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Anti-Fatigue Effect of Green Tea Polyphenols (-)-Epigallocatechin-3-Gallate (EGCG)

[Yu-song Teng](#) and [Di Wu](#)

School of Physical Education, Liaoning Normal University, Dalian, P.R. China

Correspondence: Prof. Yu-song Teng, Yellow River Road, Shahekou District, Dalian, P.R. China. E-mail:

tengyuso@sina.com

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Abstract

Background:

(-)-Epigallocatechin-3-gallate (EGCG) is the most abundant of the green tea polyphenols that exhibit a variety of bioactivities. The objective of this study was to evaluate the anti-fatigue effect of EGCG by forced swimming exercise.

Materials and Methods:

The mice were divided into one control group and three EGCG-treated groups. The control group was administered with distilled water and EGCG-treated groups were administered with different dose of EGCG (50, 100, and 200 mg/kg) by oral gavage for 28 days. On the last day of experiment, the forced swimming exercise was performed and corresponding biochemical parameters were measured.

Results:

The data showed that EGCG prolonged exhaustive swimming time, decreasing the levels of blood lactic acid, serum urea nitrogen, serum creatine kinase and malondialdehyde, which were accompanied by corresponding increase in liver and muscle glycogen contents, and superoxide dismutase, catalase, and glutathione peroxidase activities.

Conclusions:

This study indicated that EGCG had an anti-fatigue effect.

SUMMARY

- EGCG significantly prolonged exhaustive swimming time and decreased the levels of BLA, SUN, SCK and MDA, which were accompanied by corresponding increases in liver and muscle glycogen contents, and SOD, CAT, and GPx activities.
- EGCG can be used to design nutraceutical supplements aimed to facilitate recovery from fatigue and attenuate exhaustive exercise-induced oxidative damage.

Abbreviations used: EGCG: (-)-Epigallocatechin-3-gallate, ROS: reactive oxygen species, BLA: blood lactic acid, SUN: serum urea nitrogen, SOD: superoxide dismutase, GPx: glutathione peroxidase, CAT: catalase, SCK: serum creatine kinase, MDA: malondialdehyde, C: control, LET: Low-dose EGCG-treated, MET: Middle-dose EGCG-treated, HET: High-dose EGCG-treated, GTE: green tea extract.

Keywords: (-)-Epigallocatechin-3-gallate, anti-fatigue, biochemical parameters, forced swimming exercise, mice

INTRODUCTION

Fatigue, defined as physical and/or mental weariness resulting from exertion, is an inability to continue exercise at the same intensity with a resultant deterioration in performance.[1] Fatigue can be classified as secondary, physiologic, or chronic. Secondary fatigue results from disturbed sleep, depression, excess exertion, and medication side effects. Physiological fatigue is caused by inadequate rest, physical effort or mental strain.[2] Chronic or accumulated fatigue can affect an individual's performance. In addition, long-term accumulated fatigue can lead to Karoshi (death as a result of overwork).[3] During strenuous physical exercise, oxygen flux to active skeletal muscles increases, which leads to enhanced production and accumulation of excess reactive oxygen species (ROS).[4] Leakage of electrons from the mitochondrial electron transport chain, xanthine oxidase reaction, haemoglobin oxidation and activated neutrophils have been identified as major sources of intracellular ROS generation during exercise.[5] The accumulation of ROS will put the body in a state of oxidative stress and may cause injury to the body by attacking large molecules and cell organs, resulting in physical fatigue.[6] Previous studies have also shown that exogenous antioxidants from diet interact with endogenous antioxidants to form a cooperative antioxidant network, preventing exercise-induced oxidative stress and reducing physical fatigue by scavenging the free radical and ROS.[7]

Green tea, made from the harvested leaves of *Camellia sinensis* that have undergone minimal oxidation, has been widely used as both a beverage and a medicine in most countries of Asia, including China, Japan, Thailand, and Vietnam.[8] Green tea has proven to have beneficial biological effects, such as the prevention of cancers, cardiovascular diseases, dental decay, obesity, diabetes, and improvement in the immune system.[9] The beneficial effects of green tea are believed to be mediated by its polyphenols, which may account for up to 30% of the green tea dry weight.[10] Green tea polyphenols mainly include (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), and catechin. The most abundant polyphenol in green tea is EGCG, which has shown to exhibit bioactivities such as antioxidant, anticancer, anti-obesity, antibacterial, hepatoprotective, neuroprotective and others.[11,12] However, little information about the anti-fatigue effect of EGCG is currently known. Therefore, the present study was designed to evaluate the anti-fatigue effect of EGCG by forced swimming exercise of mice.

MATERIALS AND METHODS

Chemicals and reagents

(-)-epigallocatechin-3-gallate (EGCG) (>98% purity) was purchased from Meilun Biological Technology Co. Ltd. (Dalian, China). Commercial diagnostic kits used to determine blood lactic acid (BLA), serum urea nitrogen (SUN), glycogen, superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) were purchased from BioSino Bio-Technology Science Inc. (Beijing, China). Commercial diagnostic kits used to determine serum creatine kinase (SCK) and malondialdehyde (MDA) were purchased from Shuangying Biological Technology Co. Ltd. (Shanghai, China). All other chemicals used were of analytical grade.

