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Full Length Research Paper

Protective effect of flavonoids from *pericarpium citri reticulatae (chenpi)* against oxidative stress induced by exhaustive exercise

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The present study aims at exploring the effects of flavonoids from *pericarpium citri reticulatae* (FPCR) against oxidative stress induced by exhaustive exercise in rats. Male Sprague-Dawley rats were used in experimental research. Thirty-two rats were randomly divided into four groups that is control (C) group, low-dose FPCR treated (LFT) group, middle-dose FPCR treated (MFT) group and high-dose FPCR treated (HFT) group. The animals of control (C) group received an oral administration of drinking water, and the animals of treated group received FPCR (20, 50 and 100 mg/kg bodyweight, once a day) for 30 days. On the last day of treatment, rats performed an exhaustive running test on a treadmill and endurance time, blood lactate, malondialdehyde (MDA) and super oxide dismutase (SOD) levels of rats were measured. The results suggested that FPCR supplementation increased performance of exhaustive exercise, inhibited the production of blood lactate, reduced lipid per-oxidation, and up-regulated antioxidant enzymes to protect against oxidative stress-induced injury during exercise.

Key words: Flavonoids from *pericarpium citri reticulatae*, oxidative stress, exhaustive exercise.

INTRODUCTION

The role of exercise and physical activity in the prevention of chronic disease and promotion of optimal health has drawn the attention of the public (Singh, 1992; Manson et al., 1999; Chen et al., 2002; Warburton et al., 2006). However, research on dietary intervention that protects body tissues from damage during vigorous exercise is in its infancy. This damage is mostly attributed to the sharply increased reactive oxygen species (ROS) in the body during exercise (Davies et al., 1982; Suzuki et al., 1996; Peake and Suzuki, 2004). The ROS is believed to be the underlying mechanism for a series of biochemical and physiological changes that occur during exercise and are indicative of oxidative stress(Jenkins, 1988; Ji, 1995; Cooper et al., 2002). Many studies have reported that exercise contributes to oxidative stress, especially when performed at high intensity levels (Davies et al., 1982; Ji et al., 1988; Sakai et al., 1999; Leeuwenburgh and Heinecke, 2001; Belviranli and Gökbel, 2006; Vincent et al., 2006; Nikolaidis et al., 2007). Furthermore, strong evidence indicates that ROS are the primary cause of exercise-induced disturbances in muscle oxidation-reduction status that is (redox balance). Severe disturbances in cellular redox balance have been shown to contribute to oxidative injury and muscle fatigue (Shindoh et al., 1990; O'Neill et al., 1996; Powers et al., 2004; Kennedy et al., 2005; McClung et al., 2010). Two major classes of endogenous protective mechanisms, the enzymatic and non-enzymatic antioxidants, work to reduce the harmful effects of ROS in cells (Couillard et al., 2003; Powers et al., 2004). Antioxidant enzymes include super oxide dismutase, glutathione peroxidase and catalase. The main non-enzymatic antioxidants include GSH, vitamin C and vitamin E (Cooper et al., 2002; Belviranli and Gökbel, 2006). Furthermore, dietary supplement antioxidants

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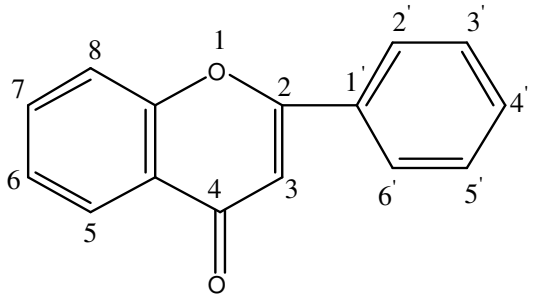

Figure 1. Basic flavonoid structure.

interact with endogenous antioxidants to form a cooperative antioxidant network (Frei, 2004; Powers and Jackson, 2008). Goldfarb et al. (1994) found that rats fed a 250 IU vitamin E/ kg diet for five weeks had lower thiobarbituric acid reactive substance (TBARS) and lipid peroxide levels in plasma and leg muscles after one hour of treadmill exercise, than rats fed a control diet. This finding suggested that antioxidant supplementation in humans and animals may be needed to protect tissues against ROS attack induced by exercise.

