

## 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.

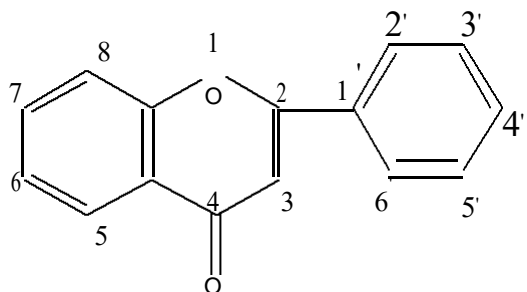
**Key words:** Flavonoids from *pericarpium citri reticulatae*, oxidative stress, exhaustive exercise.

## 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( )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



**Figure 2.** *Pericarpium citri reticulatae*.

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.

## MATERIALS AND METHODS

### Chemicals

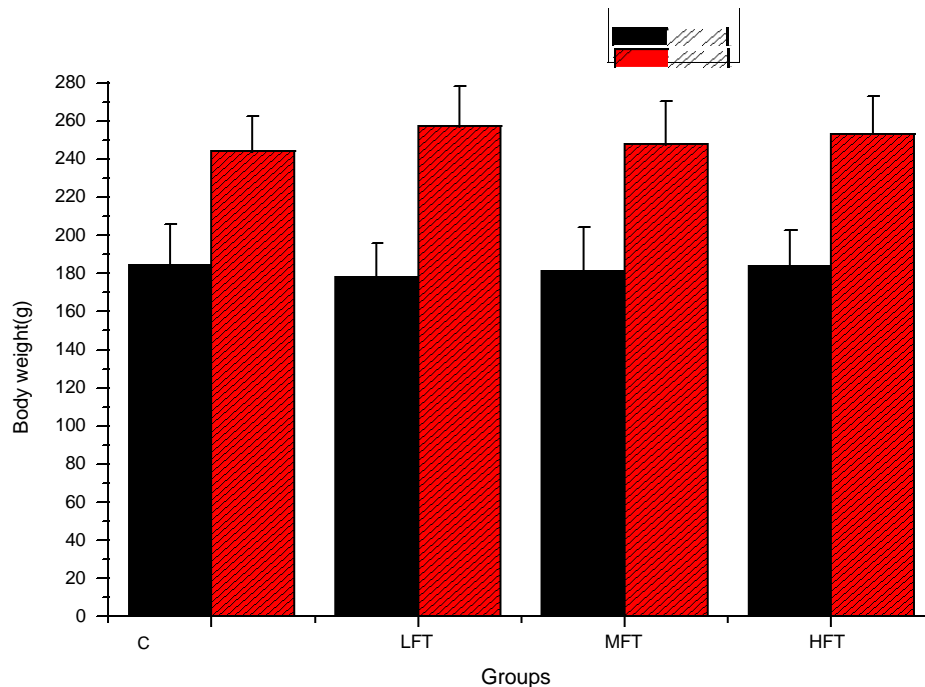
Pure standard of rutin were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All the commercial diagnostic kits were purchased from Jiancheng Biology Technology Company (NanJing, china). Other chemicals were purchased from Jilin Dihao Chemical 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 ultrasonicator 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  $\pm$  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  $24 \pm 2$  and  $50 \pm 5\%$  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%  $VO_2$ max (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 (DB030I 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  $-280^\circ\text{C}$  for further analysis. Blood samples were centrifuged at  $48^\circ\text{C}$  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  $10,000 \times g$  for 20 min at  $4^\circ\text{C}$ , and SOD activity in the supernatant were measured using commercial diagnostic kits.

### Statistical analysis

The results are expressed as means  $\pm$  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.