Experimental animals

Male Kunming mice (mean weight 20 ± 2 g) were purchased from the Laboratory Animal Center of Liaoning Normal University (Dalian, China). The animal breeding room was maintained under a constant 12-h light: 12-h dark cycle with a temperature of $23 \pm 1^\circ\text{C}$, relative humidity of $55 \pm 5\%$ and noise less than 50 dB throughout the experimental period. The mice had unlimited access to standard laboratory diet (32% being carbohydrates; 22% being proteins; 10% being lipids and 3% being vitamins) and tap water.

Local regulations related to ethical experimentation on animals and guidelines for the care and use of laboratory animals were followed in all animal procedures in this experiment. The protocols for animal experiments were approved by the Animal Ethics Committee of the Liaoning Normal University.

Experimental design

After two weeks of acclimation, animals were divided into four groups, each consisting of 12 mice.

Control (C) group: animals were administrated with distilled water (1.5 mL) by oral gavage once a day for 28 days.

Low-dose EGCG-treated (LET) group: animals were administrated with EGCG solution (50 mg/kg body weight) by oral gavage once a day for 28 days.

Middle-dose EGCG-treated (MET) group: animals were administrated with EGCG solution (100 mg/kg body weight) by oral gavage once a day for 28 days.

High-dose EGCG-treated (HET) group: animals were administrated with EGCG solution (200 mg/kg body weight) by oral gavage once a day for 28 days.

EGCG solution was prepared through dissolving it in 1.5 mL of distilled water. The body weight was measured once per week. After 28 days, the forced swimming exercise were performed and corresponding biochemical parameters such as BLA, SUN, SCK, tissue glycogen, SOD, GPx, and MDA were measured using appropriate kits.

Forced swimming exercise

One hour after the final treatment, the forced swimming exercise was performed as described previously with some modifications.[1,3] In brief, the mice exercised in acrylic plastic pool (50 cm × 50 cm × 40 cm) filled with water (25 ± 2°C) to a depth of 30 cm. A steel washer (7% of body weight) was loaded on the tail root of each mouse. Exhaustion was determined when the animals were unable to remain under the water surface for 10 s. The exhaustive swimming time was used as the index of exercise tolerance.

Analysis of biochemical parameters

After the end of the forced swimming exercise, exhausted mice were sacrificed by decapitation under ether anesthesia, and then the blood samples were collected and centrifuged (3,000 × g, 15 min) for the determination of BLA, SUN, and SCK. The spleens, hearts, livers and hind-limb skeletal muscle were dissected out, and washed in ice-cold saline patted dry. Then the spleens, hearts, and livers were weighed and their weights relative to the final body weights (organ index) were calculated. The livers and hind-limb skeletal muscle were homogenized in Tris-HCl buffer, then the homogenates were centrifuged (4,000 × g, 20 min, 4°C) and the clear supernatant was used for the determination of glycogen, SOD, GPx, CAT, MDA. All biochemical parameters were determined using commercial diagnostic kits following the manufacturer's recommended instructions.

Statistical analysis

Statistical analyses were performed using the SPSS 13.0 statistical software. Results are expressed as mean ± SD. Student's *t* test was used for two groups comparison. Multi-group comparison was performed by one-way ANOVA followed by a Tukey's test for *post hoc* analysis. Probability values $P < 0.05$ were considered significant.

RESULTS

Effects of (-)-epigallocatechin-3-gallate on body weights and organ indices of mice

As shown in [Table 1](#), during experiments, the body weights, liver index, heart index, and spleen index of the LET, MET, and HET groups was not different significantly than that of the C group ($P > 0.05$), which means the EGCG has no effects on body weight and weight ratio of organ.

Effect of (-)-epigallocatechin-3-gallate on exhaustive swimming times of mice

As shown in [Figure 1](#), compared with the C group, the exhaustive swimming times of the LET, MET, and HET groups were significantly longer ($P < 0.05$). Compared with the LET group, the exhaustive swimming times of the MET and HET groups were significantly longer ($P < 0.05$).

Effect of (-)-Epigallocatechin-3-gallate on Some Blood Biochemical Parameters Levels of Mice

As shown in [Figure 2](#), compared with the C group, the BLA and SUN levels of the LET, MET, and HET groups, as well as the SCK levels of the MET and HET groups, were significantly lower ($P < 0.05$). Compared with the LET group, the BLA levels of the MET and HET groups, as well as the SUN and SCK levels of the HET groups, were significantly lower ($P < 0.05$).

Effects of (-)-epigallocatechin-3-gallate on liver and muscle glycogen contents of mice

As shown in [Figure 3](#), compared with the C group, the liver glycogen contents of the LET, MET and HET groups, as well as the muscle glycogen contents of the MET and HET groups, were significantly higher ($P < 0.05$). Compared with the LET group, the glycogen contents of the MET and HET groups, as well as the muscle glycogen contents of the HET groups, were significantly higher ($P < 0.05$).

Effect of (-)-epigallocatechin-3-gallate on superoxide dismutase activities in liver and muscle of mice

As shown in [Figure 4](#), compared with the C group, the SOD activities in liver and muscle of the LET, MET, and HET groups were significantly higher ($P < 0.05$). Compared with the LET group, the SOD activities in liver of the MET and HET groups, as well as the SOD activities in muscle of the HET groups, were significantly higher ($P < 0.05$).