Flavonoids, a class of compounds that have the basic structural feature of a 2-phenyl benzo(γ)pyrone nucleus (Figure 1), are universally distributed among vascular plants, with over 8000 individual compounds known (Jacobs and Rubery, 1988; Jacobs and Rubery, 1993; Jiménez and García-Carmona, 1999; Havsteen, 2002; Li et al., 2008). They act in plants as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellants, and for light screening. Many studies have suggested that flavonoids exhibit biological activities, including antiallergenic, antiviral, antiinflammatory, and vasodilating actions (Yamada et al., 1999; Nishida and Satoh, 2004; Wang et al., 2006; Andres et al., 2009). However, most interest has been devoted to the antioxidant activity of flavonoids, which is due to their ability to reduce free radical formation and to scavenge free radicals (Pietta, 2000; Rice-Evans, 2001; Firuzi et al., 2004).

Pericarpium citri reticulatae (PCR) is one of the traditional Chinese medicines (TCM). Which is the dried rind of ripe Citrus reticulata Blanco fruits, and which is commercially referred to as *chenpi*. This is a widely planted and consumed species in southern China that has various therapeutic properties, including reducing fevers, soothing asthma, stimulating the appetite, and enhancing immune system function (Sheu et al., 2007; Shi et al., 2009). The major components in PCR are flavonoids, such as flavone, flavanone and flavonol (Yi et al., 2008; Sun et al., 2009). Since flavonoids from *pericarpium citri reticulatae* (FPCR) exhibit antioxidant activity *in vitro* and *in vivo* (Wang et al., 2007; Yi et al., 2008) and Sun (2009) surmised that FPCR might be responsible for the beneficial effect of lowering the incidence of diseases. The present study aims at exploring the effects of FPCR against oxidative stress induced by exhaustive exercise in rats, and endurance time, blood lactate, malondialdehyde (MDA) and super oxide dismutase (SOD) levels were measured in order to investigate its possible mechanisms.

Figure 2. *Pericarpium citri reticulatae.*

MATERIALS AND METHODS

Chemicals

Pure standard of rutin were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All the commercial diagnostic kits were purchased from Jiancheng Biology Technology Company (NanJing, china). Other chemicals were purchased from Jilin Dihao Chemical Reagent Company (Changchun, China).

Plant material and preparing flavonoids extract

Pericarpium citri reticulatae (Figure 2) used in the research was kindly supplied by Jilin Pharmaceutical Company (Changchun, China). FPCR were prepared according to the modified method of Chen et al. (2006) and Yi et al. (2008). Dried *pericarpium citri reticulatae* was ground into powder (particle diameter: 0.2 to 0.5 mm). Samples was weighted accurately and extracted by ultrasonator with ethanol solvent (ethanol concentration 80%, v/v; solid to liquid ratio 1/50, w/v) for 45 min. After that, the sample was centrifuged at 3000 rpm for 10 min to remove the insoluble and the supernatant was filtrated through 0.45 mm of filter membrane to obtain a clarified solution. Filtrate was evaporated with a rotary evaporator at 20℃ for about 6 h to afford a final flavonoids extract from *pericarpium citri reticulatae*. The flavonoids content was determined according to the modified method of Zhuang et al. (1992) and it was 225.14 mg/g in *pericarpium citri reticulatae* (dry weight).

Animal care

Thirty-two 6 week-old male Sprague-Dawley rats were purchased from the Jilin Laboratory Animal Breeding and Research Center (Changchun, China). The rats were individually housed in a room

