

## Protective effect of polysaccharides from Cortex Eucommiae on exhaustive exercise-induced oxidative stress in mice.

Rui Guo<sup>1</sup>, and Bo Qi<sup>2,\*</sup>

<sup>1</sup>Physical Education Institute, Henan University of Technology, Zhengzhou 450001, P. R. China

<sup>2</sup>Department of Physical Education, Central South University, Changsha 410083, P. R. China

### Abstract

Cortex Eucommiae is well known in traditional Chinese herbal medicine. The present study investigated the effects of polysaccharides from Cortex Eucommiae (PCE) on exhaustive exercise-induced oxidative stress in mice by measuring the changes in the activities of superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) and levels of malondialdehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHdG). The mice were randomly divided into four groups: a negative control group, a low-dose PCE intervention group, a medium-dose PCE intervention group and a high-dose PCE intervention group. The mice in the control group were given distilled water whereas those in the three intervention groups were given different doses of PCE (10, 50 and 100 mg/kg). After 28 days, the mice were made to perform an exhaustive swimming exercise. Changes in the activities of the main antioxidant enzymes and the levels of MDA and 8-OHdG in the blood, liver and muscle of the mice were measured. The results of the study showed that PCE increased the activities of SOD, GPX and CAT and decreased the levels of MDA and 8-OHdG in mice, suggesting that PCE has a protective effect on exhaustive exercise-induced oxidative stress.

**Keywords:** polysaccharides, Cortex Eucommiae, oxidative stress, exhaustive swimming exercise, superoxide dismutase, glutathione peroxidase, catalase, malondialdehyde, 8-hydroxydeoxyguanosine

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### Introduction

Cortex Eucommiae (also known as Du-Zhong or Tu-chung), the dried bark of *Eucommia ulmoides* Oliv. (family: *Eucommiaceae*), is one of the oldest tonic herbs in traditional Chinese herbal medicine [1]. It is widely used to strengthen muscles and lungs, decrease blood pressure, prevent miscarriages, improve the function of the liver and kidneys, and increase longevity [2]. Recently, several studies have shown that the extracts of Cortex Eucommiae have multiple pharmacological properties such as anti-oxidant, anti-hypercholesterolaemic, anti-obesity, anti-complementary, anti-microbial, anti-inflammatory, hypoglycaemic and hypolipidaemic properties and that they protect against ultraviolet irradiation [3-6]. Cortex Eucommiae contains many phytochemicals such as lignans, iridoids, flavonoids, polysaccharides and terpenes. To date, the pharmacological properties of Cortex Eucommiae have mainly been attributed to lignans and flavonoids. Recent studies have suggested that polysaccharides from Cortex Eucommiae (PCE) also exhibit significant pharmacological properties such as antioxidant, anti-tumour and anti-complementary proper-

ties [7,8]. However, the effects of PCE on exercise-induced oxidative stress have not yet been reported. Hence, the present study aimed to evaluate the effect of PCE on oxidative stress induced by exhaustive swimming exercise in mice.

### Materials and Methods

#### Plant material

Dried Cortex Eucommiae was purchased from a local herbal drug market (Changsha, China) and was identified by Dr Meng YJ, a botanist at the Central South University (Changsha, China). Voucher specimens (KIM-NA-9132) were preserved in the herbarium of the Central South University. The dried Cortex Eucommiae was ground to fine powder and was stored at 5 °C until further use.

#### Chemicals and reagents

Assay kits for superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and malondialdehyde (MDA) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The assay kit for 8-hydroxydeoxyguanosine (8-OHdG) was purchased

from Japan Institute for the Control of Aging (Fukuroi, Japan). All other chemicals used in this research were of analytical grade and were obtained from Hunan Chemical Reagent Co. (Changsha, China).

### **Experimental animals**

Healthy male Kunming mice (weight, 18-22 g) were obtained from the experimental animal centre of the Henan University of Technology. The mice were housed with *ad libitum* access to food and water and were maintained under constant environmental conditions (temperature, 22 °C ± 2 °C; humidity, 50% ± 5%; and 12-h light:12-h dark cycle starting at 07:00). Experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the Central South University and were approved by the Ethics Committee.

### **Extraction of PCE**

PCE was extracted as described previously [9], with slight modifications. Powdered Cortex Eucommiae was soaked in 95% ethanol to remove pigments and small lipophilic molecules. The residue was then washed thrice with distilled water (1:15, v/v) at 100 °C for 30 min to extract PCE. All the water extracts were combined, filtered, concentrated and precipitated using absolute ethanol (1:4, v/v) and stored at 4°C overnight. The precipitate was collected by centrifugation (8000 × g for 30 min) and was deproteinated using Savage method [10]. Finally, the supernatant was lyophilised to obtain crude PCE.