Effect of (-)-epigallocatechin-3-gallate on glutathione peroxidase activities in liver and muscle of mice

As shown in [Figure 5](#), compared with the C group, the GPx activities in muscle of the LET, MET and HET groups, as well as the GPx activities in liver of the MET and HET groups, were significantly higher ($P < 0.05$). Compared with the LET group, the GPx activities in liver of the HET groups, as well as the GPx activities in muscle of the MET and HET groups, were significantly higher ($P < 0.05$).

Effect of (-)-epigallocatechin-3-gallate on catalase activities in liver and muscle of mice

As shown in [Figure 6](#), compared with the C group, the CAT activities in liver and muscle of the LET, MET and HET groups were significantly higher ($P < 0.05$). Compared with the LET group, the CAT activities in liver and muscle of the MET and HET groups, were significantly higher ($P < 0.05$).

Effect of (-)-epigallocatechin-3-gallate on malondialdehyde levels in liver and muscle of mice

As shown in [Figure 7](#), compared with the C group, the MDA levels in liver of the LET, MET and HET groups, as well as the MDA levels in muscle of the MET and HET groups, were significantly lower ($P < 0.05$). Compared with the LET group, the MDA levels in liver of the HET groups, as well as the MDA levels in muscle of the MET and HET groups, were significantly lower ($P < 0.05$).

DISCUSSION

The present study aimed to evaluate the anti-fatigue effect of EGCG. A direct measure of anti-fatigue effect is the increase in exercise tolerance. The forced swimming exercise, which is perhaps one of the most commonly used animal models of behavioral despair, has been used extensively for the evaluation of the anti-fatigue properties of novel compounds.[14] Other methods of forced exercise such as the motor driven treadmill or wheel can cause animal injury and may not be routinely acceptable.[15] In this study, the data showed that EGCG significantly prolonged exhaustive swimming time of mice, which indicated that EGCG had an anti-fatigue effect.

Exhaustive swimming exercise is known to induce some blood biochemical parameters related to fatigue changes, including BLA, SUN, and SCK. Lactic acid is the glycolysis product of carbohydrate under an anaerobic condition, and glycolysis is the main source of energy for fierce exercise in a short time.[16] Many studies have shown that swimming to exhaustion results in a significantly elevated blood lactic acid level, and the rate at which lactic acid accumulates in the blood showed an inverse relation to swimming time.[17] In addition, the increased concentration of lactic acid brings about a reduction of pH in muscle tissue and blood, and causes the so called acidosis, leading to the production of fatigue.[18] Therefore, BLA is a sensitive index of fatigue status. In this study, the data showed that EGCG significantly decreased BLA levels of mice, effectively delaying the increase of BLA and postponing the appearance of physical fatigue. SUN was the end-product of protein metabolism and also the index of protein metabolism in the body. At rest, the generation and excretion of SUN were in equilibrium, while after exhaustive swimming, SUN clearly increased at this time.[18] There is a positive correlation between the urea nitrogen *in vivo* and the exercise tolerance.[6] Thus, SUN is another sensitive index of fatigue status. In this study, the data showed that EGCG significantly decreased SUN levels of mice, which indicated that EGCG could reduce protein metabolism and enhanced exercise tolerance. Serum creatine kinase (SCK) is a clinical biomarker for muscle damage and an indirect index of the damage of membrane structure.[19] The function of creatine kinase has a greater relevance to what occurs in damaged muscles. During the process of muscle degeneration, the muscle cells lyse, and their contents are released into the bloodstream. Because most of the creatine kinase in the body normally exists in the muscle, an increase in the blood levels of creatine kinase indicates that muscle damage has occurred or is being initiated.[20] The release of creatine kinase into the blood is the result of increased permeability of the cell membrane due to lipid peroxidation.[21] In this study, the data showed that EGCG significantly decreased SCK levels of mice, ameliorating muscle damage induced by exhaustive exercise. It could be considered that this amelioration contributes to EGCG improving the exercise tolerance.

Stored glycogen in the tissues is the primary source of energy during exercise since muscle cannot mobilize fat as rapidly as glycogen and fatty acids cannot be metabolized anaerobically.[22] It is well known that the depletion of glycogen severely limits energy supply and maximal power output. Energy for exercise is derived initially from the breakdown of muscle glycogen, after strenuous exercise may be depleted and at later stages the energy will be derived from liver glycogen.[23] Liver glycogen depletion might be an important factor in the development of fatigue because as liver glycogen is depleted during exercise there is an inability to maintain blood glucose level, and the ensuing hypoglycemia could result in impaired nervous function.[24] Therefore, glycogen storage directly affects exercise ability and increasing the glycogen storage conduces to enhancing the endurance capacity and locomotory capacity.[25] In this study, the data showed that EGCG significantly increased liver and muscle glycogen contents of mice, which indicated that EGCG could enhance exercise tolerance. It might be because EGCG have promoted glycogenolysis restraint and/or gluconeogenesis. However, there is experimental evidence showing that exhaustive exercise can accelerate the triglyceride (or fat) mobilization, then increase the free fatty acids released into the plasma.[26,27] Decreasing serum triglyceride concentrations and increasing the availability of fatty acids during exhaustive exercise lead to the reduction of the glycogen depletion rate and the improvement of exercise tolerance.[28,29] In this study, changes in triglycerides and fatty acids were not investigated. So, further experiments are needed in order to identify the mechanism through which EGCG might affect fat mobilization.

