International Journal of Pharmacy and Pharmacology ISSN: 2326-7267 Vol. 3 (2), pp. 001-006, February, 2012.
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Full Length Research Paper

Anti-athletic fatigue activity of saponins (Ginsenosides) from American ginseng (*Panax quinquefolium* L.)

Keji Tang[1*], Ruixin Nie[2], Longjun Jing[3, 4] and Quansheng Chen[5]

[1]Dezhou University, Dezhou, Shandong Province, 253023, People's Republic of China.
[2]Yuxi Normal University, Yuxi, Yunnan Province 653100, People's Republic of China.
[3]Qujing Normal University, Qujing, Yunnan Province, 655011, People's Republic of China.
[4]Beijing Sport University Beijing, 100084, People's Republic of China.
[5]HuangGang Polytechnic College, HuangGang, Hubei Province, 438002, People's Republic of China.

Accepted 21 November, 2011

To study the anti- athletic fatigue effects of saponins from American ginseng (SAG), male Kunming mice were randomized into 4 groups equally based on body weight after one week adoption, and they are: C group (control mice given distilled water for 14 days), LS group (mice treated SAG with 50 mg/kg for 14 days), MS group (mice treated SAG with 100 mg/kg for 14 days) and HS group (mice treated SAG with 200 mg/kg for 14 days). The C group was given distilled water and LS, MS; HS groups were given various doses of SAG (50, 100, 200mg/kg) for 14 consecutive days. The levels of lactate, serum urea nitrogen, liver glycogen, muscle glycogen, the swimming endurance time and body weight were determined before and after swimming test. Different doses of SAG significantly lengthened the swimming endurance time and increased the levels of liver glycogen and muscle glycogen, while reducing the levels of lactate significantly compared with control group, especially in the MS group. Our data demonstrated SAG has noticeable anti-athletic fatigue effect on mice. These effects were dose-dependent, and the strongest effect on most biomarkers was seen with 100 mg/kg dose.

Key words: Saponins, American ginseng, anti-athletic fatigue activity.

INTRODUCTION

Panax quinquefolium L., which grows in the United States and Canada, is known as American ginseng (AG), and is one of the 10 most commonly used herbal medicines in the United States (Kang et al., 2007). It belongs to the Panax genus of the Araliaceae (Yang et al., 2007). Also it has been used in traditional medicine in China and other countries for the treatment of various diseases (Zhang et al., 2003), including psychiatric and neurologic diseases as well as diabetes mellitus (Yuan et al., 1998; Xie et al., 2004). Saponins (ginsenosides) have been regarded as the principal components responsible for the pharmacolo-gical activities of American ginseng (Attele et al., 1999; Li, 1995; Liu et al., 2008). Saponins from American ginseng (SAG) have been reported to exhibit antioxidant and free radical scavenging activities (Li et al., 1999; Kitts et al., 2000). They also demonstrate antiulcer and immunomo-dulatory activities (Zhang et al.2005).

Athletic fatigue refers to that due to over-exercise, the body cannot maintain physiologically its specific level or cannot maintain the predetermined exercise intensity, manifested as mental and physical fatigue (Wu et al., 2003; Chen et al., 2004; Gao and Chen, 2003). Over-intensity of exercise will lead to athletic fatigue and affect sport skills brought into play. Therefore, to prevent and release athletic fatigue is the hot topic in the researches on improving exercise quality. The exercise capacity can be improved by supplementing energetic substance, rele-asing metabolic production and administrating tonics, but which brings harm to the body even though retarding the fatigue (Li and Wei, 2005). During seeking for the safe and effective anti-athletic fatigue methods, the specialty of tradi-tional herbal medicines has drawn the attention of scholars in the world. In the last decade, extensive rese-arch has been conducted on saponins from American ginseng (SAG), and has obtained many results. However, related research on athletic health protection as well as its function in anti-athletic fatigue and the improvement of athletic ability of animal or human body is less available. In the present study, the anti-athletic effect of SAG and its

*Corresponding author. E-mail: xjyangxiaojun@sina.com. Tel.: +8613853460692

Table 1. The composition of diet fed to mice.

Ingredient	Content (%)	Ingredient	Content (%)
Corn starch	20	bone meal	3
bran	19	yeast powder	2.3
rice	16	salt	0.5
soybean oil meal	20	Vitamin mix	0.1
Calcium flour	3	microelement	0.1
fish flour	16	-	-

effective dose were investigated through swimming exercise using mice.

MATERIALS AND METHODS

Plant material

The roots of American ginseng were purchased from a local drug market and authenticated by Mr Guang Li, a botanist of Dezhou University (Dezhou city, Shandong Province, China). A voucher specimen has been deposited in herbarium of Dezhou University.

Extraction of saponins from American ginseng

The dried roots of American ginseng (4000 g) were ground in a high speed disintegrator to obtain a fine powder, and then extracted with 70% EtOH (50 L) three times (2 h for each time) under reflux. After filtration, excess solvent was removed under reduced pressure. The EtOH extract was suspended in water and defatted with ether followed by partitioning with n- BuOH. The combined n- BuOH layers were concentrated to dryness (Yang et al., 2007). The dried extract was subjected to HPD100 resin column chromatography, washed with water, and eluted with EtOH to afford a total saponins fraction (1224 g).

