

Evaluation of anti-fatigue activity of total saponins of *Radix notoginseng*

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Background & objectives: Several biological activities of total saponins of *Radix notoginseng* (TSRN), a traditional Chinese medicine have been reported. The present study was carried out to investigate anti-fatigue activity of TSRN in male Kunming mice.

Methods: Mice were divided into four groups. The first group designated as control group was administered with distilled water by gavage every day. The second, third and fourth groups designated as TSRN treatment groups were administered with TSRN of 20, 40 and 80 mg/kg body weight/day, respectively. The treatment continued for 28 days. Exhaustive swimming time, blood lactate and tissue glycogen contents of mice after swimming were determined.

Results: TSRN extended exhaustive swimming time of mice, effectively delayed the increase of lactate in the blood, as well as increased the tissue glycogen contents.

Interpretation & conclusions: TSRN showed promising anti-fatigue activity in animal model. However, further study is needed to elucidate the mechanism of the effect of TSRN on fatigue.

Key words Anti-fatigue activity - lactate - swimming - total saponins of *Radix notoginseng*

Panax notoginseng (Burk.) F.H. Chen is cultivated throughout Southwest China, Burma, and Nepal. The root, the commonly used part of this plant called *Radix notoginseng* or *Sanchi*, has a long history as a remedy in oriental traditional medicine¹⁻³. In China, *R. notoginseng* is used to promote blood circulation, remove blood stasis, induce blood clotting, relieve swelling, delay fatigue and alleviate pain^{4,5}. *R. notoginseng* is reported to be beneficial for coronary heart disease, cerebral vascular disease, cancer, diabetes mellitus as well as learning and memory improvement in experimental studies⁶⁻⁸. These therapeutic effects are

attributed to its active ingredients, namely saponins, flavonoids and polysaccharides⁹⁻¹¹. Total saponins of *R. notoginseng* (TSRN) are considered to be the principal active ingredients, and are a mixture of more than 20 Dammarane type saponins, including ginsenoside Rg1, Rg2, Rb1, Rb2, Rb3, Rc, Rd, Re, Rh, F2 and notoginsenoside R1, R2, R3, R4, R6, Fa, Fc, Fe, etc¹². Although many biological activities and pharmacological functions of TSRN are known. It is known that there have been limited studies investigating its effects on physical fatigue. Therefore, the present study was designed to investigate the anti-

fatigue activity of total saponins of *R. notoginseng* in rat model.

Material & Methods

The study was conducted in the Laboratory of Biochemistry, Zhengzhou University (Zhengzhou, China).

Plant materials: Dried *R. notoginseng* sample was obtained from the Henan Chinese Herbal Medicine Company, (Zhengzhou, China), and was authenticated by the Department of Botany, Zhengzhou University (Zhengzhou, China.). The sample was identified by macro-morphological and microscopic characteristics and thin layer chromatography (TLC). Based on the Chinese Pharmacopoeia, it was identified as the root of *Panax notoginseng* (Burk.) F.H. Chen.

Preparation of total saponins of *R. notoginseng* (TSRN): Dried *R. notoginseng* was ground to powder and passed through a 40 mesh sieve. TSRN were prepared by the method of Sun *et al*¹³. In brief, the powered samples (1 kg) were extracted with 70 per cent ethanol at 100°C (3×4 l), and concentrated in vacuum (40°C) to evaporate the solvent to give a small volume. After extracting with ether (3×0.5 l), the water layer portion was extracted with n-Butanol until n-Butanol layer became colourless. The n-Butanol solution was concentrated and dried in vacuum (60°C). The dried extract was subjected to D101 resin column chromatography, washed with H₂O, and eluted with Etanol to give TSRN. TSRN contained 64.3 ± 1.15 per cent of notoginsenoside as determined by thin layer chromatography and spectrophotometric method.

Animals: Male Kunming mice, weighing between 18–22 g, were obtained from Laboratory Animal Center, Medical College of Zhengzhou University (Zhengzhou, China.), and were fed a commercial diet and water *ad libitum*. The commercial diet consisted of 12 per cent fat, 60 per cent carbohydrate, and 28 per cent protein. The animals were housed under a 12-h light/dark cycle at a temperature of $22 \pm 1^\circ\text{C}$ and a humidity of 50 ± 5 per cent. The mice were allowed to acclimate to the laboratory environment for at least 1 week before the experiments. Ethical clearance for performing the experiments on animals was obtained from Institutional Animal Ethics Committee.