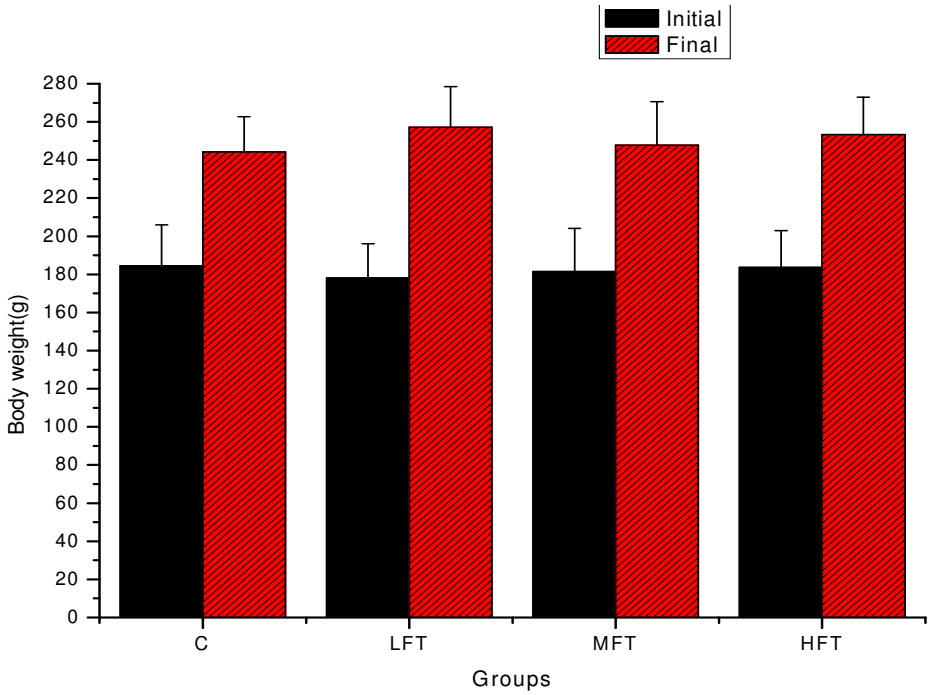

Figure 3. Effect of flavonoids from *pericarpium citri reticulatae* administration on body weight of rats. Values are means ± SE. C, control; LFT, low-dose FPCR treated; MFT, middle-dose FPCR treated; HFT, high-dose FPCR treated. *P<0.05 when compared to control group.

maintained at 24 ± 2℃ and 50 ± 5% humidity with a 12 h light-dark cycle. They were given free access to food and water throughout the experiments. The experiments were carried out in accordance with the China animal protection law and approved by Ethics Commission of Jilin Normal University.

Experimental design

Rats were randomly divided into four groups, that is control (C) group, low-dose FPCR treated (LFT) group, middle-dose FPCR treated (MFT) group and high-dose FPCR treated (HFT) group. The animals of control (C) group received an oral administration of drinking water in a volume of 0.5 ml, and the animals of treated group received with the same volume of FPCR (20, 50 and 100 mg/kg bodyweight, once a day) for 30 days.

The rats were introduced to treadmill running with 15 to 20 min exercise bouts at 15 to 30 m/min for 1 week to accustom them to running. On the day of the exercise test (the last day of treatment), rats were required to run to exhaustion on the treadmill at a final speed of 30 m/min, 10% gradient and approximately 70 to 75% VO_2max (Brooks and White, 1978; Saunders et al., 2004; Liu et al., 2005). Exhaustion was defined as the rat being unable to upright itself when placed on its back (Fielding et al., 1993; Ji and Mitchell, 1994; Liu et al., 2005; Huang et al., 2009). The treadmill was provided from Zhishuduobao Biological Technology Company (DB030l device; Beijing, china). To eliminate diurnal effects, the adaptive exercise and exercise test were performed at the same time (09.00 to 11.00 h).

Sample preparation

The rats were killed immediately after exhaustive exercise. Heparinised blood samples were collected from the abdominal aorta, hepatic and skeletal muscle tissue was carefully removed, rinsed in ice-cold normal saline, blotted dry and stored at 280℃ for further analysis. Blood samples were centrifuged at 48℃ for 10 min. The supernatant fractions (plasma) were used for the determination of lactate.

All tissues were homogenized in ice-cold buffer (0.25 M sucrose, 10 mM Tris-HCl, and 0.25 mM phenylmethylsulfonyl fluoride; pH 7.4), and a portion of the homogenate was measured immediately for MDA using a commercial diagnostic kit. Another portion of the homogenate was centrifuged at 10,000 × g for 20 min at 4℃, and SOD activity in the supernatant were measured using commercial diagnostic kits.

