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Research Article

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Total Soy Saponins Improve Antioxidant Capacity in the Liver of Exhausted Rats

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ABSTRACT

Objective: *The aim of this study was to investigate the impact of total soy saponins (TS) on the antioxidant capacity from the liver in rats exercised to exhaustion.*
Methods: *A one-time exhausted treadmill exercise session was used. Sprague-Dawley rats were divided into four groups: a control group—animals receiving no TS and no exercise (NTSNE), animals receiving TS and no exercise group (TSNE), animals receiving no TS and exercised to exhaustion group (NTSE), and animals receiving TS and exercised to exhaustion group (TSE). The TSNE and TSE groups were fed TS at a dosage of 20 mg/kg body weight, once per day for two weeks. The NTSE group was given a placebo, and the NTSNE group was not given any treatment. The NTSE and TSE groups were exercised at speed of 30m/min on treadmill until exhausted. The exercise time were recorded when the rats became exhausted and the rats were then decapitated and anatomized immediately. A 10% homogenate of the liver tissue was prepared. The liver levels of superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), glutathione peroxidase (GSH-Px), glutathione reductase (GR), reduced glutathione (GSH), total antioxidant capacity (T-AOC), and serum alanine aminotransferase (serum ALT) levels were tested.*
Results: *TS increased the exercise time by 20.62% ($p<0.05$). As compared with the NTSNE group, the SOD and GSH levels were increased whereas the MDA level decreased significantly in the TSNE group ($p<0.05$, $p<0.01$, $p<0.05$). As compared with the NTSE group, the SOD, CAT, GSH-Px, GSH and T-AOC levels increased significantly whereas the MDA level and the serum ALT activity decreased in the TSE group ($p<0.05$, $p<0.05$, $p<0.05$, $p<0.05$, $p<0.05$, $p<0.05$, $p<0.01$).*
Conclusions: *TS can significantly improve the exercised rat's antioxidant activity in their liver to varying degrees, decrease MDA and serum ALT levels, protect liver cell and liver functions, increase the exercise time and delay the occurrence of the fatigue.*

Keywords: Total soy saponins; Free radical; Exercised rat; Liver; Antioxidant capacity

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INTRODUCTION

Total soy saponins (TS) are a subset of pentacyclic triterpenoids glycosides with a variety of biological activities. According to the different sapogenins, TS can be divided into four groups: the A group, B group, E group, and 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) group. The A group can be divided into Aa-Ah, the B group can be divided into Ba, Bb, Bc, Bb', and Bc', the E group can be divided into Bd and B, and the DDMP can be divided into αg, βg, βa, γg and γa subgroups [1].
TS have a variety of biological activities, such as antioxidant, immune-enhancing, anti-cancer, anti-aging, lowering cholesterol and blood glucose regulating effects. A recent study also suggests that TS were effective in improving the exercise ability and antioxidant capacity of myocardium and quadriceps femoris in rats [2,3].

EXPERIMENTAL METHODS

Experimental Design and Subjects

Thirty-two Sprague-Dawley (SD) healthy 2-month-old male rats (SPF grade) were used weight 190-210 g and were provided care as directed by the Experimental Animal Center of the Medical School, Xi'an Jiaotong University (Animal certificate No: Shannxi Medical Animal No: 08-005). This study was performed according to the international, national, and institutional rules considering animal experiments, clinical studies and biodiversity rights, and had been approved by Xijing Hospital Ethic Committee in Fourth Military Medical University. All rats were randomly divided into four groups: a control group, animals receiving no TS and no exercise (NTSNE), animals receiving TS and no exercise group (TSNE), animals receiving no TS and exercised to exhaustion group (NTSE) and animals receiving TS and exercised to exhaustion group (TSE). Eight rats from each group were fed in divided cages. The temperature varied from 22℃ to 28℃, the relative humidity was 45%-65%, the cages were illuminated by natural light, the ambient noise was no higher than 45 dB and all rats had free access to water and basic rodent chow. North China Pharmaceutical Co., Ltd (Shijiazhuang, China) provided TS with a purity of 90% and 10% ash. The rats were started on TS gavage after 3 days of adaptation to the environment. Each rat in the supplement groups (TSNE and TSE groups) was fed a 2-mL aliquot of TS dissolved in normal saline at a fixed time of between 9:00-9:30 am, once per day for 2 weeks, with TS dosage of 20 mg/kg body weight. During the supplement gavage, the rats were weighed every 3 days and the dosage was adjusted in time according to the body weight. The NTSE group was fed with the same volume of normal saline vehicle. The NTSNE (control) group received no treatments.

