Bing and Wang **Afr J Tradit Complement Altern Med. (2010) 7(4):291-295**

# EFFECTS OF GINKGO BILOBA EXTRACT ON FREE RADICAL METABOLISM OF LIVER IN MICE DURING ENDURANCE EXERCISE

**Yan Bing* and Wang Zhaobao**

Dezhou University, Dezhou, Shandong Province, 253023, People's Republic of China.
College of Physical Education, 566 Daxue East Road . Dezhou District. Dezhou City
Dezhou University. PRC, 253023
***Email:** ybing959@sogou.com, ybing959@sina.com

## Abstract

This study investigated the effect of *Ginkgo biloba* extract on Free Radical Metabolism of Liver in mice during endurance exercise. Forty-eight mice were divided into the quiet group and the exercised group. And the two groups were both grouped again, including the control group and the drug-treated group. After exhaustive exercise, the exercised groups were subdivided into the immediate group and the recovery group. The swimming time to exhaustion significantly prolonged in the exercised drug-treated group as compared with the exercised control group ($P < 0.05$); The SOD activity of drug-treated groups significantly increased ($P < 0.05$) as compared with the control groups and MDA content was significantly lower ($P < 0.05$). The SOD activity and MDA content of exercised control groups significantly increased ($P < 0.05$) as compared with the quiet control group. The SOD activity and MDA content of exercised drug-treated groups significantly increased ($P < 0.05$) as compared with the quiet drug-treated group. The results indicated that *Ginkgo biloba* extract can obviously increase the body's endurance exercise capacity in mice and delay fatigue; *Ginkgo biloba* extract can help to increase the activity of the antioxidant enzymes in liver tissue, reduce the lipid peroxidation injury in liver tissue caused by free radicals, improve athletic ability, and promote the recovery process after exercise in mice.

**Key words:** *Ginkgo biloba* extract, Free Radicals, mice, endurance exercise

## Introduction

*Ginkgo biloba (Ginkgoaceae)* is a native tree from China that has been exhaustively cultivated in Europe, Australia, Japan, Korea, and the USA, because of its health-promoting properties (Shinozuka et al., 2002; Vilar et al., 2009). It has been used for 5000 years in traditional Chinese medicine. The leaves of the *Ginkgo biloba* tree, also known as maidenhair, are the source of this herb. *Ginkgo biloba* extract (EGB) is from green leaves of the *Ginkgo biloba* tree and the main ingredients of EGB contain 24% flavonoids (ginkgo-flavone glycosides) and 6 % terpenoids (including ginkgolides A, B, C, J and bilobalide) ( Peng et al., 2003; Zhou et al., 2006 ). EGB is well known for its antioxidant property due to its ability to scavenge free radicals and to neutralize ferry lion-induced peroxidation ( Bridi et al., 2001; He et al., 2006).

In the organism, free radicals always come from the redox intermediates. Under normal physiological conditions, the generation and removal of free radicals remained dynamic balance at low levels (Xu et al., 2002; Packer et al., 2008). Strenuous exercise can make the body ischemia and hypoxia, enhance the oxidation effect, make the free radicals rapidly increase, triggering a chain reaction and destruction of the cell structure, then cause lipid peroxidation and lead to body damage finally (Benderitter et al., 1996; Zhao et al., 2007). Both exercise and exercise-induced fatigue can cause an increase in free radicals in liver tissues of the body and cause liver cell's damage (Voces et al., 1999; Gul et al., 2006). Therefore, screening natural substances to obtain effective and non-toxic free radical scavengers is becoming a hot study spot in the field

of sports biology. The purpose of this study was to investigate the scavenge free radicals effect of EGB in order to explore the mechanism that this substance can delay the occurrence of exercise-induced fatigue and speed up the recovery process by swimming exercise.

## Materials and Methods

### Experimental animals

Purebred male Kun-Ming mice (20 ± 2 g) were purchased from the Experimental Animal Center of Shandong Province (Jinan,China). The mice were allowed to adapt to our laboratory environment for one week before the beginning of the experiment. They were housed in standard cages with free access to tap water and maintained in a room under standard conditions of feeding and temperature with a 12 h : 12 h light-dark cycle. This animal study was approved by the Ethical and Research Committee of the Dezhou University.

### Reagents

EGB were provided by Guizhou Xinbang Pharmaceutical Company, China. The contents of flavonoids and terpenoids were used as quality control standard (24 %±1 % and 6.0%±0.5 % , respectively). SOD and MDA detection kits were provided by Rongsheng Biotechnology Company, China. Test methods and formulas were used in strict accordance with the instructions of the kits.

