The Effects of Flavonoids from *Eucommia Ulmoides* on Body's Antioxidant Systems, Lipid Peroxidation and Oxidative DNA Damage after Exhaustive Exercise

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Abstract—Objective: this study examined the effects of flavonoids from Eucommia ulmoides (FEU) on antioxidant systems, lipid peroxidation and oxidative deoxyribonucleic acid (DNA) damage after exhaustive exercise. Methods: the mice were then divided into four groups, one control group and three FEU treated groups. The control group received saline solution, whilst the treated groups received different doses of FEU (5, 15 and 45 mg/kg BW) for 28 days, followed by being forced to undergo swimming test, with measurements taken of various biochemical parameters. Results showed that FEU could increase reduced glutathione (GSH) levels, GSH/oxidized glutathione (GSSG) ratio, superoxide dismutase (SOD) and catalase (CAT) activities, decrease xanthine oxidase (XO) activities, GSSG, malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels. The finding of the study suggests that FEU possess protective effects against exhaustive exercise-induced oxidative stress and oxidative injury, which might be important in preventing loss of cellular function and warrants quick recovery after sports competition.

Keywords- flavonoids from* Eucommia ulmoides*; reduced glutathione; oxidized glutathione; superoxide dismutase; catalase; malondialdehyde; 8-hydroxy-2'-deoxyguanosine

I. INTRODUCTION

It has been documented that physical exercise with increased oxygen uptake is associated with the generation of free radicals, and the productions of these deleterious free radical reactions are different depending on a variety of exercises, such as intensity, frequency, and duration (1). Indeed it has been reported that strenuous exercise increases the whole body and tissue oxygen consumption up to 20 fold, which then elevates electron leakage from the mitochondrial transport system and disturbs the intracellular pro-oxidant and antioxidant homeostasis (2). Oxidative stress, induced by the accumulation of large amounts of free radicals and an imbalance between free radicals and antioxidants, can lead to the destruction of tissue and cell macromolecules such as lipids, proteins, and DNA (3). The oxidative damage to genomic DNA by reactive oxygen species (ROS) results in DNA base modifications, single- and double-strand breaks and the formation of apurinic/apyrimidinic lesions (4). Liver is one of the most sensitive organs for exercise-induced oxidative stress, and therefore adaptation. Many studies have indicated that strenuous exercise produces a decrease in antioxidants and increases in protein, DNA oxidation and lipid peroxidation in liver (5). It has been mentioned that supplementation with antioxidants, either through an increased consumption in the diet or from supplementation, will help to prevent the accumulation of free radicals inside cells thus reducing oxidative stress (6).

The bark of *Eucommia ulmoides* has been used folk as a medicine in China for fortify the muscles and lungs, lower blood pressure, prevent miscarriage, improve the tone of liver and kidneys, and promote longevity (7). The major active ingredients in *Eucommia ulmoides* were lignans, iridoids, flavonoids, polysaccharides and triterpines (8). Evidence in the literature has shown that flavonoids from *Eucommia ulmoides* (FEU) has a large variety of bioactivities, such as anti-spasmolytic, anti-inflammatory, antioxidative, anti-hypertensive, anti-obesity, antimicrobial and hypolipidemic activities. Moreover, FEU has shown a high antioxidant activity, such as inhibition of oxidative damage in deoxyribose and DNA, and scavenging activity towards free radicals and ROS (9), which suggests that they are beneficial in counteracting exercise-induced oxidative stress. The current study aimed to demonstrate the effects of FEU on body's antioxidant systems, lipid peroxidation and oxidative DNA damage in mice liver after exhaustive exercise.

II. MATERIALS AND METHODS

A. Chemicals and reagents

Commercial kits for determination of reduced glutathione (GSH), oxidized glutathione (GSSG), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) were purchased from Jiancheng Bioengineering Institute (Nanjing, China). Commercial kit for determination of xanthine oxidase (XO) were purchased from Beyotime institute of Biotech (Shanghai, China). Commercial kit for determination of 8-hydroxy-2'-deoxyguanosine (8-OHdG) were purchased from Control of Aging, Nikken SEIL Co., Ltd. (Shizuoka, Japan). All

other chemicals used were of good quality and analytical grade.

B. Collection of plant material and extraction

The dried bark of *Eucommia ulmoides* were purchased from Changsha Medical Company (Changsha, China). Botanical identification was carried out at the Herbarium of Hunan Normal University (Changsha, China), where a voucher specimen has been deposited. Extraction of flavonoids from *Eucommia ulmoides* (FEU) was conducted as we described previously (10). The resulting crude FEU was purified using a column packed with polyamide resin. The 95 % (v/v) ethanol was used for desorption solvent. The purified FEU was collected, and then dried at reduced pressure. the dry FEU powder was sealed in polyester bottles and stored at 4 °C.