Exhaustive Exercise Protocol

An acute exhaustive exercise session was completed. The rats were not given any prior training, the NTSE and TSE groups underwent an acute exhaustive exercise session on the treadmill only before dissection. The treadmill was horizontal and gradually increased to the predetermined exercise intensity (30 m/min) within 3 min. The treadmill speed was set at 10 m/min for the first minute, 20 m/min for the second minute and 30 m/min for the third minute. The exercise time to exhaustion and exercise distance for each rat was recorded. We judged whether the rats exercised to exhaustion according to the following criteria: the rats could not maintain a predetermined treadmill speed; squatted against the back wall of the treadmill lane on their buttocks and both the current stimulus and the brush driving could not force the rats to continue exercising. The exhaustive behavior was characterized by shortness of breath, mental fatigue, and a prone nutation.

Dissection and Index Test

The rats were anesthetized with ether immediately after reaching a state of exhaustion and killed by decapitation. Blood was collected and the serum was separated after coagulation. The liver was removed immediately and the remaining blood was washed away with 4℃ normal saline and placed in a clean culture dish, marked according to each group. The weight of the liver was measured and the liver was ground in 4℃ normal saline. Liver tissue homogenates of 10% mass concentration were prepared and the supernatant was separated after centrifugation (7.99 × g, 5 min). Finally, antioxidant indicators and serum ALT were assayed accordingly.

Methods of Testing

The antioxidant indicators were tested with reagent kits provided by Nanjing Jiancheng Bioengineering Institute (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). SOD was tested by the xanthine oxidase method, malondialdehyde (MDA)was tested by the thiobarbituric method (TBA method), CAT was tested by the ultraviolet spectroscopy method, GSH-Px, GR, and GSH were tested by the dithiobis nitrobenzoic acid method, total antioxidant capacity (T-AOC) was tested by a spectrophotometry method, the serum ALT were tested by an automatic biochemistry analyzer (Hitachi 7060, Hitachi Corporation of Japan, LabStar 2.5, Beijing Zhifang Technology Development Co. Ltd., Beijing, China). A 721B spectrophotometer (Shanghai Jingke Instrument Co., Ltd, Shanghai, China), a 752B spectrophotometer (Shanghai Jingke), an FJ - 2008Pγ radioimmunoassay counter (Xi'an Nuclear Instrument Factory, Xi’an, China), a Hitachi 7060 automatic biochemical analyzer (Hitachi 7060), a TGL-16G refrigerated centrifuge (Flying Pigeon, Shanghai Anting Scientific Instrument Factory, Shanghai, China), a DK-98-1A water bath (Taisite, Tianjin City Taisite Instrument Co., Ltd., Tianjin, China) and a DSPT-202 treadmill (Duanshi, Shanghai Xinruan Information Technology Co. Ltd., Hangzhou, China) were used.

Data Processing

The experimental data were processed with statistical software SigmaStat 3.5 (Version 3.5; SYSTAT Software Inc., San Jose, CA, USA) and the results were shown (mean ± SD). A $p<0.05$ or $p<0.01$ was considered statistically significant after a one-way analysis of variance (ANOVA) Student-Newman-Keuls test (S-N-K test).

The exhaustive time was processed by a *t* test and the results of the *t* test were measured by Cohen's *d* value.

