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*Full Length Research Paper*

# Antifatigue activity of water extracts of *Toona sinensis* Roemor leaf and exercise-related changes in lipid peroxidation in endurance exercise

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**This study evaluated the antifatigue activity of water extracts of *Toona sinensis* Roemor leaf (ETL) and exercise-related changes in lipid peroxidation in swimming exercise of mice. Male Kunming mice were studied by being divided into four groups (n = 30 mice per group): low, medium, high-dose ETL treated groups and control group. Swimming endurance experiment were conducted after 21 days of ETL supplementation and the result showed that ETL was found to possess an antifatigue activity, extend the swimming time of the mice, prevent the increase in lactic acid, increasing content of liver and muscle glycogen of mice and may decrease the contribution of exercise-induced oxidative stress.**

**Key words:** Antifatigue activity, water extracts of *Toona sinensis* leaf, lipid peroxidation, endurance exercise.

## INTRODUCTION

*Toona sinensis* Roemor (Cedrela sinensis A. Juss) is an upland tree that is widely distributed at the higher altitude eastwards from India and Nepal through China, Burma, Thailand and Malaysia to Java and was first introduced to Europe in 1862 (Edmonds and Staniforth, 1998). A number of compounds including retinoid, vitamins B and C, o-coumaric acid, kaempferol, methyl gallate, quercetin, afzelin, quercitrin, isoquercitrin, rutin, cedrellin and phytol derivatives have been isolated from this plant (Park et al., 1994; Park et al., 1996; Luo et al., 2000). Its bark, oil, seed, flower and leaf have been used in traditional Chinese medicine. The fresh young leaves and shoots, known as Chinese toon and a tree vegetable, is one of the most popular vegetables in China. The leaves of *T. sinensis* were used medicinally for the treatment of heliosis, vomitting, dysentery, lack of appetite and enteritis in the folk in China, due to their effects in detoxification, antiinflammation (Wang et al., 2007). Water extracts of *T. sinensis* leaf (ETL) has been proved to possess antiproliferative effects and also to promote the apoptosis of human lung cancer cells (Chang et al., 2001). Hsu et al. (2003) reported that ETL could increase lipolytic activity in 3T3-L1 adipocytes by mediating the protein kinase C pathway. In addition, studies have pointed out that ETL is able to enhance glucose uptake in 3T3-L1 adipocytes (Yang et al., 2003), increase the level of GLUT 4 protein and mRNA expression and reduce blood sugar level in alloxan-induced diabetes mellitus mice (Liao et al., 2006). Previous phytochemical investigations showed that the leaves of *T. sinensis* were rich in flavonoids, alkaloids, terpenes and anthraquinones (Chen et al., 2000; Luo et al., 2001). However, no detailed study has been reported on the antifatigue activity of water extracts of *T. sinensis* leaf (ETL). This study evaluated the antifatigue activity of water extracts of *T. sinensis* leaf (ETL) by swimming exercise of mice. Furthermore, exercise-related changes in lipid peroxidation were also determined. The results obtained from this study may offer further information to provide scientific evidence for development of ETL as a potential natural antifatigue agent in food.

## MATERIALS AND METHODS

### Plant material

The leaves of *T. sinensis* were purchased locally in Nanchong, Sichuan Province in September, 2008 and identified in the Depart-

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ment of Botany of China West Normal University (Voucher number: 08641; deposited in: Herbarium; Director: Dr. Wang).

### Preparation of the leaves water extract

*T. sinensis* leaves were cut into small pieces, weighed and extracted with reverse osmosis water (1:4 w/v) at 100℃ for 30 min and then cooled down slowly without further boiling for 2 h at room temperature. The leaves were then removed and the remaining liquid was concentrated over low heat and was filtered with a sieve (70-mesh). The filtrated concentrate was designed as crude water extract of *T. sinensis* leaves (ETL). The ETL was then centrifuged at 3000 rpm at 4℃ and the suspension portion was further lyophilized with a Virtis apparatus to obtain the lyophilized powder (ETL-l) (Li et al., 2002; Liao et al., 2007). Through this procedure, the ETL-l was obtained at yields of 5.84%. Each dose of ETL-l testing sample was prepared by dissolving lyophilized powder with distilled water through the experiment.