Statistical analysis

The results are expressed as means ± SE, and statistical analyses were done by one-way ANOVA. Newman-Keuls posttest for multiple comparison among means was used to compare inter-group differences. $P < 0.05$ was accepted as significant.

RESULTS

Body weight of rats

As shown in Figure 3, at the end of 30 day, there was no significant difference in body weight between each group ($P > 0.05$).

Endurance time of rats

As shown in Figure 4, high-dose and middle-dose FPCR

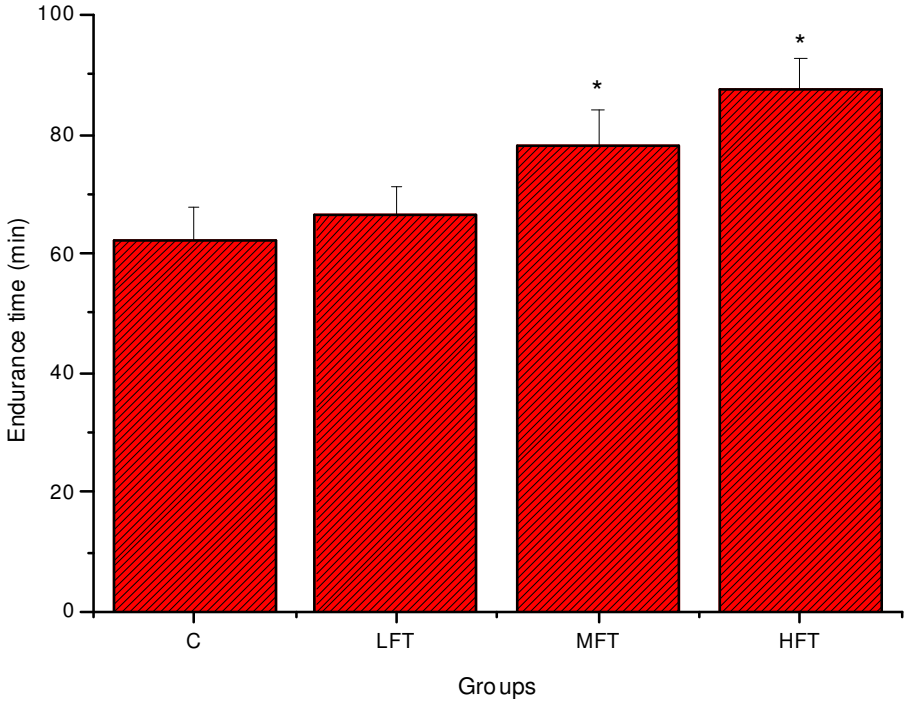

Figure 4. Effect of flavonoids from *pericarpium citri reticulatae administration* on endurance time of rats after exhaustive exercise.Values are means ± SE. C, control; LFT, low-dose FPCR treated; MFT, middle-dose FPCR treated; HFT, high-dose FPCR treated. *P<0.05 when compared to control group.

treated groups showed a significant increase endurance time of treadmill running to exhaustion compared with the control group (P<0.05). However, endurance time in low-dose FPCR treated group showed no significant changes compared to control group.

Blood lactate level of rats

As shown in Figure 5, after exhaustive exercise, blood lactate level of FPCR treated groups were significantly lower than that of control group (P<0.05).

Malondialdehyde (MDA) and super oxide dismutase (SOD) levels of rats

Table1 shows the MDA and SOD levels of rats. After exhaustive exercise, MDA levels of hepatic were all significantly lower in FPCR treated group compared to control group (P<0.05). Compared to control group, MDA levels of skeletal muscle were significantly lower in high-dose and middle-dose FPCR treated groups (P<0.05). However, there were no differences between control group and low-dose FPCR treated group in MDA levels of skeletal muscle (P>0.05). In comparison with control group, SOD level in hepatic and skeletal muscles in FPCR treated group were all significantly higher (P<0.05)

DISCUSSION

The purpose of this research was to study the effects of FPCR against oxidative stress induced by exhaustive exercise in rat model. In this research, high-dose and middle-dose FPCR treated groups showed a significant increase endurance time of treadmill running to exhaustion compared with the control group (P<0.05). The results showed that FPCR supplementation increased performance of exhaustive exercise in rats.