There is an evidence that ROS exceeds the normal physiological coping range during exhaustive exercise, accumulation of ROS and decrease in antioxidant status could be resulted.[30] This scenario increased oxidative stress and leads to lipid peroxidation and oxidative modifications of proteins and DNA.[31] The antioxidant enzymes such as SOD, CAT, and GPx may have an important function in mitigating the toxic effects of ROS, and the improvement in the antioxidant enzyme activities can help to fight against fatigue. [6] However, many studies have reported a decrease tendency in antioxidant enzyme activities after exhaustive exercise,[32] and the decrease in antioxidant enzyme activities possibly owe to their use against the free radicals and their inhibition by free radical species.[33] In this study, the data showed that EGCG significantly increased SOD, CAT, and GPx activities of mice, which indicated that EGCG is capable to up-regulate antioxidant enzyme activity to protect against oxidative stress induced by exhaustive exercise, again supporting that EGCG had anti-fatigue effect.

Lipid peroxidation represents oxidative tissue damage caused by hydrogen peroxide, superoxide anions and hydroxyl radicals, resulting in structural alteration of the membrane, release of cell and organelle content and loss of essential fatty acids with formation of cytosolic aldehyde and peroxide products.[12] The MDA, a metabolite of phospholipid peroxidation, is a popular index of first condition on living body oxidative damage.[34] In this study, the data showed that EGCG significantly decreased MDA levels of mice, which indicated that EGCG could reduce lipid peroxidation and attenuate exhaustive exercise-induced oxidative damage.

In recent years, some researchers have endeavored to study the anti-fatigue effect of green tea extract and green tea polyphenols. Yu *et al.*[35] discovered that green tea beverage concentrate can significantly lengthen the swimming time, reduce the lactate acid level, and increase the content of liver glycogen. Liang *et al.*[36] reported that Yunnan green tea extract reduced the exhaustive swimming time and improved the liver and muscle glycogen contents. Fan *et al.*[37] found that green tea polyphenols extract could significantly prolong the exhaustive swimming time, which demonstrated that green tea polyphenols extract possessed anti-fatigue effect. Murase *et al.*[38] investigated the effects of catechin-rich green tea extract (GTE) on running endurance and energy metabolism during exercise in BALB/c mice, and found that the endurance-improving effect of GTE were mediated, at least partly, by increased metabolic capacity and utilization of fatty acid as a source of energy in skeletal muscle during exercise. Huang *et al.*[39] found that EGCG could extend the climbing pole time, exhaustive swimming time, running wheel time, and survival time of hypoxia tolerance of the mice, as well as increasing the LDH activity and MG and LG contents, but decrease the BLA and BUN contents. Sachdeva *et al.*[40] reported that chronic treatment with EGCG significantly restored all the behavioural deficits including anxiety and hyperalgesia in the chronic fatigued mice in a dose-dependent manner. Tanaka *et al.*[41] suggested that EGCG was effective for attenuating fatigue. EGCG given orally appears to have an antioxidant effect on the oxidatively damaged liver of fatigued animals. In this study, we also found that EGCG prolonged exhaustive swimming time and decreased the levels of BLA, SUN, SCK and MDA, which were accompanied by corresponding increases in liver and muscle glycogen contents, and SOD, CAT, and GPx activities. Therefore, the present results further support that EGCG had anti-fatigue effect in a dose-dependent manner and at the dose of 200 mg/kg exhibited the optimal effect. Combined with previous studies, anti-fatigue mechanisms of EGCG may possibly be due to its protective effects on corpuscular membrane by prevention of lipid oxidation via modification of several antioxidant enzyme activities.[42] Further study is warranted to elucidate its molecular mechanism and anti-fatigue-related gene regulation.

The finding of the study suggests that EGCG can be used to design nutraceutical supplements aimed to facilitate recovery from fatigue and attenuate exhaustive exercise-induced oxidative damage.