### Experimental design

Forty-eight mice were randomly divided into two groups, the quiet group (Q group, 16 mice) and the exercised group (E group, 32 mice). The mice of quiet group were not exercised, while the mice of exercised group were exercised to swim for 4 weeks. The swimming exercise was carried out in a tank (30×50×30cm), filled with water to 25 cm depth and maintained at a temperature of 30± 1 □. During the first week, the swimming exercise time was 30 min every day; during the following three weeks, the swimming exercise time was respectively 35 min, 40 min and 45min.

The two groups (Q group and E group) were both grouped again, including the control group (C group) and the drug-treated group (D group). The 4 groups were the quite control group (QC group, 8 mice), the exercised control group (EC group, 16 mice), the quite drug-treated group (QD group, 8 mice), and the exercised drug-treated group (ED group, 16mice). The drug-treated groups received EGB in doses of 100 mg/kg by stomach every morning. In the control groups of mice, saline was given instead of EGB at the same dose for 4 weeks.

At the end of a four-week period, the mice were killed by using ether anesthesia. Before being killed the mice were weighed and then were forced to swim without a load until being exhausted and the mice were considered to be exhausted when they failed to rise to the surface of the water to breathe within a 7-s period( Fushiki et al., 1995). After exhaustive exercise, the exercised group (E group) were subdivided into two groups, including the immediate group (IE group, 16 mice) and the recovery group (IR group, 16 mice). The 4 groups were the immediate exercised control group (IEC group, 8 mice), the recovery exercised control group (REC group, 8 mice), the immediate exercised drug-treated group (IED group, 8 mice), and the recovery exercised drug-treated group (RED group, 8 mice). The mice of immediate group were killed immediately after exhaustive exercise and the mice of recovery group were killed after recovering for 24h after exhaustive exercise. Liver was excised from the mice and placed in ice-saline to clear off blood, then the liver were dried with filter paper and weighed. Then the liver was homogenized in ice-cold 0.15M Tris-KCl buffer (pH 7.4) to yield a 10% (w/v) homogenate. The latter was next subjected to high-speed centrifugation at 15000 r/min for 30 minutes at 4 □. The resulting supernatant was used as such

for assaying Superoxide dismutase (SOD) and Malondialdehyde (MDA).

**Statistical analyses**

All the data is expressed as a mean ± standard deviation (S.D.). A one-way ANOVA using SPSS ver. 10.0 software was used for multiple comparisons. A value of $p < 0.05$ was considered to be significant.

## Results

**Effects of EGB on forced swimming capacity**

The forced swimming capacities are shown in Fig. 1.There are significant differences in the swimming time to exhaustion between the ED group and the EC group（P <0.05）.

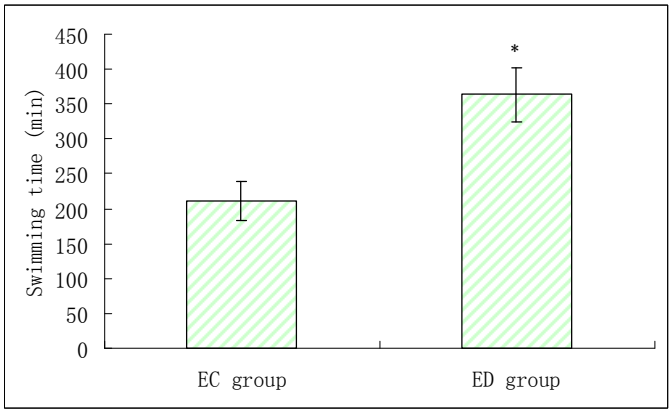


**Figure 1:** Effects of EGB on swimming time to exhaustion in mice

n =16; Mean ± S.D. # p<0.05 compared with the EC group.

**Effects of EGB on the SOD activity and MDA content of Liver in mice**

The effects of EGB on the SOD activity and MDA content of Liver in mice are shown in Table 1. The SOD activity of D groups (QD, IED, RED) significantly increased (P <0.05) as compared with their respective control groups (QC, IEC, REC) and MDA content were significantly lower (P <0.05). The SOD activity and MDA content of EC groups (IEC, REC) significantly increased (P <0.05) as compared with the QC group. The SOD activity and MDA content of ED groups (IED, RED) significantly increased (P <0.05) as compared with the QD group.

**Table 1:** Effects of EGB on the SOD activity and MDA content of Liver in mice

| Group | SOD(U/mg pro) | MDA(nmol/mg pro) |
|---|---|---|
| QC | 272.21±6.79 | 8.63±1.26 |
| QD | 324.25±11.24 [a] | 6.52±0.87 [a] |
| IEC | 301.24±7.15 [b] | 17.67±0.92 [b] |
| IED | 394.42±9.25 [a c] | 14.96±1.21 [a c] |
| REC | 327.58±8.79 [b] | 12.24±1.16 [b] |
| RED | 374.19±9.69 [a c] | 9.19±0.95 [a c] |

n =8; Mean ± S.D. [a] p<0.05 compared with the control groups (QC, IEC, REC); [b] p<0.05 compared with the QC group; [c] p<0.05 compared with the QD group.