Previous studies indicated that blood lactate is the glycolysis product of carbohydrate under an anaerobic condition, and glycolysis is the main energy source for intense exercise in a short time. The accumulation of blood lactate is a reason of fatigue during the physic excise (Jacobs, 1981; Maughan, 1988; Korzeniewski and Liguzinski, 2004; Cairns, 2006). In this research, blood lactate level of FPCR treated groups were significantly lower than that of control group (P<0.05), which suggested that FPCR supplementation inhibited the production of blood lactate during exercise. Oxidative stress induced by acute exercise can significantly elevate markers of tissue per-oxidative damage because physical exercise promotes the production of ROS due to a substantial increase in oxygen consumption (Ayres et al., 1998; Chen et al., 2002). The MDA, a metabolite of phospholipid per-oxidation, is a popular index of first condition on living body oxidative damage

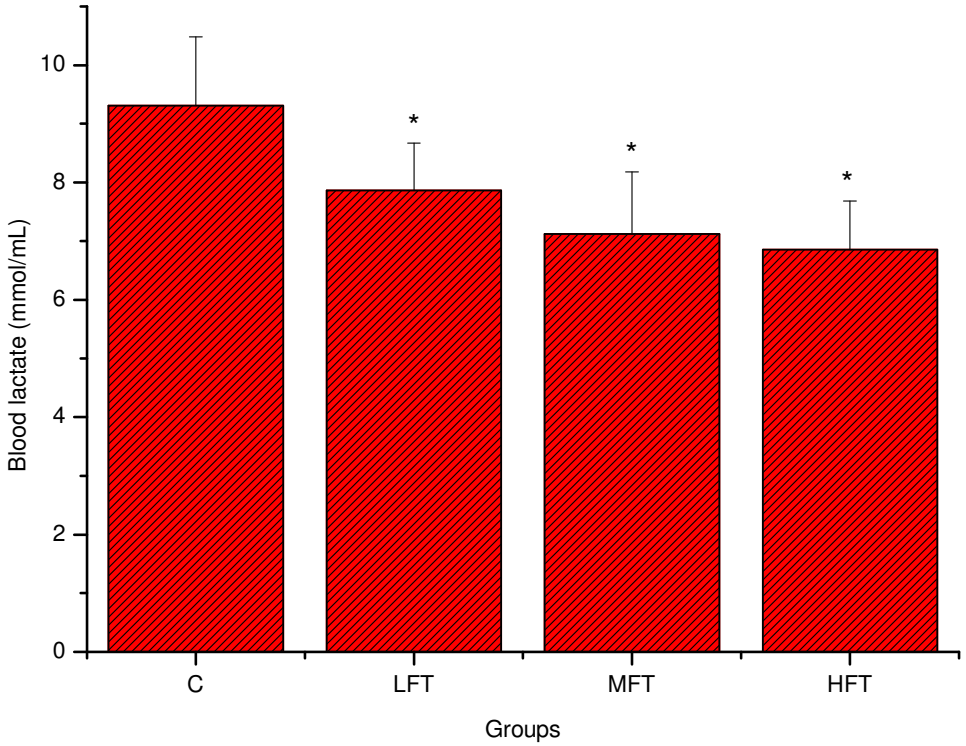

Figure 5. Effect of flavonoids from *pericarpium citri reticulatae* administration on blood lactate level of rat after exhaustive exercise. Values are means ± SE. C, control; LFT, low-dose FPCR treated; MFT, middle-dose FPCR treated; HFT, high-dose FPCR treated. *P<0.05 when compared to control group.

Table 1. Effect of flavonoids from *pericarpium citri reticulatae* administration on MDA and SOD level of rat after exhaustive exercise.

Groups	MDA (nmol/mg·pro)		SOD (NU/mg·pro)	
	Hepatic	Skeletal muscle	Hepatic	Skeletal muscle
C	4.72 ± 0.83	2.87 ± 0.31	44.26 ± 6.13	8.96 ± 3.34
LFT	3.02 ± 0.46*	2.48 ± 0.18	53.47 ± 5.29*	16.25 ± 3.02*
MFT	2.75 ± 0.89*	2.08 ± 0.32*	58.69 ± 7.11*	19.84 ± 4.17*
HFT	2.28 ± 0.74*	2.01 ± 0.25*	54.94 ± 5.73*	20.16 ± 3.28*

Values are means ± SE. C, control; LFT, low-dose FPCR treated; MFT, middle-dose FPCR treated; HFT, high-dose FPCR treated. *P<0.05 when compared to control group.