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Nil

Conflicts of interest

There are no conflicts of interest

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Figures and Tables

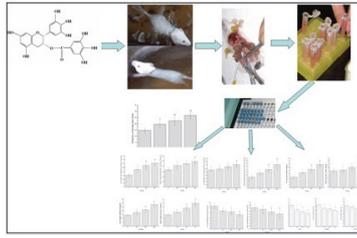


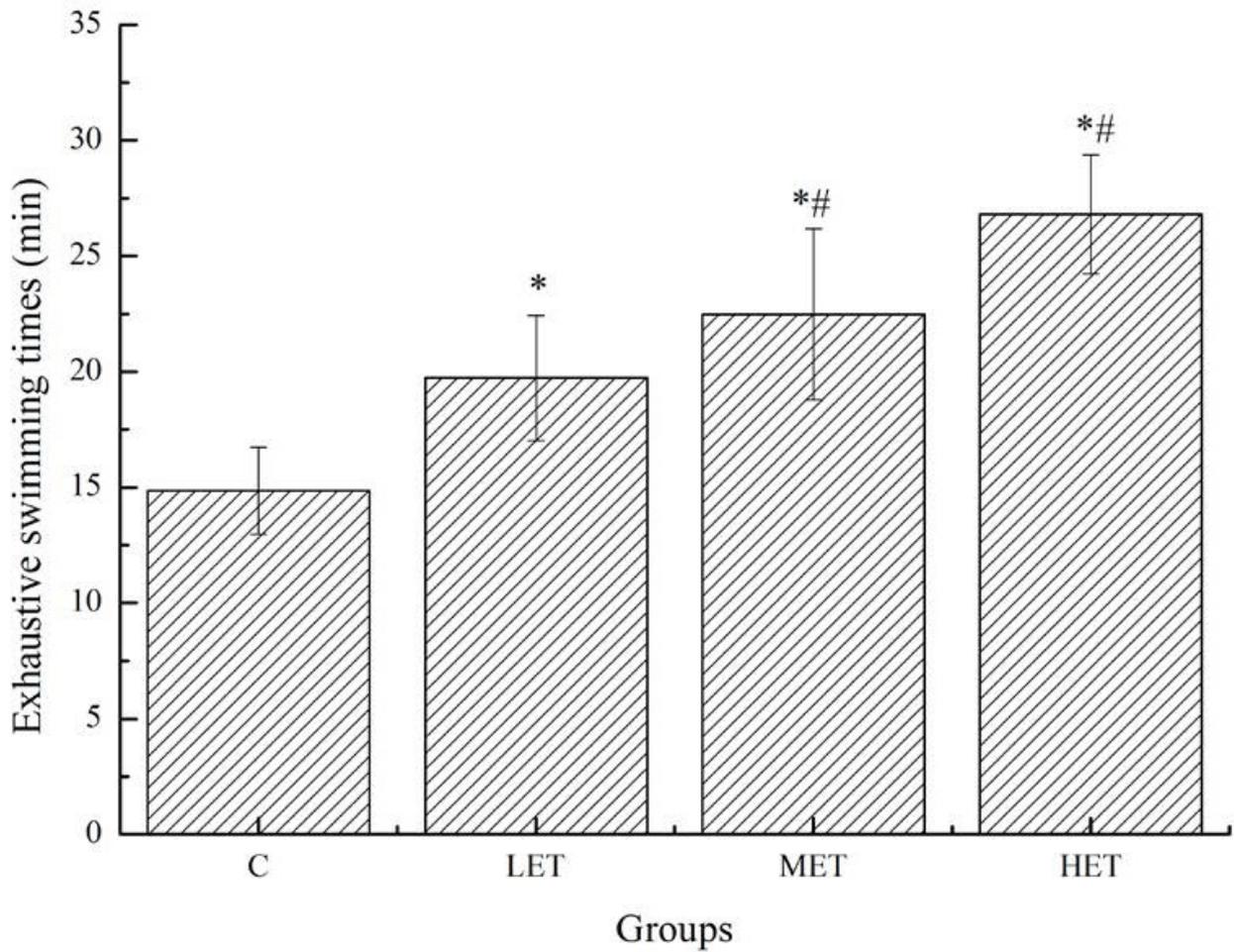
Table 1

Effects of EGCG on body weights and organ indices of mice Group

Body weights (g)	Organ indices							
	Initial	Week 1	Week 2	Week 3	Week 4	Liver	Heart	Spleen
C	25.32 ± 3.41	28.36 ± 4.21	30.51 ± 2.87	32.51 ± 4.28	34.91 ± 3.96	4.62 ± 0.31	0.56 ± 0.12	0.57 ± 0.06
LET	26.11 ± 2.75	27.87 ± 3.26	29.69 ± 3.74	32.74 ± 3.16	34.78 ± 4.23	4.74 ± 0.52	0.53 ± 0.07	0.58 ± 0.08
MET	25.98 ± 3.86	27.56 ± 3.19	30.78 ± 3.31	33.69 ± 3.93	35.96 ± 5.16	4.85 ± 0.43	0.58 ± 0.09	0.52 ± 0.09
HET	25.34 ± 3.07	27.84 ± 3.79	30.89 ± 4.13	33.42 ± 4.01	35.17 ± 4.82	4.81 ± 0.49	0.61 ± 0.07	0.55 ± 0.07

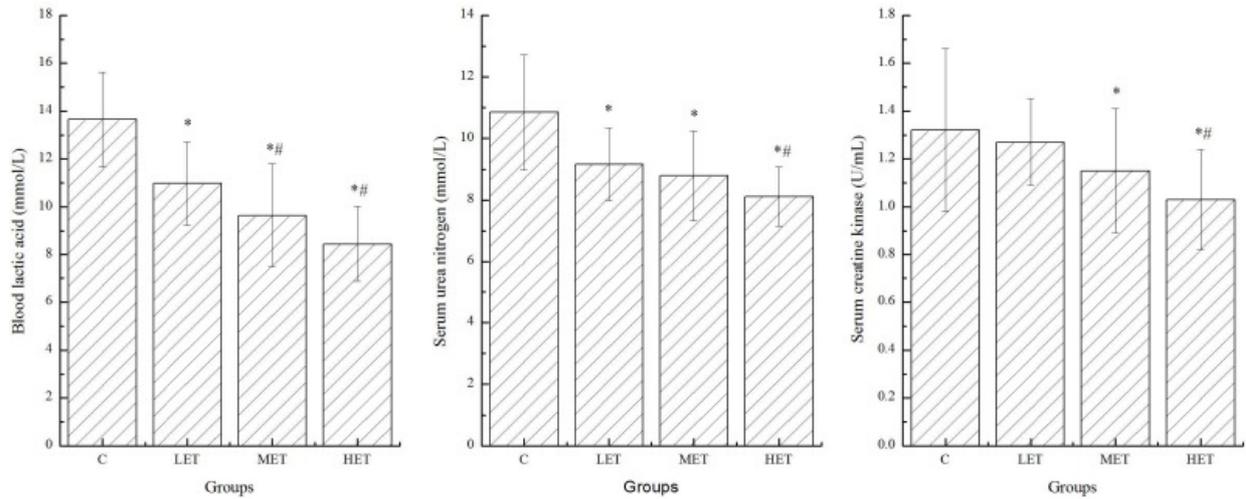
C, Control; HET, High-dose EGCG-treated; LET, Low-dose EGCG-treated; MET, Middle-dose EGCG-treated.