C. Experimental animals

Male Kun-Ming mice (18 - 22 g) were purchased from Laboratory Animal Center of Hunan (Changsha, China), and fed with standard laboratory feed and purified drinking water *ad libitum*. The temperature of the experimental room was maintained at 23 ± 2°C. Relative humidity was controlled to be within 55% and a 12 h light/12 h dark cycle was maintained. Mice were treated according to the National Institutes of Health guidelines for the care and use in experimental animals. The experimental protocol was approved by the Animal Studies Committee of Central South University (Changsha, China).

D. Animals grouping

The animals were kept in these facilities for at least 1 week before the experiment. Mice were randomly divided into four groups, each consisting of eight mice. One group was the control group (I). The others were FEU treated groups (groups II, III, IV).

1) Group I: Mice were allowed free access to standard laboratory feed, water and orally administrated with the same volume of saline solution for 28 consecutive days.

2) Group II: Mice were allowed free access to standard laboratory feed, water and were treated by oral administration with FEU at a dose of 5 mg/kg BW/day dissolved in saline solution for that same period.

3) Group III: Mice were allowed free access to standard laboratory feed, water and were treated by oral administration with FEU at a dose of 15 mg/kg BW/day dissolved in saline solution for that same period.

4) Group IV: Mice were allowed free access to standard laboratory feed, water and were treated by oral administration with FEU at a dose of 45 mg/kg BW/day dissolved in saline solution for that same period

E. Exercise protocol

Mice were pretreated with the saline solution or FEU for 28 continuous days, followed by an forced swimming test which began thirty min after the last administration. Details of the forced swimming test were as previously described (11) . In brief, the mice were placed in an acrylic plastic pool (50 cm × 50 cm × 40 cm). The water depth and temperature were 30 cm and 25 ± 0.5°C, respectively. Mice were loaded 5% of the body weight of lead threads at the bases of the tails. Exhaustion was determined by observing loss of coordinated movements and failure to return to the surface within 10 s.

F. Analysis of biochemical parameters

All animals were anesthetized with ether and sacrificed immediately after the forced swimming test. The liver and gastrocnemius muscle were dissected out quickly from the mice, washed with saline solution, blotted dry and stored at -80°C for GSH, GSSG, SOD, CAT, XO, and 8-OHdG analysis. All biochemical parameters were determined using commercial kits following the manufacturer's instructions.

G. Statistical analysis

Data from experiments were analyzed by SPSS 16.0 and expressed as mean ± SD. The significance of the mean difference between the control group and each treatment group was determined by Student's t-test. Values of $p<0.05$ were regarded as significant.

III. Results and Discussion

A. Effect of FEU on reduced and oxidized glutathione in liver

To protect against exercise-induced oxidative stress, body's antioxidant defenses, consisting of enzymatic and non-enzymatic systems, works in a coordinated fashion to resist redox disturbances in the cell (12). Glutathione is one of the most important non-enzymic antioxidants that protects mammalian cells against oxidative processes, and the liver is the largest producer and exporter of this antioxidant (13). Clear evidence has shown that strenuous physical exercise can promote reduced glutathione (GSH) oxidation to oxidized glutathione (GSSG) and decreased the GSH/GSSG ratio in liver. The GSH/GSSG ratio is considered an index of the cellular redox state (14). As shown in Figure 1, GSH levels in liver in the LF, IF and HF groups were significantly higher compared with that of the C group ($p<0.05$). In contrast, LF, IF and HF groups exhibited significantly lower GSSG levels in liver ($p<0.05$).

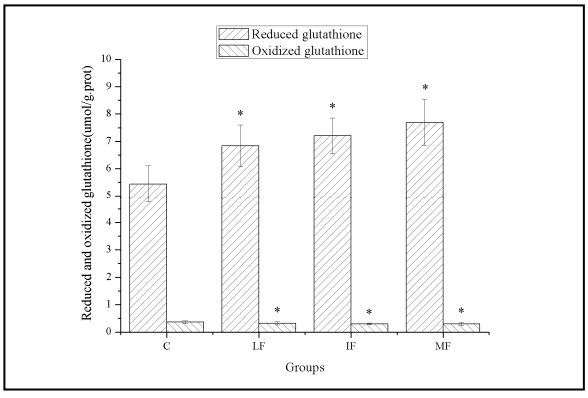

Figure 1. Effect of FEU on reduced and oxidized glutathione in liver. Data were presented as means ± SD. *, $p<0.05$ as compared with the control (C) group.