RESULTS AND DISCUSSION

The Exhaustion Time of the Rats

Table 1: Impact of TS on the exhaustion time in exhausted rats (n = 8, mean ± SD)

Indicators\ groups	NTSE	TSE	Difference in exercise time to exhaustion
Velocity (m/min)	30	30	
Average exhaustive time (min)	86 ± 12	103 ± 19	20.62%

Note: By S-N-K test of a one way ANOVA, as compared with the NTSE group, the treadmill exhaustion time increased significantly in the TSE group ($p<0.05$, Cohen's d = 1.07). TS = total soy saponins; NTSNE = animals receiving no TS and no exercise; TSE = animals receiving TS and exercised to exhaustion group

Antioxidant and Serum Enzyme Indices

As shown in Table 1, TS can significantly increase exhaustion time of the rats by 20.62% ($p < 0.05$, Cohen's d = 1.07). According to the Cohen's standards, the *t* test has a small effect size, medium effect size and large effect size when Cohen's *d* value = 0.2, 0.5, and >0.8. The Cohen's *d* value of present *t* test was 1.07 > 0.8, which indicated that the present *t* test was trustworthy.

Table 2: Impact of TS on the liver antioxidant capacity and serum ALT in exercised rats (n = 8, mean ± SD)

Indices\ groups	NTSNE	TSNE	NTSE	TSE
SOD (U/mgprot)	424.24 ± 22.59	463.09 ± 41.97* Cohen's d = 1.15	378.99 ± 66.99* Cohen's d = 0.17	425.51 ± 36.55$^{\#}$ Cohen's d = 0.86
MDA（nmol/mgprot）	4.96 ± 0.31	4.50 ± 0.38* Cohen's d =1.33	6.50 ± 1.23* Cohen's d = 1.72	5.26 ± 0.87$^{\#}$ Cohen's d = 1.16
CAT (U/gprot)	32.67 ± 8.97	33.17 ± 10.80	25.94 ± 3.75	30.04 ± 8.20$^{\#}$ Cohen's d = 0.64
GSH-Px（U/0.1ml）	36.94 ± 3.61	39.11 ± 11.38	29.61 ± 9.38	41.54 ± 3.65$^{\#}$ Cohen's d = 1.68
GR（U/gprot）	7.41 ± 2.54	9.67 ± 3.41	6.66 ± 1.93	7.01 ± 1.29
GSH（mg/mgprot）	2.75 ± 0.16	2.97 ± 0.26** Cohen's d =1.02	2.58 ± 0.37* Cohen's d = 0.60	2.85 ± 0.33$^{\#}$ Cohen's d = 0.77
T-AOC（U/mgprot）	0.64 ± 0.38	0.74 ± 0.14	0.56 ± 0.13* Cohen's d = 0.28	0.78 ± 0.20$^{\#}$ Cohen's d = 1.30
Serum ALT（U/ml）	45.33 ± 5.61	43.67 ± 7.55	222.33 ± 200.12	107.00±10.60$^{\#\#}$ Cohen's d = 0.81

Note: by S-N-K test of a one way ANOVA: * $p < 0.05$, $^{**}p < 0.01$, compared with the NTSNE group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared with the NTSE group. Abbreviations: TS = total soy saponins; NTSNE = animals receiving no TS and no exercise; TSNE = animals receiving TS and no exercise group; NTSE = animals receiving no TS and exercised to exhaustion group; TSE = animals receiving TS and exercised to exhaustion group; SOD = superoxide dismutase; MDA = malondialdehyde; CAT = catalase; GSH-Px = glutathione peroxidase; GR = glutathione reductase; GSH = reduced glutathione; T-AOC = total antioxidant capacity; ALT = alanine aminotransferase

Table 2 showed that TS can improve rats' antioxidant capacity of their liver to varying degrees. As compared with the NTSNE group, the enzyme activities of SOD and GSH were all significantly enhanced whereas the MDA level decreased in the TSNE group ($p<0.05$, $p<0.01$, $p<0.05$); the enzyme activities of SOD, GSH and T-AOC were enhanced whereas the MDA level decreased in the NTSE group ($p<0.05$, $p<0.05$, $p<0.05$, $p<0.05$). As compared with the NTSE group, the enzyme activities of SOD, CAT, GSH-Px, GSH and T-AOC were significantly enhanced whereas the MDA level and the serum ALT activity decreased in the TSE group ($p<0.05$, $p<0.05$, $p<0.05$, $p<0.05$, $p<0.05$, $p<0.05$, $p<0.01$). As compared with the TSNE group, the enzyme activities of SOD, CAT, GR, GSH showed a tendency to decrease, but a tendency to increase was observed for MDA, GSH-Px, and ALT in the TSE group (not statistically significant).