Determination of the totals saponins from American ginseng

The measurement of total saponins from American ginseng followed the colorimetric method described in Ref. with modifications (Li et al., 2008). The method was based on a color reaction of the acid-hydrolysis products of the saponins, sapogenins, with vanillin. The purified American ginseng extract solution in acetonitrile (10ul) was applied to a TLC plate (Silica Gel 60, UV254, 0.25 mm layer) with chloroform-methanol -water at the ratio of 15:12:2 as the mobile phase. The total saponins spot was located with an American ginseng standard, then scratched off and mixed with 0.2 ml of acetic acid containing 5% vanillin and 0.8 ml of perchloric acid at 60°C for 15 min. The concentration of total saponins was determined with a spectrophotometer at 560 nm against a calibration curve established with a panaxtriol standard. According to above method (Wu et al., 2001), it was calculated that purity of total saponins was 91.44% and extraction yield of total saponins was 22.38%.

Animals and diets

Kunming male mice weighing approximately 18 - 20 g were obtained from Research Institute of Surgery Experimental Animal Center (Jinan, China). The animals were housed in a room maintained at 25 - 30°C with relative air humidity of 45 - 55% on a 13 h light/ 11 h dark cycle. Mice were provided the basal diet (the Disease Control Center, Jinan China) and water ad libitum. The ingredient and nutrient composition of the basal diet fed to mice are given in Table 1. The approval of this experiment was obtained from the Institutional Animal Ethics Committee of Dezhou University.

Experimental design

The mice were randomized into 4 groups equally based on body weight after one week adoption:

Group 1. Control mice given distilled water for 14 days (C).
Group 2. Mice treated SAG (50 mg/kg) for 14 days (LS).
Group 3. Mice treated SAG (100 mg/kg) for 14 days (MS).
Group 4. Mice treated SAG (200 mg/kg) for 14 days (HS).

SAG was dissolved in distilled water and fed by gavage to mice once a day. The control group was given distilled water and the treated groups were given different doses of SAG (50, 100, 200 mg/kg). The above method of grouping and feeding was repeated to determine related indicators.

The swimming exercise of mice was measured with acrylic plastic pool (90 × 45 × 45) filled with water to a depth of 35 cm (Matsumoto et al., 1996; Kamakura et al., 2001). The temperature of the water was maintained at 34 ± 1°C.

Determination of swimming endurance time

Eight male mice were taken out from each group to make swimming endurance exercise. The mice were loaded with a lead block weighing approximately 5% of their body weight attached to the tail. It was reported that this arrangement forced the mouse to maintain continuous rapid leg movement (Bostrom et al., 1974). The end point of the swimming endurance was taken as when the mouse remained at the bottom for more than 10 s, when swimming endurance time was measured.

Biochemical analysis

Eight male mice were taken out from each group for blood lactate analyses. The mice made swimming exercise and were loaded with a lead block weighing approximately 2% of their body weight attached to the tail. 20 L of blood were collected from the veins of the tails of mice after the last administration of SAG. Another 20 µL of blood samples were collected immediately after mice had been swimming for 10 min. The third batch of blood samples were collected after mice had rested for 20 min. The levels of blood lactate were determined using a commercial diagnostic kit obtained from Jiancheng Diagnostic Systems (Nanjing, China). The increase ratio of blood lactate was calculated as using the following equation (Yu et al., 2008):

Increase ratio = (a- b)/b
Reduce ratio = (a- c)/c
Where,
a=the blood lactate concentration of mice after swimming

Table 2. Effect of the SAG on body weight of mice.

Group	Body weight (g)	
	Initial	Final
C	25.8 ± 1.6	36.4 ± 2.7
LS	25.7 ± 1.8	34.7 ± 3.2
MS	25.5 ± 1.2	35.9 ± 2.4
HS	25.6 ± 1.7	36.1 ± 3.5

N = 24; (means ± S.E).

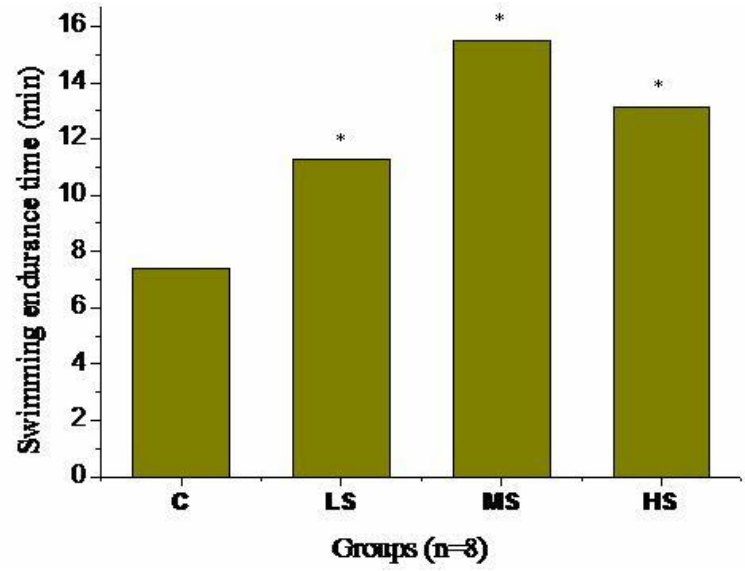

Figure 1. Effect of SAG on swimming endurance time of mice N = 8; (means ± S.E); P < 0.05 as compared with C group.

immediately.
b=the blood lactate concentration of mice before swimming.
c=the blood lactate concentration of mice after resting for 20 min.