As shown in Figure 2, GSH/GSSG ratio in liver in the LF, IF and HF groups were significantly higher compared with that of the C group ($p<0.05$). These results indicate that FEU promoted a strong alteration of the liver redox state and increased body's antioxidant defenses, as evidenced by the increased GSH/GSSG ratio. Increasing

GSH/GSSG ratio can happen by either increasing GSH biosynthesis or activating GSH-recycle enzyme activity (15). The exogenous antioxidants from FEU may act directly or interact with endogenous antioxidants for synergistic effects to defend against exercise-induced oxidative stress.

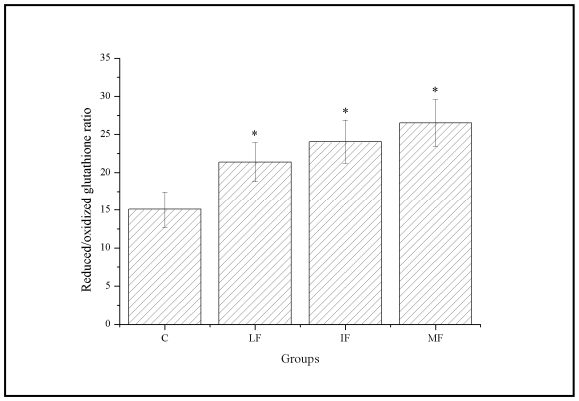

Figure 2. Effect of FEU on reduced/oxidized glutathione ratio in liver. Data were presented as means ± SD. *, $p<0.05$ as compared with the control (C) group.

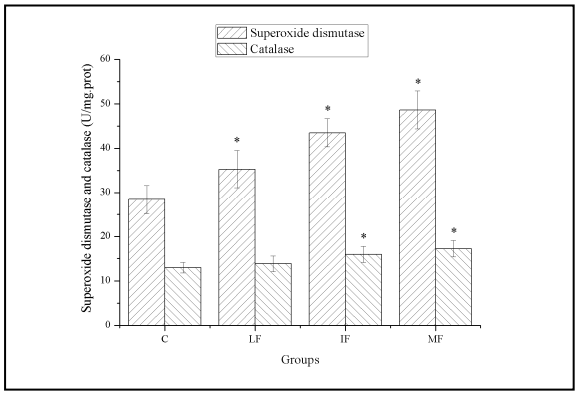

Figure 3. Effect of FEU on superoxide dismutase and catalase in liver. Data were presented as means ± SD. *, $p<0.05$ as compared with the control (C) group.

B. Effect of FEU on superoxide dismutase and catalase in liver

Principal antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). SOD forms the first line of defense against superoxide radicals as SOD dismutates superoxide radicals to form hydrogen peroxide (H_2O_2) and oxygen (O_2). CAT serves several biochemical functions, but the principal purpose of CAT is to catalyze the break-down of H_2O_2 into H_2O and O_2 (12). As shown in Figure 3, SOD activities in liver in the LF, IF and HF groups were significantly higher compared with that of the C group ($p<0.05$). Similarly CAT activities in liver in the IF and HF groups were also significantly higher than those observed in the C group ($p <0.05$). Although CAT activities in liver in the LF group were also higher than that in the C group, this difference was not significant ($p > 0.05$). These results indicate that FEU were able to up-regulate antioxidant enzyme activities to protect against exercise‐induced oxidative stress. This is probably due to the per se antioxidant activities of FEU.

C. Effect of FEU on xanthine oxidase in liver

Xanthine oxidase (XO), a metalloflavoprotein, is an important source of oxygen free radicals (14). It uses molecular oxygen as the electron acceptor, generating the superoxide anion as a by-product, contributing to oxidative stress during exercise (15). Many studies have indicated that XO activities were significantly increased in the circulation and tissue after exhaustive exercise (16). As shown in Figure 4, XO activities in liver in the LF, IF and HF groups were significantly lower compared with that of the C group ($p<0.05$). These results indicate that FEU could inhibit the elevation in XO activities, and affords protection against free radical production and liver oxidative injury after exhaustive exercise.

