6): 801-809.