### Selection of animals

Male Kunming mice (6 - 8 weeks old and 18 - 22 g weight) were from the Experimental Animal Center of China West Normal University. The mice were housed in colony cages (five mice per cage) at an ambient temperature of 25 ± 2℃ with 12 h light and 12 h dark cycle. The mice were fed normal diets purchased commercially from vendors. Animal procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and approved by the Animal Ethics Committee of the University. The mice were allowed to acclimatize to the laboratory environment for 1 week and then randomly divided into four groups (n = 30 mice per group) based on body weight as given below: low, medium, high-dose ETL treated groups and control group. The control group was fed with distilled water by gavage and the 3 treated groups were fed different doses of ETL-l (40, 80 and 160 mg/kg) by gavage for 21 consecutive days, respectively.

### Swimming endurance experiment

Ten mice were taken out from each group to make swimming test after being administrated with different dose of ETL for 21 days. Each mouse's tail was loaded with galvanized wire, which was 5% of its body weight, then they were pulled into different swimming boxes (80 × 50 × 50 cm), respectively, which were filled with water (temperature: 25 ± 1℃, depth: 30 cm), the swimming endurance of the mouse was observed. The endurance time was defined as the time mice kept swimming activity until it them sank into the bottom of swimming boxes and stopped moving for at least 10 s (Abe et al., 1995; Yu et al., 2008). The time of each group of mice was averaged and the data of the different groups was analyzed with T-test.

### Determination of body weight, blood lactic acid, hepatic and muscle glycogen

Body weights were measured by electronic balance before and after the experiment.

After a period of 21 days, ten mice were taken out from each group for blood lactic acid (BLA) analyses. The blood samples were collected from eye sockets of mice 30 min after intragastric administration and 30 min after weight loading swimming (2% body weight), respectively (Ma et al., 2008; Wang and Zhu, 2008). Then BLA was tested according to the recommended procedures provided by the kit (Jiancheng Chem. Co., St. Nanjing, Jiangsu, China).

After a period of 21 days, ten mice were forced to swim for 90 min without a load. After an hour's resting, the mice were killed to collect liver and gastrocnemius muscle for liver glycogen and muscle glycogen analyses (Ma et al., 2008; Wang and Zhu, 2008; Zhang et al., 2008). Hepatic and muscle glycogen were tested according to the recommended procedures provided by the kits (Jiancheng Chem. Co., St. Nanjing, Jiangshu, China).

### Determination of serum lipid peroxidation

Serum lipid peroxidation was measured by using superoxide dismutase (SOD) and Malondiadehycle (MDA) (Li and Wen, 2006; Hu et al., 2008). After a period of 21 days, ten mice were forced to swim for 90 min without a load. The mice were killed to collect plasma samples. The concentration of serum SOD and MDA were tested according to the recommended procedures provided by the kits (Jiancheng Chem. Co., St. Nanjing, Jiangshu, China).

### Data analysis

Statistical analysis was carried out using ANOVA followed by post-hoc Turkey test (SPSS 15.0 for Windows). The rejection of null hypothesis was set at 5%.

## RESULTS

### Effects of ETL on body weight

As shown in Figure 1, the body weight of mice in 3 treated groups was not different significantly from that in the control group before and after experiment ($p > 0.05$), which means the ETL has no effect on body weight.

### Effect of ETL on the swimming endurance time

The swimming endurance of the mouse is shown in Figure 2. There are significant differences in the swimming endurance time between the control group and each treatment group. The swimming endurance time of the control, low-dose, medium-dose and high-dose groups were 927 ± 114, 1845 ± 186, 2471 ± 221 and 2813 ± 267 s, respectively. Thus, the swimming endurance times of the treated groups were significantly longer than that of the control group ($P < 0.05$).