Grouping of animals: Ninety six mice were randomly divided into four groups, each consisting of 24 mice. The first group designated as control group (Control)

was administered with distilled water by gavage every day. The second, third and fourth groups designated as TSRN treatment groups were administered with TSRN of 20, 40 and 80 mg/kg body/weight day, respectively. The administration of distilled water or TSRN was continued for 28 days. The doses of TSRN and 28 days treatment time used in this study were confirmed to be suitable and effective in tested mice, according to preliminary experiments.

Exhaustive swimming test: After the final treatment with TSRN or distilled water, the mice were allowed to rest for 30 min. Then, eight mice were taken out from each group for exhaustive swimming test. The animals were placed in the swimming tank (50 × 50 × 40 cm) 30 cm deep with water maintained at $25 \pm 2^\circ\text{C}$. The tail of each mouse was loaded with a bundle of lead pieces, which was 10 per cent of its body weight. Exhaustion was determined by observing loss of coordinated movements and failure to return to the surface within 10 sec¹⁴. The swimming time was immediately recorded.

Measuring biochemical parameters related to fatigue: After the final treatment with TSRN or distilled water, the mice were allowed to rest for 30 min. Then, eight mice were taken out from each group for blood lactate analyses. Mice were forced to swim for 30 min after weight loading (2% body weight), and blood was collected from the tail vein before and after swimming. Blood lactate contents were measured according to the recommended procedures provided by the commercial diagnostic kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

The remaining eight mice were taken out from each group for tissue glycogen analyses. Mice were forced to swim for 90 min without loads. After resting for an hour, mice were killed by cervical dislocation under anaesthesia. The liver and gastrocnemius muscle were collected¹⁵. Tissue glycogen contents were tested according to the recommended procedures provided by the commercial diagnostic kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

Statistical analysis: All the tests were conducted in triplicate. The experimental data were expressed as mean \pm standard deviation. One-way analysis of variance (ANOVA), LSD and Dunnett's T3 tests were performed to determine the significant difference between samples within the 95% confidence interval, using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA).

Results

Effects of TSRN on exhaustive swimming time of mice: Exhaustive swimming time of the third and fourth groups increased significantly ($P < 0.05$) when compared with the control group. However, exhaustive swimming time of the second group showed no significant changes compared with the control group (Fig. 1). The swimming time of the second, third and fourth groups increased by 21.15, 27.41 and 34.01 per cent, respectively.

Effects of TSRN on blood lactate contents of mice after swimming: There was no significant difference in the

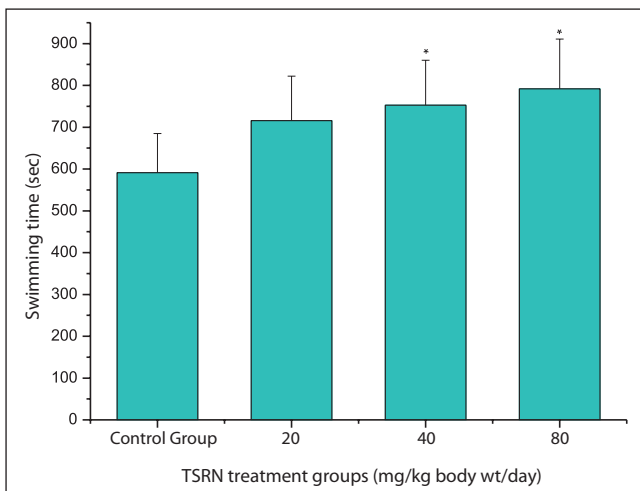


Fig. 1. Effects of total saponins of *R. notoginseng* on exhaustive swimming time of mice. Values are expressed as mean \pm SD of 8 mice per group. * $P < 0.05$ compared to the control group.