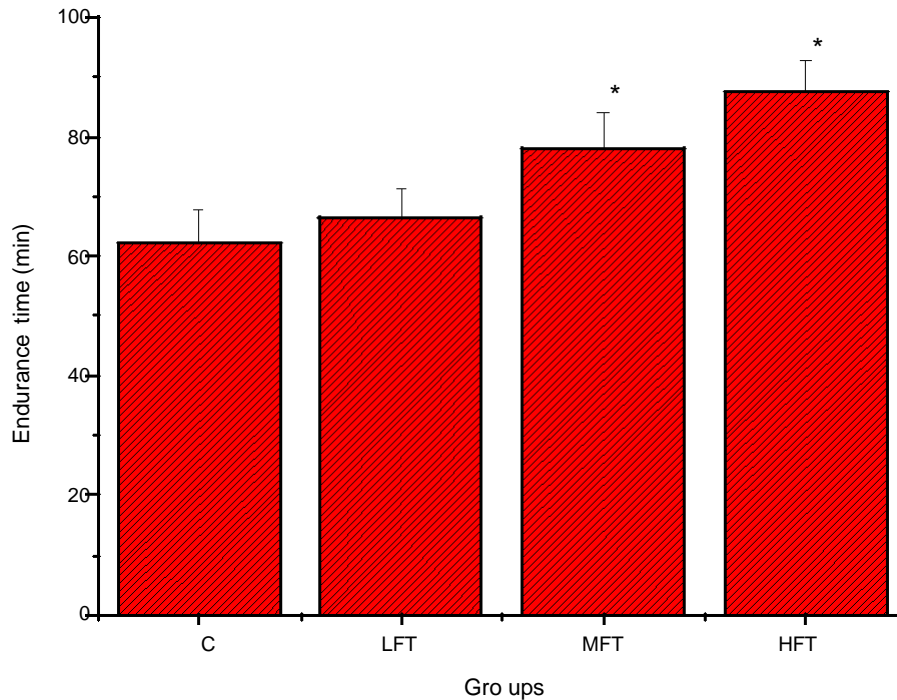
## RESULTS

### 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  $\pm$  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 ( $P < 0.05$ ).

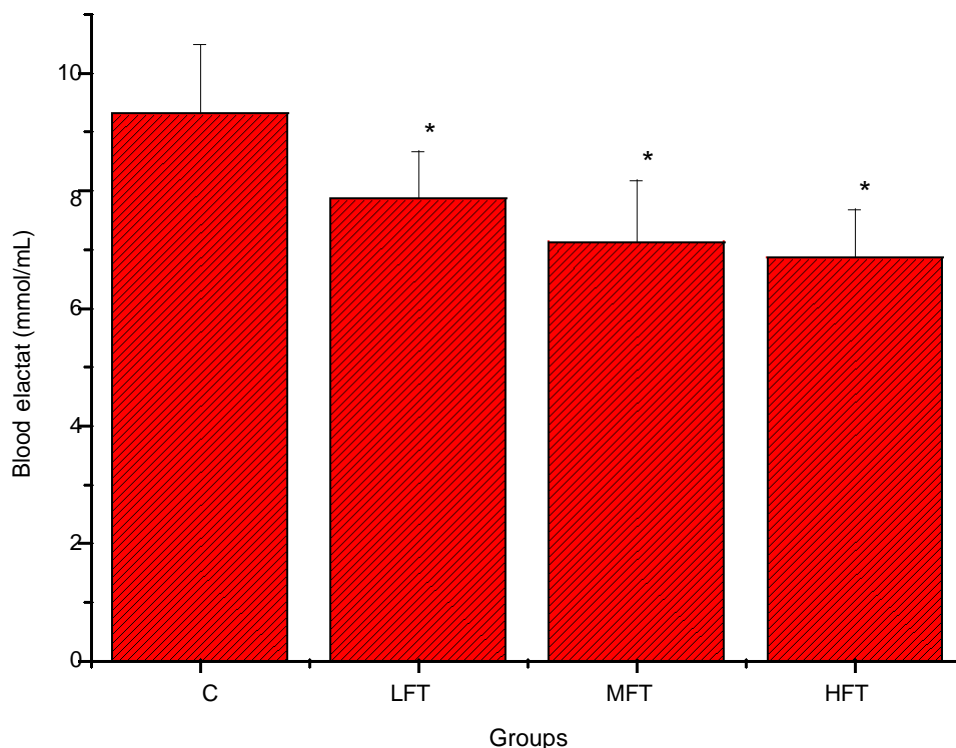
#### Malondialdehyde (MDA) and super oxide dismutase (SOD) levels of rats

Table 1 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 physical exercise (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  $\pm$  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 $\pm$ 0.83	2.87 $\pm$ 0.31	44.26 $\pm$ 6.13	8.96 $\pm$ 3.34
LFT	3.02 $\pm$ 0.46*	2.48 $\pm$ 0.18	53.47 $\pm$ 5.29*	16.25 $\pm$ 3.02*
MFT	2.75 $\pm$ 0.89*	2.08 $\pm$ 0.32*	58.69 $\pm$ 7.11*	19.84 $\pm$ 4.17*
HFT	2.28 $\pm$ 0.74*	2.01 $\pm$ 0.25*	54.94 $\pm$ 5.73*	20.16 $\pm$ 3.28*

Values are means  $\pm$  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.

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