### Effect of ETL on the level of blood lactic acid

As shown in Figure 3, there was no significant difference in the levels of blood lactic acid between each treatment group and the control group before swimming ($P > 0.05$). After swimming, the levels of blood lactic acid of treatment groups were significantly lower than that of control group ($p < 0.05$). These results hinted that ETL could prevent the increase of blood lactic acid of mice after swimming.

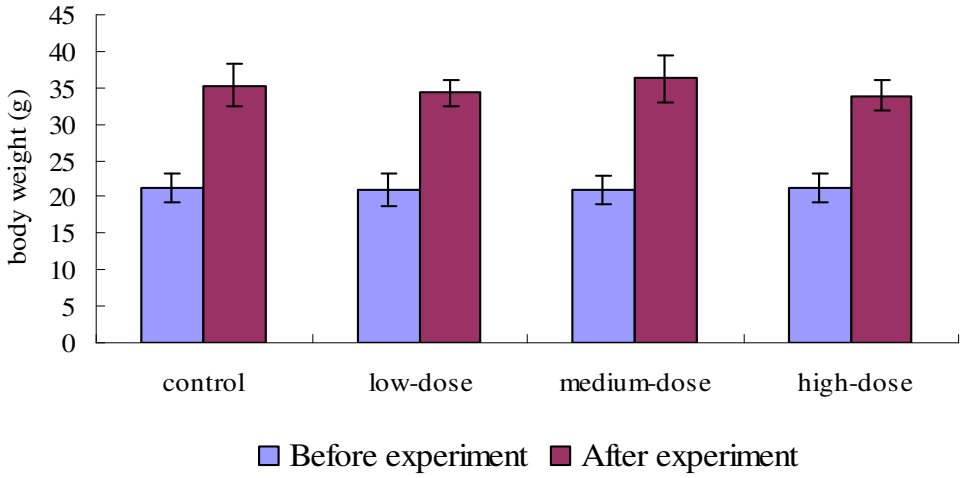


**Figure 1.** Effects of ETL on body weight of mice (mean ± SD, n = 30).

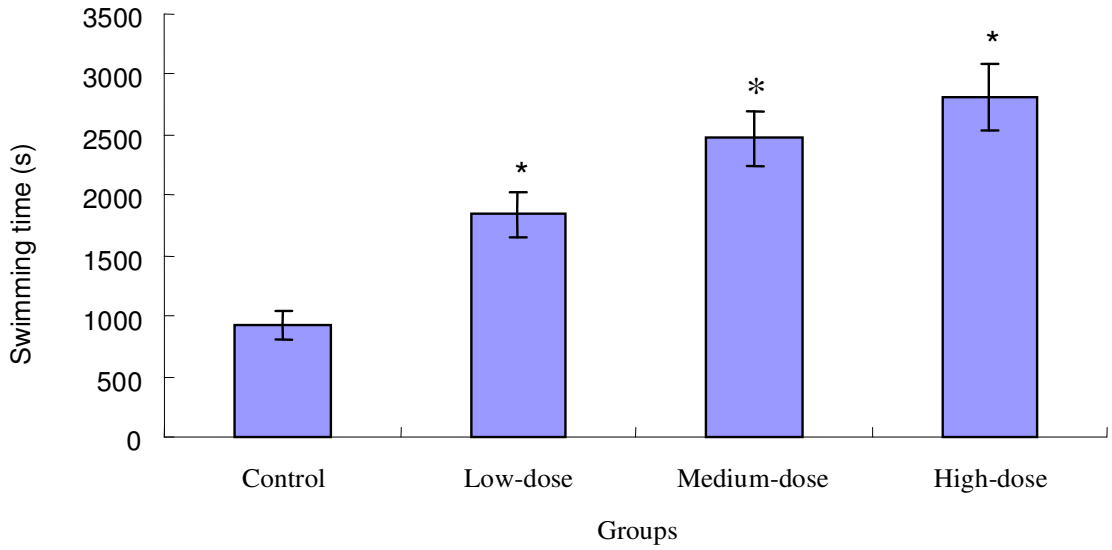


**Figure 2.** Effects of ETL on the swimming endurance time of mice (mean ± SD, n = 10). *p < 0.05 as compared with the control group.