(Lu et al., 2006). The current study showed that MDA levels of hepatic and skeletal muscle were significantly lower in high-dose and middle-dose FPCR treated group compared to control group after exhaustive exercise (P<0.05). As per the above findings, it is suggested that FPCR supplementation reduced lipid per-oxidation during exercise.

It is well known that SOD is regarded as the first line of defense by the antioxidant enzyme system against ROS generated during exhaustive exercise (Manna et al., 2004; Huang et al., 2009). The increase in SOD in both hepatic and muscle would indicate an up-regulation of the defense mechanism to try to cope with an enhanced production of super oxide anion radicals. This in turn might help to down-regulate the production of lipid peroxides or oxidative stress (Lee et al., 2009). The current study showed that SOD levels of hepatic and skeletal muscle were significantly lower in FPCR treated group compared to control group after exhaustive exercise (P<0.05), which suggested that FPCR supplementation was able to up-regulate antioxidant enzymes to protect against oxidative stress-induced injury during exercise.

In conclusion, this study is to directly verify the effect of flavonoids from *pericarpium citri reticulatae (chenpi)* against oxidative stress induced by exhaustive exercise.

The experiment results indicated that FPCR supplementation increased performance of exhaustive exercise, inhibited the production of blood lactate, reduced lipid per-oxidation, and up-regulated antioxidant enzymes to protect against oxidative stress-induced injury during exercise.

REFERENCES

Andres A, Donovan SM, Kuhlenschmidt MS (2009). Soy isoflavones and virus infections. J. Nutr. Biochem., 20(8): 563-569.

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.

Belviranli M, Gökbel H (2006). Acute exercise induced oxidative stress and antioxidant changes. Eur. J. General Med., 3: 126-131.

Brooks GA, White TP (1978). Determination of metabolic and heart rate responses of rats to treadmill exercise. J. Appl. Physiol., 45: 1009-1015.

Cairns SP (2006). Lactic acid and exercise performance: culprit or friend?. Sports Med., 36: 279-291.

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. Pak. J. Nutr., 1(1): 1-7.

Chen FS, Zuo JJ, Yao YZ, Li L (2006). Study on the conditions of refining citrus flavonoid. Food Res. Dev., 27(9): 38-41.

Cooper CE, Vollaard NB, Choueiri T, Wilson MT (2002). Exercise, free radicals and oxidative stress. Biochem. Soc. Trans., 30(2): 280-285.

Couillard A, Maltais F, Saey D, Debigaré R, Michaud A, Koechlin C, LeBlanc P, Préfaut C (2003). Exercise-induced quadriceps oxidative stress and peripheral muscle dysfunction in patients with chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care Med., 167(12): 1664-1669.

Davies KJ, Quintanilha AT, Brooks GA, Packer L (1982). Free radicals and tissue damage produced by exercise. Biochem. Biophys. Res. Commun., 107(4): 1198-1205..

Fielding RA, Manfredi TJ, Ding W, Fiatarone MA, Evans WJ, Cannon JG (1993). Acute phase response in exercise. III. Neutrophil and IL-1 beta accumulation in skeletal muscle. Am. J. Physiol. Regulat. Integrat. Comp. Physiol., 265: 166-172.

Firuzi O, Mladenka P, Petrucci R, Marrosu G, Saso L (2004). Hypochlorite scavenging activity of flavonoids. J. Pharm. Pharmacol., 56(6): 801-807.

Frei B (2004). Efficacy of dietary antioxidants to prevent oxidative damage and inhibit chronic disease. J. Nutr., 134(11): 3196S-3198S.

Goldfarb AH, McIntosh MK, Boyer BT, Fatouros J (1994). Vitamin E effects on indexes of lipid peroxidation in muscle from DHEA-treated and exercised rats. J. Appl. Physiol., 76(4): 1630-1635.