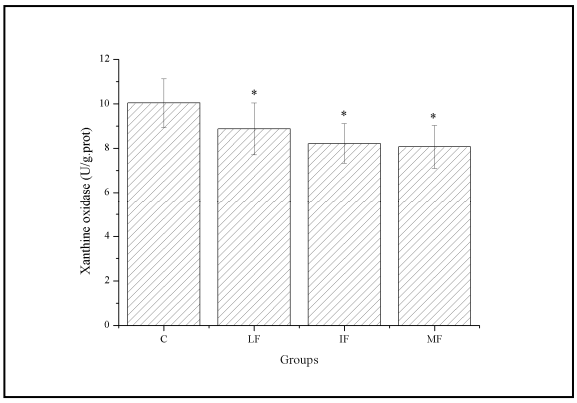

Figure 4. Effect of FEU on xanthine oxidase in liver. Data were presented as means ± SD. *, $p<0.05$ as compared with the control (C) group.

D. Effect of FEU on malondialdehyde in liver

It has been reported that strenuous exercise can accelerate lipid peroxidation due to oxidative stress. Malondialdehyde (MDA), a by-product of lipid peroxidation, is the most frequently studied marker of oxidative tissue damage during exercise (17). As shown in Figure 5, MDA levels in liver in the LF, IF and HF groups were significantly lower compared with that of the C group ($p<0.05$). These results indicate that FEU could reduce lipid per-oxidation and prevent the exercise-induced oxidative injury in liver.

E. Effect of FEU on 8-hydroxy-2'-deoxyguanosine in liver

Several studies have demonstrated that oxidative stress-induced DNA damage may play a significant role in processes that cause many chronic diseases. 8-hydroxy-2′-deoxyguanosine (8-OHdG) has been used widely in many studies not only as a biomarker for the measurement of oxidative DNA damage but also as a risk factor for many diseases. DNA can be oxidized to produce many oxidative products, however oxidation of the C-8 of guanine is one of the more common oxidative events, and results in a mutagenic lesion that produces predominantly G-to-T transversion mutations (18). As shown in Figure 6, 8-OHdG levels in liver in the LF, IF and HF groups were significantly lower compared with that of the C group ($p<0.05$)). These results indicate that FEU could reduce oxidative DNA damage in liver after exhaustive exercise.

This may be one of the mechanisms of Protective effects of FEU against exercise-induced oxidative stress.

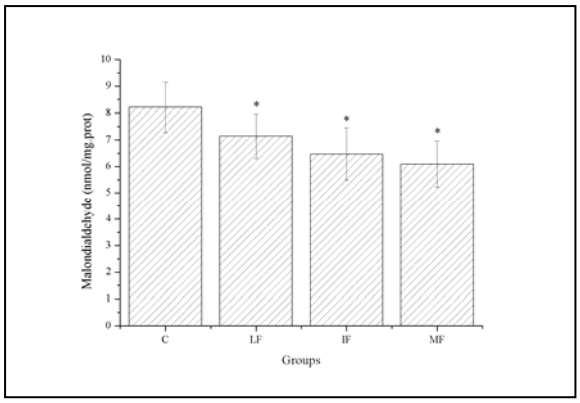

Figure 5. Effect of FEU on malondialdehyde in liver. Data were presented as means ± SD. *, $p<0.05$ as compared with the control (C) group.

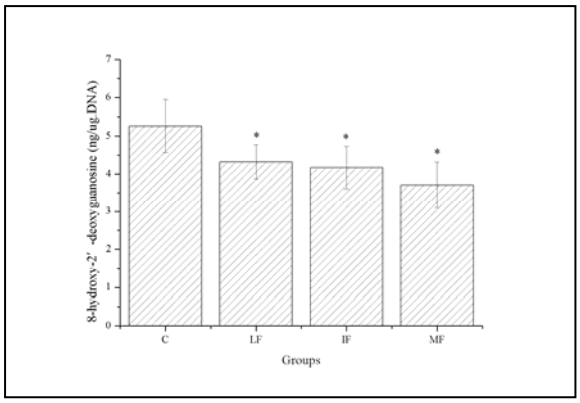

Figure 6. Effect of FEU on 8-hydroxy-2′-deoxyguanosine in liver. Data were presented as means ± SD. *, $p<0.05$ as compared with the control (C) group.

IV. Conclusions

The present study clearly demonstrates that FEU could increase body's antioxidant defenses, which consisting of non-enzymatic (GSH and GSH/GSSG ratio) and enzymatic (SOD and CAT) systems, inhibit the elevation in XO activities, decrease lipid peroxidation (MDA) and oxidative DNA damage (8-OHdG) in liver after exhaustive exercise. The finding of the study suggests that FEU possess protective effects against exhaustive exercise-induced oxidative stress and oxidative injury, which might be important in preventing loss of cellular function and warrants quick recovery after sports competition.

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