Figure 1

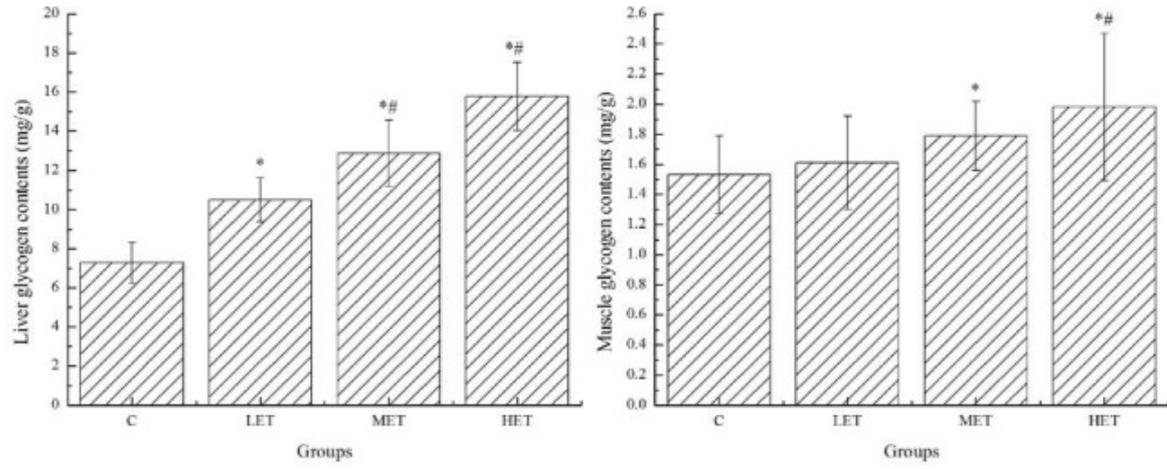


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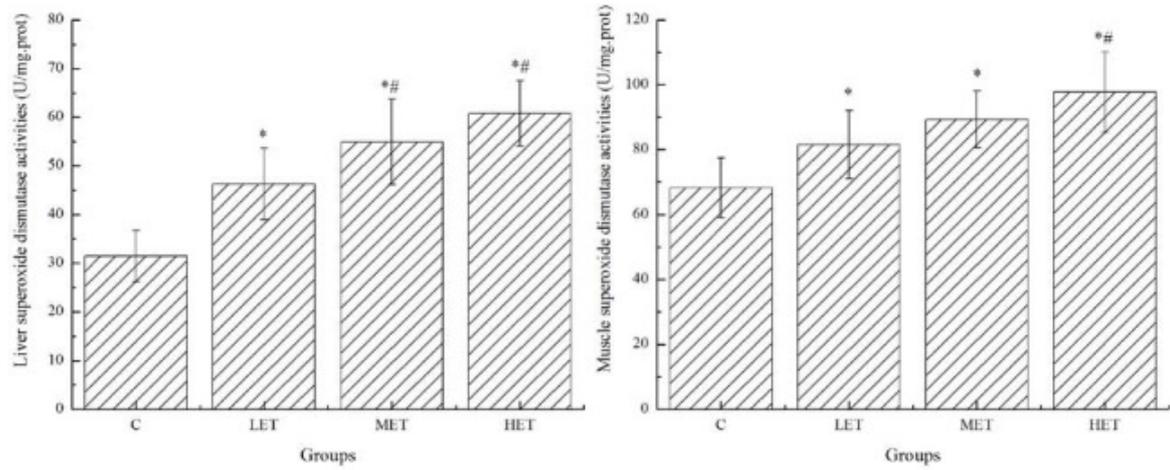
Effects of EGCG on exhaustive swimming times of mice. Data are expressed as mean \pm SD. * $P < 0.05$ compared with C group; # $P < 0.05$ compared with LET group