### Effect of ETL on contents of hepatic and muscle glycogen

As shown in Table 1, after swimming, the content of hepatic and muscle glycogen of treatment groups were higher than that of control group ($p < 0.05$). These data indicated that ETL could significantly increase the content of glycogen of mice after swimming.

### Effect of ETL on contents of serum SOD and MDA

As shown in Table 2, after swimming, the content of serum SOD of treatment groups was higher than that of control group ($p < 0.05$). The content of serum MDA of treatment groups were significantly lower than that of the control group ($p < 0.05$). Thus, it is evident that ETL possesses the ability of reducing lipid peroxide and exerted beneficial antioxidant defense actions after swimming.

## DISCUSSION

Medicinal properties have been attributed to many plants for thousands of years (Mau et al., 2001; Jung et al., 2004). The medicinal plant extracts are widely sold as nutritional supplements or tonic and touted as beneficial for health (Borchers et al., 1999). In particular, medicinal plants are useful against some kinds of cancers, lipid

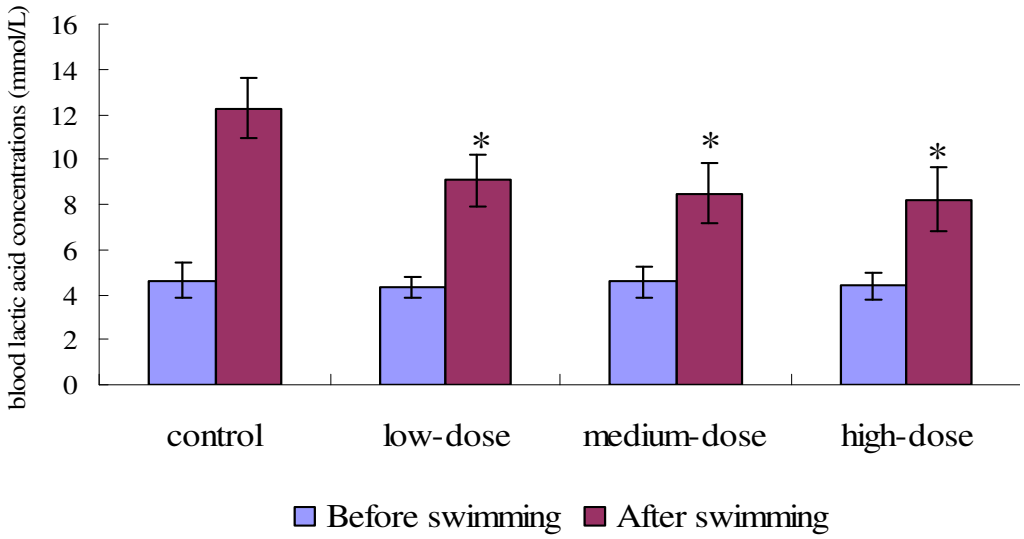


**Figure 3.** Effects of ETL on blood lactic acid concentrations of mice (mean ± SD, n = 10). *p < 0.05 as compared with the control group.

**Table 1.** Effects of ETL on hepatic and muscle glycogen of the mice after swimming (mean ± SD, n =10).

| Groups | Muscle glycogen (mg/g) | Hepatic glycogen (mg/g) |
|---|---|---|
| control | 0.39 ± 0.11 | 1.12 ± 0.37 |
| low-dose | 0.57 ± 0.19* | 3.08 ± 0.69* |
| medium-dose | 0.66 ± 0.13* | 3.92 ± 0.74* |
| high-dose | 0.74 ± 0.12* | 4.12 ± 0.83* |

*p < 0.05 as compared with the control group.