## Discussion

Medicinal properties have been attributed to many plants for thousands of years (Mau et al., 2001; Ramesh et al., 2008). The medicinal plant extracts are widely sold as nutritional supplements or tonic, and touted as beneficial for health (Borchers et al.,1999; Jung et al., 2004). In the present study, the anti-fatigue effect of *Ginkgo biloba* extract (EGB) was investigated in male Kun-Ming mice by using the forced swimming capacity test (Long et al., 2008). The swimming time to exhaustion significantly prolonged in the ED group as compared with the EC group. ($P < 0.05$), which indicated that EGB can significantly increase the body's endurance exercise capacity, and delay the fatigue obviously.

ROS may damage body tissues, if their production is not controlled precisely and adequately. To combat the deleterious effects of free radicals and ROS, the body has some complex internal protective mechanisms like enzymatic defenses and non-enzymatic defenses (Gupta et al., 2009). SOD is the main antioxidant enzymes in the body, and its main function is to disproportion superoxide anion into $H_2O_2$ and $O_2$ and the functions in the body become particularly important (Liu et al., 2004; Zeng et al., 2006). In the present study, the data showed that after having been exercised for 4 weeks, the SOD activity in the exercised groups significantly increased ($P < 0.05$) as compared with the quiet group, which can be seen as positive adaptation changes in the body to training; in another words, exercise can improve the SOD activity; the SOD activity of liver tissue in the drug-treated group significantly increased ($P < 0.05$) as compared with the control group, which indicated that EGB can improve the activity of antioxidant enzymes in liver tissue, reduce the lipid peroxidation damage caused by free radicals, thereby protect the integrity of the liver cell membrane, and keep the physiology of the liver operating normally, finally enhance the athletic ability and promote the recovery process after exercise in mice.

During strenuous exercise or high-intensity endurance exercise, the generation of oxygen free radicals increased heavily, and the MDA is one of the main products of lipid peroxidation induced by the free radicals. Therefore, determining the MDA content in tissue can evaluate the degree of lipid peroxidation and indirectly assess the body's antioxidant capacity (Wang et al., 2008; Lyle et al., 2009). In the present study, the data showed after exercised for 4 weeks, the MDA content of liver tissue in the exercised group significantly increased ($P < 0.05$) as compared with the quiet group. This shows that when doing high-intensity endurance exercise, the body is in a strong oxidative stress state, and the generation and elimination of free radicals in body can not keep balance, leading to the increase of free radicals and oxidation of the liver tissue; the MDA activity of liver tissue significantly reduced in the drug-treated group as compared with the control group, which indirectly reflected the strong anti-lipid peroxidation effects of EGB, indicating that the EGB can help to improve the activity of antioxidant enzymes in exercised body, diminish the exercise-induced lipid peroxidation on a certain degree, and have significant effects on getting rid of free radicals.

In conclusion, *G. biloba* extract can obviously increase the body's endurance exercise capacity in mice and delay fatigue; it can help to increase the activity of the antioxidant enzymes in liver tissue, reduce the lipid peroxidation injury in liver tissue caused by free radicals, improve athletic ability, and promote the recovery process after exercise in mice.

## References

1.Benderitter, M., Hadj-Saad, F., Lhuissier, M., Maupoil, V., Guilland, J.C. and Rochette, L. (1996). Effects of exhaustive exercise and vitamin b6 deficiency on free radical oxidative process in male trained rats. Free Radical Biol. Med. **21:** 541-549.

2.Banister, E.W., Allen, M.E., Mekjavic, I.B., Singh, A.K., Legge, B., Mutch, B.J.C. (1999). The time course of ammonia and lactate accumulation in blood during bicycle exercise. European J. Applied Physiol., **51:** 195–202.

3.Bridi, R., Crossetti, F.P., Steffen, V.M. and Henriques, A.T. (2001). The antioxidant activity of standardized extract of Ginkgo biloba (EGb 761) in rats. Phytother. Res., **15:** 449-451.

4.Fushiki, T., Matsumoto, K., Inoue, K., Kawada, T. and Sugimoto, E. (1995). Swimming endurance capacity of mice is increased by chronic consumption of medium-chain triglycerides. Nutrient Metabolism, **125:** 531-539.