Havsteen BH (2002). The biochemistry and medical significance of the flavonoids. J. Pharmacol. Ther., 96(2-3): 67-202.

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.

Jacobs I (1981). Lactate concentrations after short, maximal exercise at various glycogen levels. Acta. Physiol. Scand., 111(4): 465-469.

Jacobs M, Rubery PH (1988). Naturally occurring auxin transport regulators. Science, 241(4863): 346-349.

Jenkins RR (1988). Free radical chemistry: Relationship to exercise. Sport Med., 5: 156-170.

Ji LL (1995). Oxidative stress during exercise: implication of antioxidant nutrients. Free Radic. Biol. Med., 18(6): 1079-1086.

Ji LL, Mitchell EW (1993). Effects of adriamycin on heart mitochondrial function in rested and exercised rats. Biochem. Pharmacol., 47: 877-885.

Ji LL, Stratman FW, Lardy HA (1988). Antioxidant enzyme systems in rat liver and skeletal muscle. Influences of selenium deficiency, chronic training, and acute exercise. Arch. Biochem. Biophys., 263(1): 150-160.

Jiménez M, García-Carmona F (1999). Oxidation of the flavonol quercetin by polyphenol oxidase. J. Agric. Food Chem., 47(1): 56-60.

Kennedy G, Spence VA, McLaren M, Hill A, Underwood C, Belch JJ (2005). Oxidative stress levels are raised in chronic fatigue syndrome and are associated with clinical symptoms. Free Radic. Biol. Med., 39(5): 584-589.

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.

Lee SP, Mar GY, Ng LT (2009). Effects of tocotrienol-rich fraction on exercise endurance capacity and oxidative stress in forced swimming rats. Eur. J. Appl. Physiol., 107(5): 587-595.

Leeuwenburgh C, Heinecke JW (2001). Oxidative stress and antioxidants in exercise. Curr. Med. Chem., 8(7): 829-838.

Li FL, Li QW, Gao DW, Feng CN, Shao JJ (2008). Research development on the isolation and purification of natural flavonoid. Jiangsu Condim. Subsidiary Food., (5): 20-24.

Liu CC, Huang CC, Lin WT, Hsieh CC, Huang SY, Lin SJ, Yang SC (2005). Lycopene supplementation attenuated xanthine oxidase and myeloperoxidase activities in skeletal muscle tissues of rats after exhaustive exercise. Br. J. Nutr., 94(4): 595-601.

Lu HK, Hsieh CC, Hsu JJ, Yang YK, Chou HN (2006). Preventive effects of Spirulina platensis on skeletal muscle damage under exercise-induced oxidative stress. Eur. J. Appl. Physiol., 98(2): 220-226.

Manna I, Jana K, Samanta PK (2004). Intensive swimming exercise-induced oxidative stress and reproductive dysfunction in male wistar rats: protective role of alpha-tocopherol succinate. Can. J. Appl. Physiol., 29(2): 172-185.

Manson JE, Hu FB, Rich-Edwards JW, Colditz GA, Stampfer MJ, Willett WC, Speizer FE, Hennekens CH (1999). A prospective study of walking as compared with vigorous exercise in the prevention of coronary heart disease in women. N. Engl. J. Med., 341(9):650-658.

Maughan RJ (1988). Effects of prior exercise on the performance of intense isometric exercise. Br. J. Sports Med. 22(1):12-15.

Mcclung JM, Deruisseau KC, Whidden MA, Van Remmen H, Richardson A, Song W, Vrabas IS, Powers SK (2010). Overexpression of antioxidant enzymes in diaphragm muscle does not alter contraction-induced fatigue or recovery. Exp. Physiol. 95(1): 222-231.

Nguyen TD, Canada AT (1993). Flavonoids Stimulate Secretion by Human Colonic T84 Cells. J. Nutr., 123: 259-268.

Nikolaidis MG, Kyparos A, Hadziioannou M, Panou N, Samaras L, Jamurtas AZ, Kouretas D (2007). Acute exercise markedly increases blood oxidative stress in boys and girls. Appl. Physiol. Nutr. Metab., 32(2): 197-205.

Nishida S, Satoh H (2004). Comparative vasodilating actions among terpenoids and flavonoids contained in Ginkgo biloba extract. Clin. Chim. Acta., 339(1-2): 129-133.