Figure 2

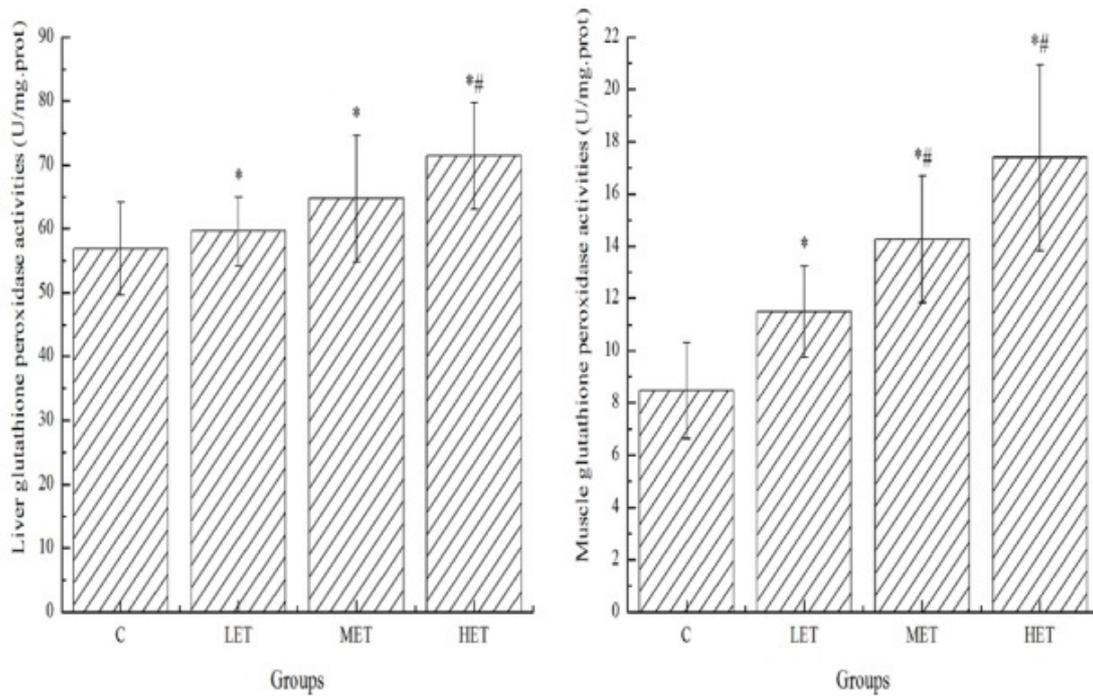
Effects of EGCG on some blood biochemical parameters levels of mice. Data are expressed as mean \pm SD. * $P < 0.05$ compared with C group; # $P < 0.05$ compared with LET group

Figure 3

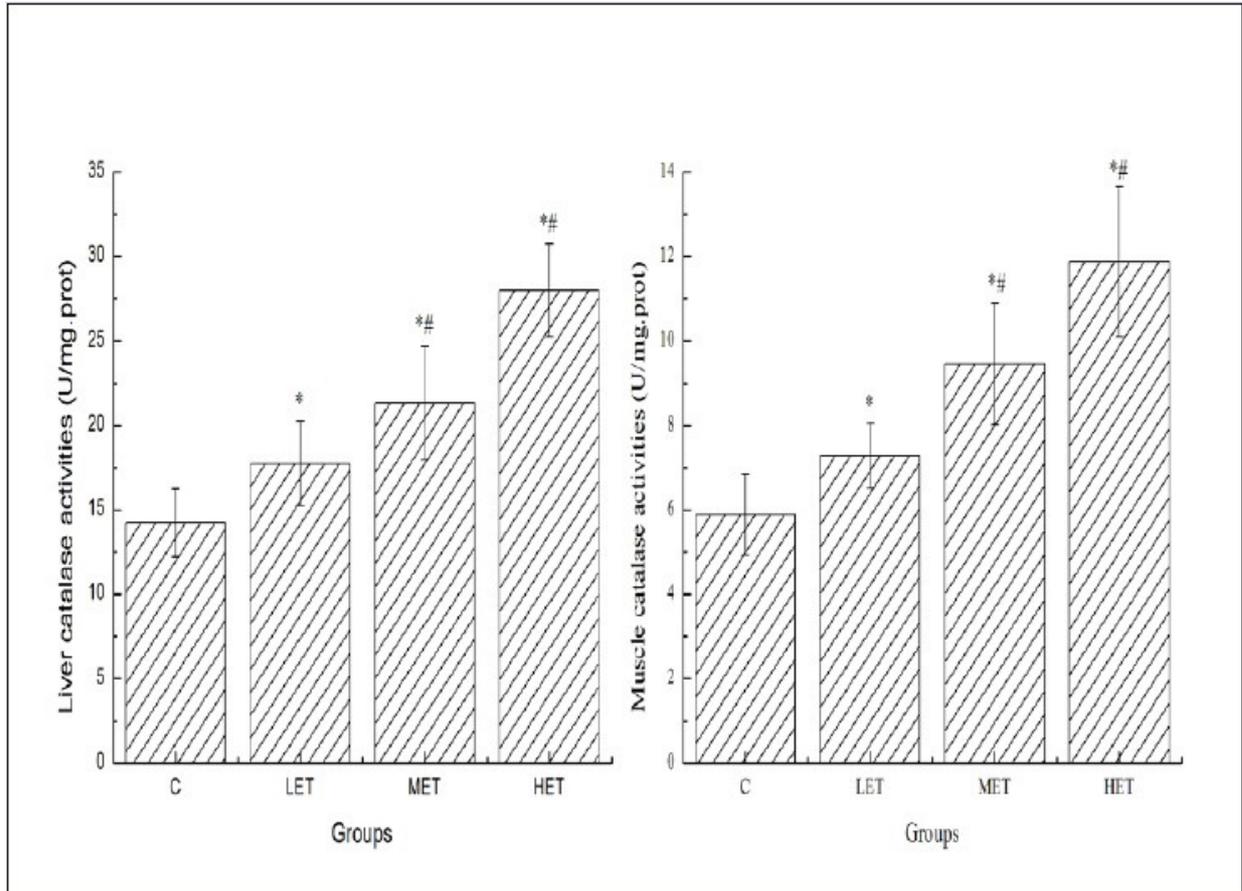
Effects of EGCG on liver and muscle glycogen contents of mice. Data are expressed as mean \pm SD. * $P < 0.05$ compared with C group; # $P < 0.05$ compared with LET group