DISCUSSION

The liver is the main place for body metabolism, such as the synthesis and storage of glycogen, gluconeogenesis, and glucose uptake and release. Meanwhile the liver is the main organ of detoxification. The metabolic waste generated in movement like ammonium synthesizes urea or converts into non-toxic glutamine. As the experimental data shown in Table 2, exhaustive exercise caused a significant increase in MDA level in rat liver cells, which indicated that exhaustive exercise increased the FR metabolism and the oxidative stress of rat liver, the FR and FR metabolites MDA can cause damage to the liver cells. Such as lipid peroxidation, nucleic acids and proteins damage, MDA also makes protein cross-linking degeneration, which affects the biological function of the liver cells. A large number of FR in the body produced in the movement not only consume antioxidant enzymes but also directly attack the enzyme molecule, and thus affect the synthesis and regeneration of the

enzymes. Data found in Table 2, after exhaustive exercise, the enzyme activities of SOD, CAT, GSH, GSH-Px, GR and T-AOC in rat liver show a tendency to decrease while the serum ALT increase, which is consistent with the experimental expectations. The serum ALT is mainly derived from the liver cells, thus the serum ALT increases usually result from the damaged liver cell and the increased permeability of the liver cell membrane. By the intervention of TS, the rising trend of MDA level and serum ALT activity and the downtrend of antioxidant enzymes activity caused by exhaustive exercise were inhibited significantly. The experimental data shows significant differences and has a large effect size, which indicated that TS can significantly reduce the liver FR production of exercised rats and improve the liver antioxidant capacity. As a result of decreased production of FR, the cell membranes, membrane proteins, nucleic acids and ribosomes were protected from oxidative damage and thus the cell functions were maintained and the liver energy metabolism during exercise was enhanced. Therefore, the exercise ability was improved (Table 1).