O'Neill CA, Stebbins CL, Bonigut S, Halliwell B, Longhurst JC (1996). Production of hydroxyl radicals in contracting skeletal muscle of cats. J. Appl. Physiol., 81(3): 1197-1206.

Peake J, Suzuki K (2004). Neutrophil activation, antioxidant supplements and exercise-induced oxidative stress. Exerc. Immunol. Rev., 10: 129-141.

Pietta PG (2000). Flavonoids as antioxidants. J. Nat. Prod., 63(7): 1035-1042.

Powers SK, DeRuisseau KC, Quindry J, Hamilton KL (2004). Dietary antioxidants and exercise. J. Sports Sci., 22(1): 81-94.

Powers SK, Jackson MJ (2008). Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiol. Rev., 88(4): 1243-1276.

Rice-Evans C (2001). Flavonoid antioxidants. Curr. Med. Chem. 8(7):797-807.

Sakai Y, Iwamura Y, Hayashi J, Yamamoto N, Ohkoshi N, Nagata H (1999). Acute exercise causes mitochondrial DNA deletion in rat skeletal muscle. Muscle Nerve., 22(2): 258-261.

Saunders MJ, Kane MD, Todd MK (2004). Effects of a Carbohydrate-Protein Beverage on Cycling Endurance and Muscle Damage. Med. Sci. Sports Exerc., 36(7): 1233-1238.

Sheu F, Chuang WI, Chien PJ (2007). Citri Reticulatae Pericarpium extract Suppress Adipogenesis in 3T3-L1 Preadipocytes. J. Sci. Food Agric., 87(13): 2382-2389.
Shi Q, Liu Z, Yang Y, Geng P, Zhu YY, Zhang Q, Bai F, Bai G (2009). Identification of anti-asthmatic compounds in Pericarpium citri reticulatae and evaluation of their synergistic effects. Acta. Pharmacol. Sin., 30(5): 567-575.
Shindoh C, DiMarco A, Thomas A, Manubay P, Supinski G (1990). Effect of N-acetylcysteine on diaphragm fatigue. J. Appl. Physiol., 68(5): 2107-2013.
Singh VN (1992). A current perspective on nutrition and exercise. J. Nutr., 122: 760-765.
Sun YS, Liu ZB, Wang JH, Zhu LX, Li LL (2009). Preparative isolation and purification of flavones from Pericarpium Citri Reticulatae by high-speed counter-current chromatography. Chinese J. Chromatogr., 27(2): 244-247.
Suzuki K, Sato H, Kikuchi T, Abe T, Nakaji S, Sugawara K, Totsuka M, Sato K, Yamaya K (1996). Capacity of circulating neutrophils to produce reactive oxygen species after exhaustive exercise. J. Appl. Physiol., 81(3): 1213-1222.
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 L, Tu YC, Lian TW, Hung JT, Yen JH, Wu MJ (2006). Distinctive antioxidant and antiinflammatory effects of flavonols. J. Agric. Food Chem., 54(26): 9798-9804.
Wang WD, Zhao ZH, Zhang XJ, Chen FS (2007). Study on extraction of flavonoids from orange peel and its antioxidation activity. Sci. Tech. Food Ind., 28(9): 48-57.
Warburton DE, Nicol CW, Bredin SS (2006). Health benefits of physical activity: the evidence. CMAJ, 174(6): 801-809.
Yamada K, Shoji K, Mori M, Ueyama T, Matsuo N, Oka S, Nishiyama K, Sugano M (1999). Structure-activity relationship of polyphenols on inhibition of chemical mediator release from rat peritoneal exudate cells. *In vitro* Cell Dev. Biol. Anim., 35(3): 169-174.
Yi ZB, Yu Y, Liang YZ, Zeng B (2008). *In vitro* antioxidant and antimicrobial activities of the extract of pericarpium citri reticulatae of a new citrus cultivar and its main flavonoids. LWT-Food Sci. Technol., 41: 597-603.
Zhuang XP, Lu YY, Yang GS (1992). Extraction and determination of flavonoid in ginkgo. Chin. Herb. Med., 23: 122-124.