

Original article

# Effects of total soy saponins on free radicals in the quadriceps femoris, serum testosterone, LDH, and BUN of exhausted rats

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## Abstract

**Purpose:** The aim of this study was to investigate the impact of total soy saponins (TS) on the free radical metabolism from the quadriceps femoris muscle, serum testosterone, lactate dehydrogenase (LDH), and blood urea nitrogen (BUN) in rats exercised to exhaustion.

**Methods:** A one-time exhausted treadmill exercise session was used. Sprague-Dawley rats were divided into 4 groups: a control group—animals receiving no TS and no exercise (NTSNE), animals receiving TS but no exercise group (TSNE), animals receiving no TS but 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 2 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 30 m/min on treadmill until exhausted. The exercise time and exercise distance were recorded when the rats became exhausted and the rats were then decapitated and anatomized immediately. A 10% homogenate of the quadriceps femoris tissue was prepared. The levels of superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), glutathione peroxidase (GSH-Px), glutathione reductase (GR), reduced glutathione (GSH), total antioxidant capacity (T-AOC), LDH, BUN, and serum testosterone were tested.

**Results:** TS significantly increased the exercise time to exhaustion by 20.62% ( $p < 0.05$ ). The MDA levels were decreased significantly in the TSNE group than in NTSNE group ( $p < 0.05$ ); the T-AOC levels increased significantly in the TSNE group than in the other 3 groups ( $p < 0.01$ ,  $p < 0.05$ ,  $p < 0.05$ ). The LDH activity significantly increased in the NTSE group than in TSNE group ( $p < 0.05$ ). The BUN levels significantly increased in the NTSE group than in the other 3 groups ( $p < 0.01$ ,  $p < 0.01$ ,  $p < 0.05$ ), and significantly increased in the TSE group than in NTSNE and TSNE groups (both  $p < 0.01$ ). The serum testosterone levels increased significantly in the TSNE group than in the other 3 groups (all  $p < 0.01$ ). SOD, CAT, GSH-Px, GR, and GSH were not statistically different among the groups.

**Conclusion:** TS can significantly improve the exercised rats' serum testosterone level and antioxidant activity in their quadriceps femoris to varying degrees, decrease MDA and serum LDH and BUN levels, increase the exercise time, and delay the occurrence of the fatigue.

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**Keywords:** Exercised rat; Free radical; Quadriceps femoris; Serum enzymes; Testosterone; Total soy saponins

## 1. Introduction

Total soy saponins (TS) are a subset of pentacyclic triterpenoid glycosides with a variety of biological activities. According to the different sapogenins, TS can be divided into 4 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 Be; the DDMP group can be divided into  $\alpha$ g,  $\beta$ g,  $\beta$ a,  $\gamma$ g, and  $\gamma$ a subgroups (Figs. 1 and 2).

There are 2 free radical (FR) defense systems in the human body. One type is an enzymatic defense system such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), and glutathione reductase (GR). The other is a non-enzymatic defense system such as vitamin C, vitamin E, and glutathione (GSH). 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 increases significantly. When the level of lipid

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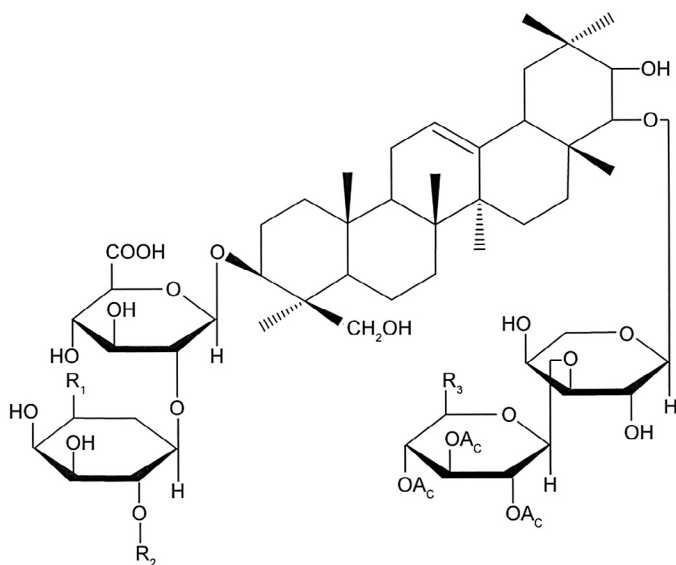