Typically, the body keeps a dynamic balance between the generation and the removal of FR. However, under the condition of exhausted exercise, FR in the body can increase significantly. When the level of lipid peroxidation exceeds the body's antioxidant capacity, this imbalance can cause the occurrence of oxidative stress and directly caused biofilm injuries, the degeneration of intracellular proteins and lead to cell death, apoptosis, tissue damage and disease [4]. SOD, CAT and GSH-Px are common antioxidative enzymes which can eliminate FR and reduce this imbalance. Under normal physiological conditions and appropriate exercise work rates, the antioxidant enzymes system of the body, by way of their respective roles, can maintain a dynamic balance between the FR generation and removal. During exhausted exercise, the body's oxygen uptake increased substantially, of which approximately 2% is converted into FR [5]. Therefore, FR and especially oxygen FR levels increased significantly; this constituted one of the major factors of body injury and prompted the occurrence of exercise fatigue. Data presented in Table 2 show that the exhaustive exercise significantly increased serum ALT activity, which indicated that the liver cell membrane damaged or an increased membrane permeability. The possible mechanisms can be explained: 1). As a result of exercise-induced redistribution of the blood flow *in vivo*, the liver suffers ischemic-reperfusion injury and further develops calcium overload, which lead to the uncoupling of oxidative phosphorylation and causes a decrease of ATP level. 2) The enzyme activity of creatine kinase (CK) decreased while the adenosine monophosphate (AMP) level increased, and a mounts of AMP, catalyzed by adenine deaminase, converted into inosine. Then catalyzed by 5'-nucleotidase and nucleosidase, inosine converted into hypoxanthine. The increased hypoxanthine catalyzed by hypoxanthine oxidase, generated a mounts of superoxide anion. The superoxide anion radicals can cause membrane lipid peroxidation level increased significantly and damage the liver cell membrane functions. 3) During exhausted exercise, the oxygen FR produced lipid peroxidation metabolin MDA which cause nucleic acids and membrane proteins cross-linking and further damage membrane functions of the liver cell. Excessive FR constantly attack unsaturated fatty acids of the biofilm, causing lipid peroxidation, a reduced flowability and an increased brittleness of the membrane, and finally leading to membrane dynamic changes. 4) The changes of the membrane lipid molecules can expand the pores of membrane and results in membrane permeability increase and may cause membrane damage. Followed by membrane damage, the subcellular organelles (such as mitochondria, Golgi apparatus, endoplasmic reticulum) structure has been destroyed, and finally led to a series of cell dysfunction. 5) In the process of lipid peroxidation, the arachidonic acid metabolic pathway was activated and the bioactivator leukotrienes and prostaglandins were generated. Meanwhile, the oxygen FR were produced and the membrane lipid peroxidation was initiated again therefore forms a positive feedback. As a result, the further damage of the liver cells appeared. 6) FR can also lead to pairing error of the nucleic acid base and induce gene mutations, which cause the changes of proteins and enzymes structure and function, thus cause a reduction of the enzyme activity. 7) Exhaustive exercise consume a numerous hepatic glycogen reserve, furtherly reduce the detoxify ability of the liver. Increased protein metabolisms produce excessive NH_4^+ exceeds the liver's detoxification capacity and bring toxic effects. A mounts of ketone generated from incomplete oxidation of fatty acids can lead to ketoacidosis. The experimental data also show that, TS significantly increased activity of antioxidant enzymes in rat liver and decreased the MDA levels. This may be related to the hepatoprotective effect of TS. A study had revealed that, TS shows a protective effect on hepatic injury induced by carbon tetrachloride (CTC). Together with glucuronic acid, the hepatoprotective effect of TS increased, while the separate glucuronic acid did not have this effect [6]. This indicated that TS can significantly inhibit the abnormity of cell structure and function in rat liver caused by CTC. The experimental results show a significant reduction of serum ALT activity in the TSE group than that in the NTSE group, which also indicated the hepatoprotective effect of TS. As the experimental data shown, after exhaustive exercise, TS significantly improved the enzyme activity of SOD, CAT, GSH-Px, GSH and T-AOC ($p<0.05$) while the MDA level decreased ($p<0.05$), which indicated that TS has a significant antioxidant capacity. It can enhance the body's antioxidant enzymes activity and decrease MDA level. We speculated that this may be related to the effects of TS on scavenging FR and its multiple biological functions. From the structural perspective, the mother nucleus of TS molecule which is rich in phenolic hydroxyl group can combine with the FR and form a resonance stabilized semiquinone structure. So that TS can break off the chain reaction of FR and clear FR directly. It has been reported that TS has a strong antioxidant capacity and the ability

of anti-active oxygen *in vitro*. TS can inhibit the lipid peroxidation in the liver tissue and relieve mitochondria swelling of the liver cell [7]. TS also can significantly inhibit lipid peroxidation of liver cells induced by Fe^{2+}-H_2O_2 and the formation of lipid peroxidation in liver cells [8]. Yoshiki et al. analyzed the soyasaponin of DDMP by molecular orbital method. They considered that the antioxidant activity of DDMP was mainly related to the C-6 region of the TS molecule where the main part of the active oxygen was eliminated [9]. TS increase the level of T-AOC in the liver of exhausted rats, was probably partly due to the antioxidant enzymes, partly due to the reduced oxidative stress in liver and a promotion of glycometabolism. The enhanced glycometabolism provides a number of reducing substances and reduces the consummation of reducing vitamins, amino acids and proteins.

CONCLUSION

TS can significantly improve the exercised rat's antioxidant activity in their liver to varying degrees, decrease MDA and serum ALT levels, protect liver cell and liver functions, increase the exercise time and delay the occurrence of the fatigue.

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