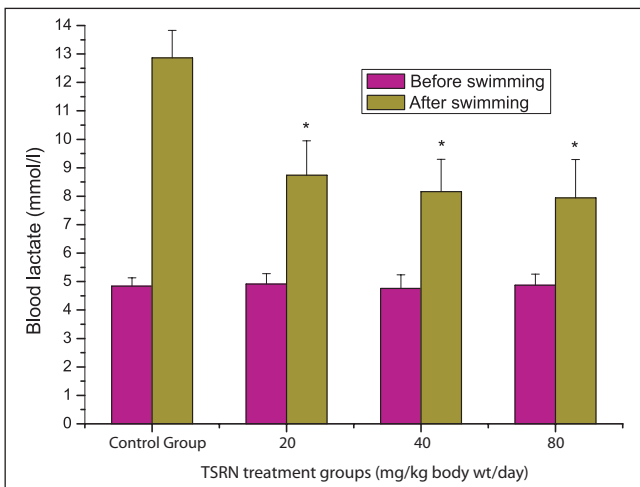


Fig. 2. Effects of total saponins of *R. notoginseng* on blood lactate contents of mice after swimming. Values are expressed as mean \pm SD of 8 mice per group. * $P < 0.05$ compared to control group.

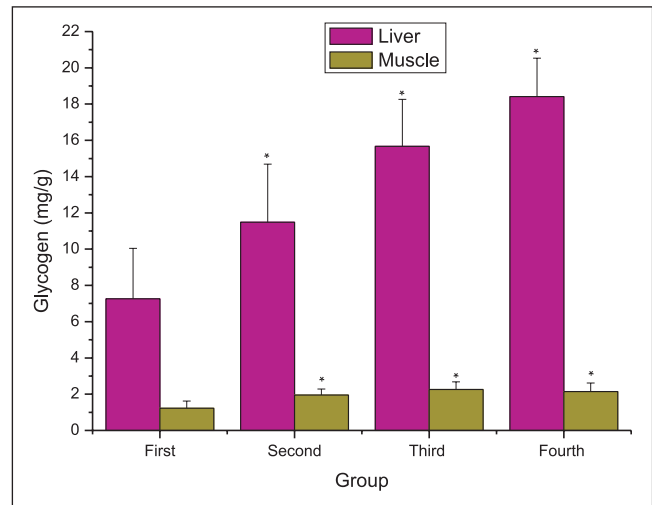


Fig. 3. Effects to total saponins of *R. notoginseng* on liver and muscle glycogen contents of mice after swimming. Values are expressed as mean \pm SD of 8 mice per. * $P < 0.05$ when compared to first group.

blood lactate contents between TSRN treatment groups and control group before swimming. After swimming, blood lactate contents of each TSRN treatment groups decreased significantly ($P < 0.05$) when compared to control group (Fig. 2). The results indicated that the blood lactate contents of the second, third and fourth groups decreased by 47.14, 57.59 and 61.96 per cent, respectively.

Effects of TSRN on tissue glycogen contents of mice after swimming: After swimming, liver and muscle glycogen contents of each TSRN treatment groups increased significantly ($P < 0.05$) when compared with that of control group (Fig. 3). The liver glycogen contents of TSRN treatment groups increased by 58.12, 115.84 and 153.58 per cent, respectively. The muscle glycogen contents of treatment groups increased by 58.54, 83.74 and 73.98 per cent, respectively.

Discussion

The present study was designed to investigate the anti-fatigue activity of total saponins of *R. notoginseng* (TSRN). Forced swimming of animals has been employed as a criterion of their physical work capacity. Many studies pointed out that swimming has advantages over other forms of exercise, including the treadmill¹⁶⁻¹⁹. To standardize the workload and reduce the swimming time, weights at specific body weight percentages were added to the chest or tail of the animal^{17,20}. The present study showed that TSRN extended exhaustive

swimming time of mice, which indicated that TSRN had anti-fatigue activity and could elevate the exercise tolerance.

To explore the mechanisms, some biochemical parameters were determined in the mice after swimming. Blood lactate is the glycolysis product of carbohydrate under an anaerobic condition, and glycolysis is the main energy source for intense exercise in a short time^{21,22}. Thus, blood lactate is one of the important indicators for judging the degree of athletic fatigue. In the present study, the TSRN effectively delayed the increase of lactate in the blood and the appearance of fatigue. Energy for exercise is derived initially from the breakdown of glycogen, after strenuous exercise muscle glycogen will come exhaust, and later, energy will come from circulating glucose released by the liver. Thus, the liver and muscle glycogen contents are sensitive parameters related to fatigue²³⁻²⁵. In the present study, the TSRN significantly increased tissue glycogen contents of mice after swimming.

In conclusion, the results suggested that TSRN had anti-fatigue activity, which extended exhaustive swimming time of mice, effectively delayed the increase of lactate in the blood, as well as increased the tissue glycogen contents. Further study is needed to elucidate the exact mechanism of the effect of TSRN on fatigue.

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