### **Experimental design**

After one week of accommodation, the mice were randomly divided into the following four groups (10 mice in each group) based on their body weight: negative control (C) group, low-dose PCE intervention (LP) group, medium-dose PCE intervention (MP) group and high-dose PCE intervention (HP) group. The mice in the C group were given distilled water (2.0 mL) whereas those in the three intervention groups were given different doses of PCE (10, 50 and 100 mg/kg). Samples were administered once a day for 28 consecutive days by gavage using a feeding needle. PCE solutions used in intervention groups were prepared by dissolving PCE in 2.0 mL distilled water.

After 28 days, the mice were made to perform an exhaustive swimming exercise, as described previously [11]. Briefly, 30 min after the last treatment, the mice were placed individually in acrylic plastic tanks (50 cm × 50 cm × 40 cm) containing 30-cm deep water that was maintained at 25°C ± 1°C. The mice were loaded 7% of the body weight of lead threads at the bases of the tails. The mice were identified as being exhausted when they sunk into the water and could not rise to the surface within a 10-s period.

### **Assay of biochemical parameters**

After completing the exhaustive swimming exercise, all the mice were immediately anaesthetised using ethyl ether and sacrificed by exsanguination via the abdominal aorta. Blood samples were collected and serum was immediately separated by centrifugation at room temperature (2000 × g for 10 min). Next, the liver and hindlimb skeletal muscles were carefully removed and rinsed in ice cold physiological saline solution (0.9% NaCl), blotted dry and stored at -80°C until biochemical parameters were analysed. Next, the activities of SOD, GPX, and CAT and the levels of MDA and 8-OHdG in the blood, liver and muscle were measured using procedures recommended in the commercial assay kits.

### **Statistical analysis**

All data are expressed as mean ± standard deviation (SD). Statistical comparisons were made using one-way analysis of variance. P values of <0.05 were considered statistically significant. SPSS software version 17.0 (SPSS Inc., Chicago, Illinois) was used for all analyses.

## **Results**

### **Effects of PCE on the activities of SOD in the blood, liver and muscle**

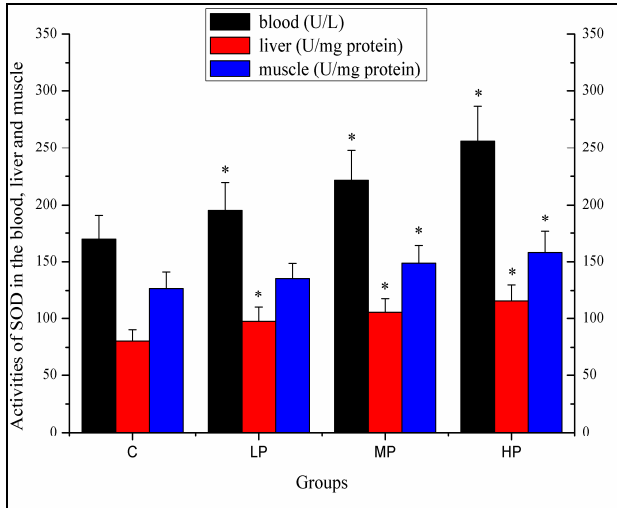
Effects of PCE on the activities of SOD in the blood, liver and muscle are shown in Figure 1. The activities of SOD in the blood and liver of mice in the LP, MP and HP groups were significantly higher ( $P < 0.05$ ) than those of mice in the C group. The activity of SOD in the muscle of mice in the MP and HP groups was significantly higher ( $P < 0.05$ ) than that of mice in the C group. Although the activity of SOD increased in the muscle of mice in the LP group, the increase was not significant ( $P > 0.05$ ).

### **Effects of PCE on the activities of GPX in the blood, liver and muscle**

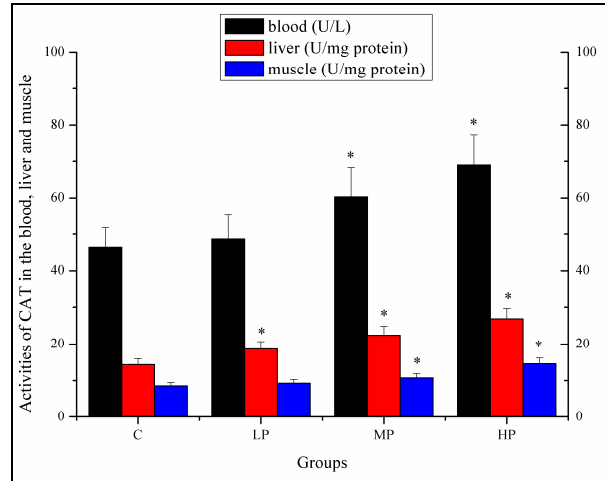
Effects of PCE on the activities of GPX in the blood, liver and muscle are shown in Figure 2. The activities of GPX in the blood, liver and muscle of mice in the LP, MP and HP groups were significantly higher ( $P < 0.05$ ) than those of the mice in the C group.