Figure 4

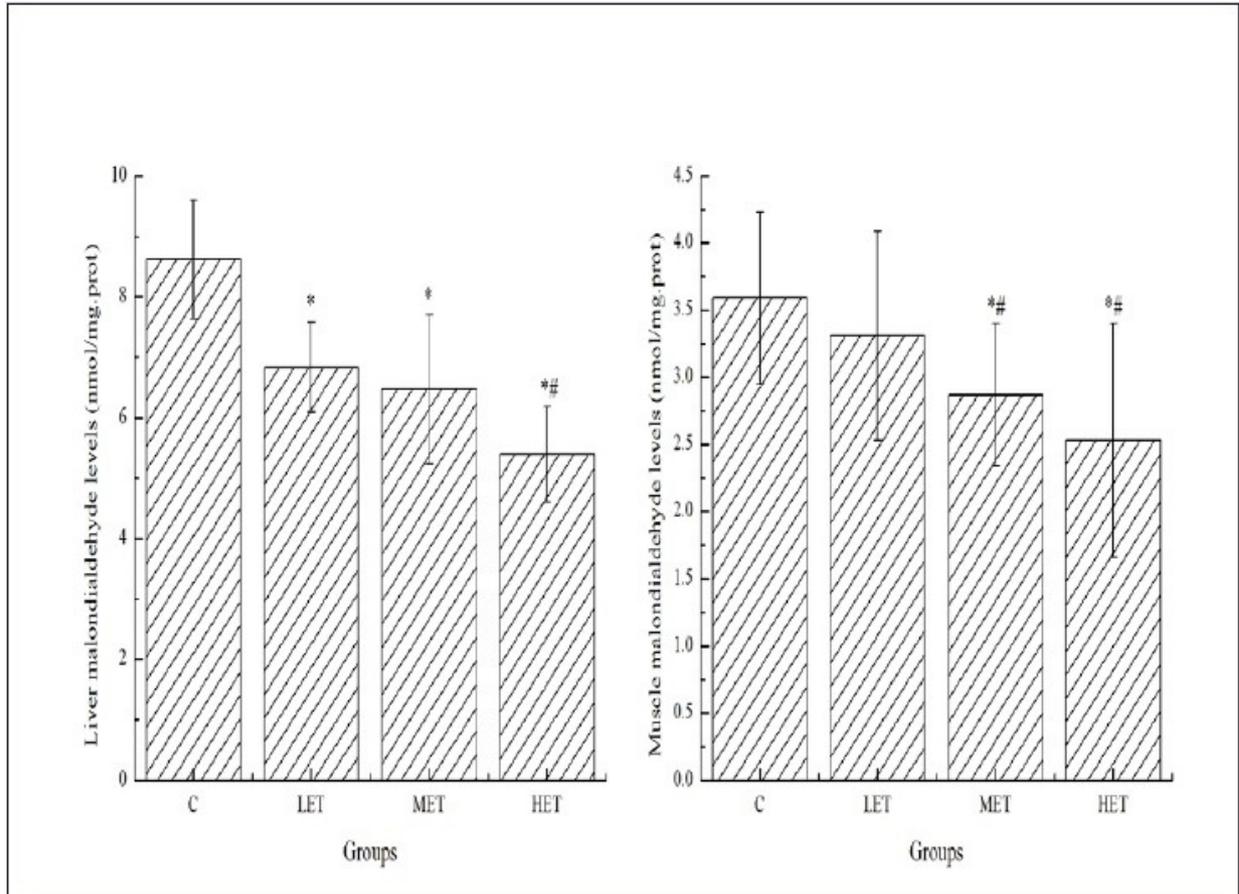
Effects of EGCG on superoxide dismutase activities in liver and muscle of mice. Data are expressed as mean \pm SD. * $P < 0.05$ compared with C group; # $P < 0.05$ compared with LET group

Figure 5

Effects of EGCG on glutathione peroxidase activities in liver and muscle of mice. Data are expressed as mean \pm SD. * $P < 0.05$ compared with C group; # $P < 0.05$ compared with LET group.

Figure 6

Effects of EGCG on catalase activities in liver and muscle of mice. Data are expressed as mean \pm SD. * $P < 0.05$ compared with C group; # $P < 0.05$ compared with LET group

Figure 7

Effects of EGCG on malondialdehyde levels in liver and muscle of mice. Data are expressed as mean \pm SD. * $P < 0.05$ compared with C group; # $P < 0.05$ compared with LET group.

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