Fig. 1. Total soy saponins structure of A group.

peroxidation exceeds the body's antioxidant capacity, this will result in the occurrence of oxidative stress and directly cause biofilm injury, the degeneration of intracellular proteins, and lead to cell death, apoptosis, tissue damage, and disease.<sup>1</sup>

TS have a variety of biological activities, such as antioxidant<sup>2</sup> and immune-enhancing activity.<sup>3,4</sup> They can also improve the rats' macrophage phagocytic capacity<sup>5</sup> and humoral and cellular immunity.<sup>3</sup> By inhibiting the activity of  $\alpha$ -glucosidase<sup>6</sup> and  $\alpha$ -amylase,<sup>7</sup> TS significantly reduced the level of blood sugar in diabetic rats, and significantly improved the glucose tolerance in both the diabetic and healthy rats.<sup>8</sup> TS also have significant effects on anti-aging<sup>9</sup> and the inhibition of tumor cell DNA,<sup>10</sup> herpes simplex virus (HSV-1), human cytomegalovirus

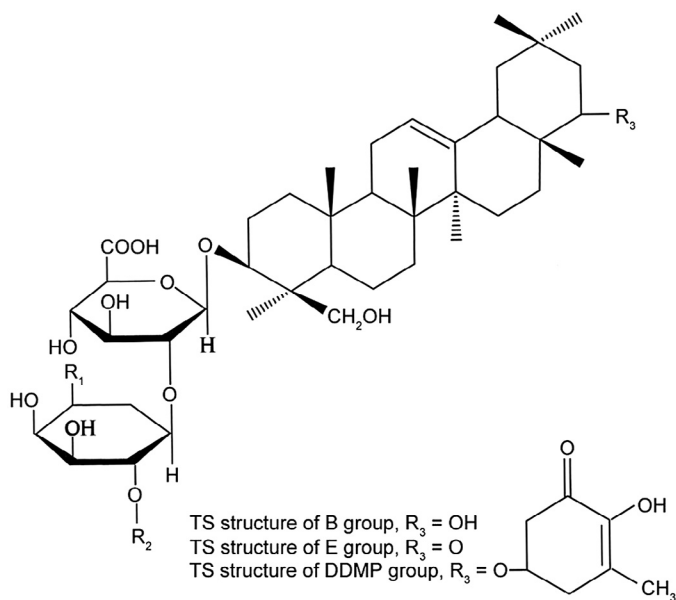


Fig. 2. Total soy saponins structure of B, E, and DDMP groups. DDMP = 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one; TS = total soy saponins.

(HCMV), polio virus, influenza virus, measles virus, mumps virus, and coxsackie virus.<sup>11-14</sup> Further anti-aging studies on human embryonic lung diploid fibroblasts *in vitro* confirmed that the cells treated with TS can grow extended to 80 generations, whereas the longest survival time of the control group was only until 51 generations.<sup>15</sup> TS also have a significant capacity of anti-lipid peroxidation activity on plasma lipoprotein<sup>16</sup> and can prevent low-density lipoprotein cholesterol (LDL-C) from oxidizing and decrease their susceptibility to oxidation, thus hindering the conversion of LDL-C to oxidized low-density lipoprotein, which is an important factor in atherosclerosis risk. TS protected not only the heart, but also the vascular smooth muscle. TS can significantly reduce the generation of lipid peroxides, protect endothelial integrity, and maintain the normal cardiovascular function. The aim of this study was to investigate the impact of TS on the free radical metabolism from the quadriceps femoris muscle, serum testosterone, lactate dehydrogenase (LDH), and blood urea nitrogen (BUN) in rats exercised to exhaustion.

## 2. Methods

### 2.1. Experimental design and subjects

Thirty-two Sprague-Dawley (SD) healthy 2-month-old male rats were used (weight 190–210 g) and were provided care as directed by the Experimental Animal Center of the Medical School, Xi'an Jiao Tong University (animal certificate No.: Shaanxi 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 4 groups: a control group—animals receiving no TS and no exercise (NTSNE), animals receiving TS but no exercise group (TSNE), animals receiving no TS but 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°C to 28°C, 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 between 9:00 a.m. and 9:30 a.m., 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 the same volume of normal saline vehicle. The NTSNE (control) group received no treatments.

### 2.2. 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

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