### **Effects of PCE on the activities of CAT in the blood, liver and muscle**

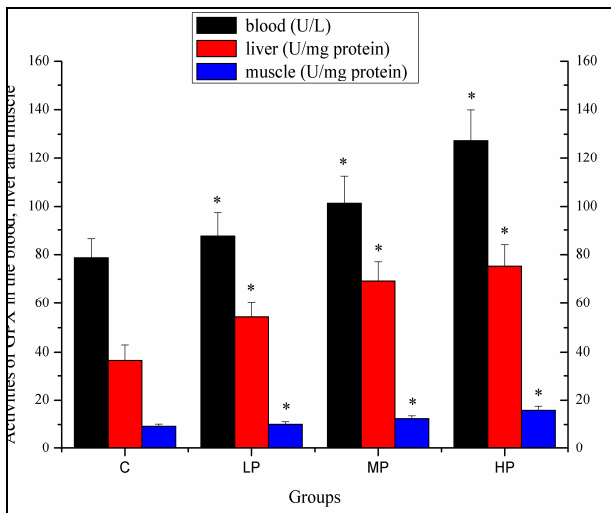
Effects of PCE on the activities of CAT in the blood, liver and muscle are shown in Figure 3. The activity of CAT in the liver of mice in the LP, MP and HP groups was significantly higher ( $P < 0.05$ ) than that of mice in the C group. The activities of CAT in the blood and muscle of mice in the MP and HP groups were significantly higher ( $P < 0.05$ ) than those of mice in the C group. Although the activities of CAT increased in the blood and muscle of mice in the LP group, the increase was not significant ( $P > 0.05$ ).



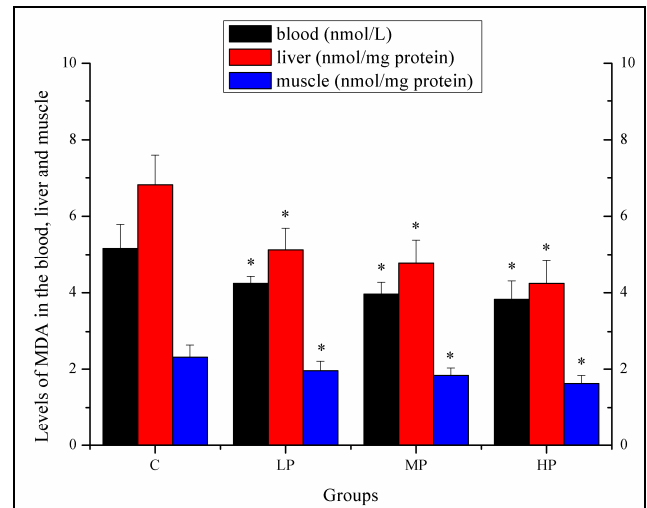
**Figure 1.** Effects of PCE on the activities of SOD in the blood, liver and muscle. All data are expressed as mean  $\pm$  SD. \*P < 0.05 vs control



**Figure 3.** Effects of PCE on the activities of CAT in the blood, liver and muscle. All data are expressed as mean  $\pm$  SD. \*P < 0.05 vs control



**Figure 2.** Effects of PCE on the activities of GPX in the blood, liver and muscle. All data are expressed as mean  $\pm$  SD. \*P < 0.05 vs control



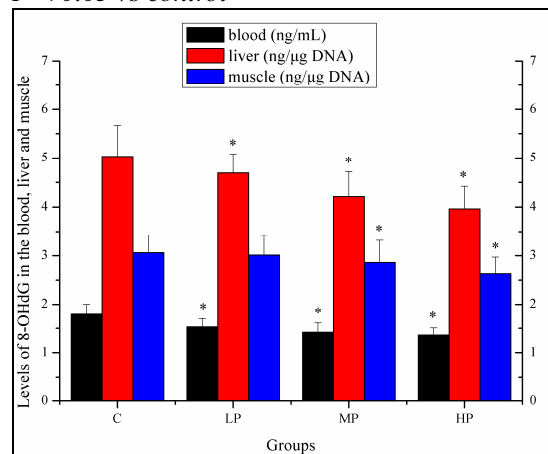
**Figure 4.** Effects of PCE on the levels of MDA in the blood, liver and muscle. All data are expressed as mean  $\pm$  SD. \*P < 0.05 vs control

**Effects of PCE on the levels of MDA in the blood, liver and muscle**

Effects of PCE on the levels of MDA in the blood, liver and muscle are shown in Figure 4. The levels of MDA in the blood, liver and muscle of mice in the LP, MP and HP groups were significantly lower (P < 0.05) than those of mice in the C group.