5.Gul, M., Demircan, B., Taysi, S., Oztasan, N., Gumustekin, K., Siktar, E., Polat, M.F., Akar, S., Akcay, F. and Dane, S. (2006). Effects of endurance training and acute exhaustive exercise on antioxidant defense mechanisms in rat heart. Comp. Biochem. Physiol. - Part A: Mol. & Integrative Physiol., **143:** 239-245.

6.Gupta, C., Gupta P.H. and Singh, B. (2009). Effect of Vitamin Supplementation on Exercise Induced Oxidative Stress in Trained Elite Indian Cyclists. Am J. Biomed. Sci., **1:** 166-170.

7.He, S.X., Luo, J.Y., Wang, Y.P., Wang, Y.L., Fu, H., Xu, J.L., Zhao, G. and Liu, E.Q. (2006). Effects of extract from Ginkgo biloba on carbon tetrachloride-induced liver injury in rats. Wld J.Gastroenterol., **12:** 3924-3928.

8.Jung, K., Kim, I.H., Han, D. (2004). Effect of medicinal plant extracts on forced swimming capacity in mice. J. Ethnopharmacol., **93:** 75–81.

9.Liu, H., Zhang, D., Zhao, B. and Zhao, J. (2004). Superoxide anion, the main species of ROS in the development of ARDS induced by oleic acid. Free Radical Res., **38:** 1281-1287.

10.Long, B.B., Zhang, X.D. and Shu, J.W. (2008). Effect of Gvnoslemma penlaphllum on training rat liver's free radical metabolism and serum enzymein. J. Hainan Normal University(Natural Science), **21:** 193-195.

11.Lyle, N., Gomes, A., Sur, T., Munshi, S., Paul, S., Chatterjee S. and Bhattacharyya, D. (2009). The role of antioxidant properties of Nardostachys jatamansi in alleviation of the symptoms of the chronic fatigue syndrome. Behavioural Brain Res., **202:** 285-290.

12.Mau, J.L., Lin, H.C., Chen, C.C. (2001). Non-volatile components of several medicinal mushrooms. Food Re. Intern., **34:** 521–526.

13.Packer, L., Cadenas, E. and Davies, K.J.A. (2008). Free radicals and exercise: An introduction. Free Radical Biol. Med., **44:** 123-125.

14.Peng, H., Li, Y.F. and Sun, S.G. (2003). Effects of Ginkgo biloba extract on acute cerebral ischemia in rats analyzed by magnetic resonance spectroscopy. Acta Pharmacologica Sinica, **24:** 467-471.

15.Ramesh., Putheti., Okigbo, R.N. (2008). Effects of plants and medicinal plant combinations as anti-infectives. Afr J. Pharm. Pharmacol., **2:** 130-135.

16.Shinozuka, K., Umegaki, K., Kubota, Y. and Tanaka, N. (2002). Feeding of Ginkgo biloba extract (GBE) enhances gene expression of hepatic cytochrome P-450 and attenuates the hypotensive effect of nicardipine in rats. Life Sciences, **70:** 2783-2792.

17.Vilar, J.B., Leite, K.R. and Chen, L.C. (2009). Antimutagenicity protection of Ginkgo biloba extract (Egb 761) against mitomycin C and cyclophosphamide in mouse bone marrow. Genetics Molecular Res. **8:** 328-333.

18.Voces, J., Alvarez, A.I., Vila, L., Ferrando, A., Cabral de Oliveira, C. and Prieto, J.G. (1999). Effects of administration of the standardized Panax ginseng extract G115 on hepatic antioxidant function after exhaustive exercise. Comp. Biochem. Physiol. Part C: Pharmacol., Toxicol. Endocrinol., **123:** 175-184.

19.Wang, L., Zhang, H.L., Zhou, Y.J., Ma, R., Lv, J.Q., Li, X.L., Chen, L.J. and Yao, Z. (2008). The decapeptide CMS001 enhances swimming endurance in mice. Peptides, **29:** 1176-1182.

20.Xu, D.Q., Xia, S.Y. and Cao, F. (2002). Development of Study on Exercise and Metabolism of Free Radical. J. PLA Institute of Physical Education, **21:** 39-43.

21.Zeng, M., Li, S.H., Guo, C.C., Li, Z.E., Wang, P. and Liu, X.W. (2006). Research on the Effects of the Extract of Pine Needles on Free Radicals Metabolism of the Skeletal Muscle of Rats. J. Beijing Sport University, **29:** 484-488.

22.Zhou, Y.H., Yu, J.P., Liu, Y.F., Teng, X.J., Ming, M., Lv, P., An, P., Liu, S.Q. and Yu, H.G. (2006). Effects of Ginkgo biloba Extract on Inflammatory Mediators (SOD,MDA, TNF-α, NF-κBp65, IL-6) in TNBS-Induced Colitis in Rats. Mediators of Inflammation, **10:** 1-9.