**Table 2**. Effects of ETL on serum SOD and MDA of the mice after swimming (mean ± SD, n =10).

| Groups | SOD (U/mL) | MDA (nmol/mL) |
|---|---|---|
| control | 184.27 ± 27.62 | 30.23 ± 5.48 |
| low-dose | 247.56 ± 16.35* | 21.29 ± 6.17* |
| medium-dose | 253.21 ± 21.34* | 22.47 ± 5.84* |
| high-dose | 230.41±19.63* | 25.38±5.39* |

*p < 0.05 as compared with the control group.

peroxidation, hypercholesterolemia, immune function, etc. as known in China, Russia, Japan, Korea, as well as the USA and Canada (Jung et al., 2004). Unfortunately, the database on the tonic effects of medicinal plants is still rather limited.

In the present study, the antifatigue activity of water extracts of *T. sinensis* leaf (ETL) was observed by swimming exercise of mice. It is commonly accepted that swimming is an experimental exercise model (Lapvetelainen et al., 1997). The data showed that different doses of ETL especially medium-dose and high-dose could significantly lengthen the longest swimming time, which indicated that ETL could elevate the exercise tolerance of mice.

Blood lactate is the glycolysis product of carbohydrate under an anaerobic condition and glycolysis is the main energy source for fierce exercise in a short time. Therefore, blood lactate is closely related to workload intensity and is one of the important indicators for judging the intensity of the exercise or the degree of fatigue. In other

words, blood lactate represents the degree of fatigue after exercise and the condition of recovery (Wang et al.,2006). In this study, the data showed that different doses of ETL could effectively delay the increasing of lactate in the blood.

Energy for exercise is derived initially from the breakdown of glycogen and later from circulating glucose released by the liver (Suh et al., 2001). So liver and muscle glycogen are sensitive parameters related to fatigue (Ma et al., 2008). The data mentioned above revealed that the content of liver and muscle glycogen of mice in ETL treatment groups were higher than that of control group after swimming. However, its detailed mechanism is not clear. The possible reason is that ETL may increase the content of liver and muscle glycogen of mice post-exercise by improving glycogen reserve, or by reducing the consume of glycogen during exercise, or both (Ma et al., 2008). It still needs further studies.

Oxygen free radical was believed to have something to do with the sickness and aging of humankind. During the metabolism process of a normal person, one can produce many reactive oxygen species (ROS), and the toxic product would gradually accumulate on the cell and the tissue, which causes irreversible oxidized damage. Besides, strenuous exercise caused oxidative stress to the body and resulted in tissue damage (Armstrong, 1990). During exercise, a large amount of oxygen was consumed and the rate of oxygen-free-radical generation was accelerated due to the electron transport chain activity (Kanter, 1994). Also, earlier studies showed that lipid peroxidation was increased after physical exercise (Davies et al., 1982). Lipid peroxidation was frequently used as an indication of tissue oxidative stress as a result of a free radical attacking the cell membrane (Cho and Choi, 1994). The metabolite produced during the lipid peroxidation process can be used as the method to judge oxidized injury (Wang et al., 2006). For example, the formation of an end product during the lipid peroxidation process such as aldehydes and the increase in SOD concentration; can all be used to judge the degree of lipid peroxidation. In this study, the data showed that the content of serum SOD of treatment groups were higher and the content of serum MDA of treatment groups were lower than that of the control group. The SOD activity increase may be the result of a self-protective mechanism which was developed from superoxide-generated radicals operating in aging or fatigue. It can be infer that ETL possesses the ability of reducing lipid peroxide and exerted beneficial antioxidant defense actions.

In conclusion, ETL was found to possess an antifatigue activity, extend the swimming time of the mice, prevent the increase in lactic acid and increasing content of liver and muscle glycogen of mice. In addition, the result suggested that the ETL supplementation may decrease the contribution of exercise-induced oxidative stress. However, further study is needed to elucidate the more exact mechanism of the effect of ETL on fatigue and exercise durability.

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