**Effects of PCE on the levels of 8-OHdG in the blood, liver and muscle**

Effects of PCE on the levels of 8-OHdG in the blood, liver and muscle are shown in Figure 5. The levels of 8-OHdG in the blood and liver of mice in the LP, MP and HP groups were significantly lower (P < 0.05) than those mice in the C group. The level of 8-OHdG in the muscle of mice in the MP and HP groups was significantly lower (P < 0.05) than that of mice in the C group. Although the



**Figure 5.** Effects of PCE on the levels of 8-OHdG in the blood, liver and muscle. All data are expressed as mean  $\pm$  SD. \*P < 0.05 vs control

level of 8-OHdG decreased in the muscle of mice in the LP group, the decrease was not significant (P > 0.05).

## Discussion

It is well-established that physical exercise is associated with increased free radical generation, primarily due to a dramatic increase in oxygen uptake both at the whole body level and at local tissue levels [12]. Production of these deleterious free radicals differs based on the intensity, frequency and duration of various exercises [13]. There is evidence that acute, intense physical exercise induces oxidative stress due to increased generation of reactive oxygen species (ROS) and/or reduced antioxidant capacity [14]. These excessive ROS then attack vital biomolecules such as plasma membrane lipids and proteins, thus deteriorating normal cellular functions. Moreover, exercise-induced oxidative stress may be associated with muscle fatigue, muscle damage and decreased physical performance [15,16]. More than three decades have elapsed since the first findings related to exercise-induced oxidative stress, and the topic is still of interest to researchers in different scientific fields [17].

Previous studies have shown that exercise-induced oxidative stress can be counteracted by functional food ingredients such as polysaccharides, flavonoids, saponins and phenolics [11,18-20]. Therefore, the present study was designed to verify the possible protective effects of PCE on exhaustive swimming exercise-induced oxidative stress by measuring the activities of antioxidant enzymes and levels of MDA and 8-OHdG in mice receiving PCE intervention and those not receiving PCE intervention (control group).

The antioxidant defence systems (AOS) of a living body include antioxidant enzymes and nutrients, which may be involved in reducing oxidative stress. Because antioxidant enzymes play an important role in providing protection against free radical damage, a decrease in the activity or expression of these enzymes may predispose tissues to free radical damage [21]. SOD, CAT and GPX are important components of the AOS. CAT catalyses the detoxification of  $H_2O_2$  to  $H_2O$ , GPX activates GSH-scavenging reactions of free radicals ( $\cdot OH$ ) and singlet oxygen species ( $^1O_2$ ), and SOD catalyses the dismutation of two superoxide anions to form  $H_2O_2$  and  $O_2$  [22]. The present study showed that the activities of SOD, CAT and GPX in the blood, liver and muscle of mice in the MP and HP groups were significantly higher ( $P < 0.05$ ) than those of mice in the C group. The increase in the activities of antioxidant enzymes in mice receiving PCE intervention may be because of the antioxidant activities of PCE itself.

Exercise-induced oxidative stress is characterised by ROS-induced lipid peroxidation, DNA damage and protein degradation [23]. MDA is a secondary product generated during the oxidation of polyunsaturated fatty

acids and is frequently used as an indicator of lipid peroxidation and oxidative stress in vivo [24]. Increased MDA levels confirm the increase in exhaustive exercise-induced oxidative stress. Increased MDA levels can be normalised using functional food ingredients [15,19,24]. In the present study, levels of MDA in the blood, liver and muscle of mice in the LP, MP and HP groups were significantly lower ( $P < 0.05$ ) than those of mice in the C group, indicating that PCE could effectively reduce lipid peroxidation.

Oxidative damage of DNA usually involves damage to single bases. It is estimated that ROS alter at least 35 different bases. Base damage differs depending on the ROS that damages the DNA [25]. Because 8-OHdG is not an intermediate of normal nucleotide metabolism, it is used as an important indicator of DNA damage and repair. The present study showed that the levels of 8-OHdG in the blood, liver and muscle of mice in the MP and HP groups were significantly lower ( $P < 0.05$ ) than those of mice in the C group, indicating that PCE could prevent DNA damage and attenuate oxidative stress. However, the detailed mechanism of PCE action is unclear. This may be because PCE also increases DNA repair.

## Conclusions

The results of this study indicate that PCE has a protective effect on exhaustive exercise-induced oxidative stress in mice because it increases the activities of antioxidant enzymes and decreases the levels of MDA and 8-OHdG in the blood, liver and muscle. Although the detailed mechanism of the protective effect of PCE remains to be elucidated, this study provides evidence that supports the use of PCE as an effective ergogenic aid for athletes.

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**\*Correspondence to:**

Bo Qi  
Department of Physical Education  
Central South University  
Changsha 410083  
P. R. China