Eight male mice were taken out from each group for liver glycogen, muscle glycogen and serum blood urea nitrogen (BUN) analyses. The mice made swimming exercise for 90 min without a load. After an hour's resting, the mice were killed to collect liver, gastrocnemius muscle, and plasma samples. The levels of liver glycogen and muscle glycogen were determined using commercial diagnostic kit obtained from Jiancheng Diagnostic Systems (Nanjing, China). The levels of serum blood urea nitrogen (BUN) were determined by an SABA/18 automatic. Biochemistry analyzer from Italy.

Statistical analysis

All values were expressed as means ± S.E. Data were analyzed by one-way ANOVA, and then differences among means were analyzed using Fisher's protected least significant differences (LSD) multi-comparison test. Differences were considered significant at $p < 0.05$.

RESULTS

Effect of the SAG on body weight of mice

Change of body weight, during the experimental period is shown in Table 2. Body weight was recorded before experiment (initial) and after 14 days (final).

Effect of the SAG on swimming endurance time of mice

Swimming endurance time of mice is shown in Figure 1. The average swimming time of mice of the treated groups were all remarkably longer than that of the control group (C group) ($P < 0.05$).

Effect of the SAG on blood lactate of mice

The levels of blood lactate of mice with different treatment were measured before swimming, after swimming and after 20 min rest as described in the methods. The data of increase ratio and reduce ratio of blood lactate were shown in Table 3.

Effect of the SAG on serum BUN of mice

The levels of serum BUN of mice are shown in Figure 2.

Table 3. Effect of the SAG on blood lactate of mice.

Group	The levels of blood lactate (mmol/l)			Increase ratio	Reduce ratio
	Before swimming		After swimming	After 20 min rest	
C	4.64 ± 1.23	9.59 ± 1.45	7.91 ± 1.04	1.04 ± 0.01	0.21 ± 0.02
LS	4.78 ± 0.87	8.44 ± 1.13	5.98 ± 0.92	0.77 ± 0.01	0.41 ± 0.01
MS	4.85 ± 1.14	7.15 ± 0.96	4.94 ± 1.25	0.47 ± 0.02	0.45 ± 0.01
HS	4.69 ± 1.06	7.76 ± 1.17	5.43 ± 1.01	0.65 ± 0.01	0.43 ± 0.01

N = 8; (means ± S.E); P < 0.05 as compared with C group.

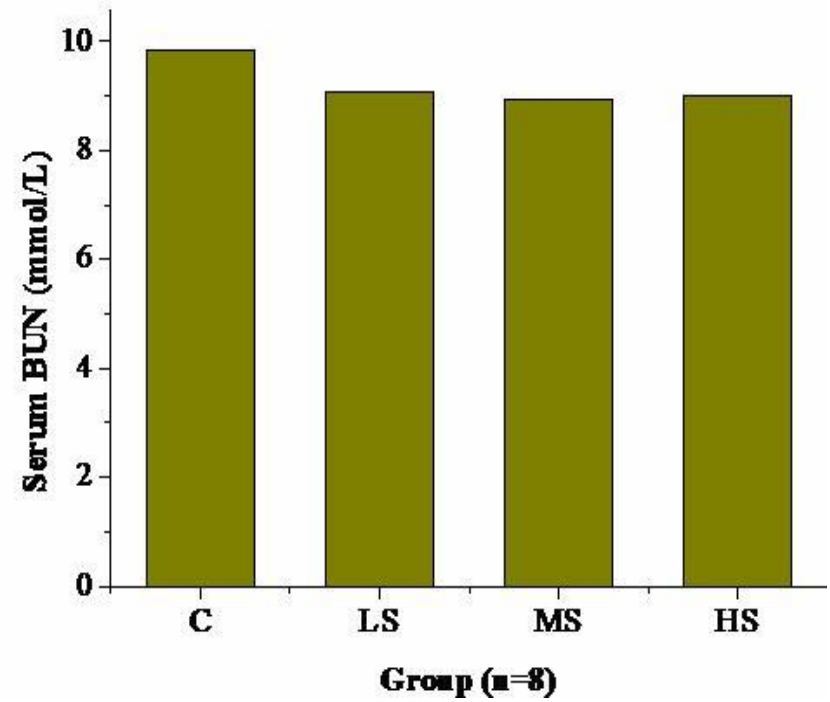

Figure 2. Effect of SAG on s serum BUN of mice N = 8; (means ± S.E).

Effects of SAG on liver glycogen and muscle glycogen of mice

The levels of liver glycogen and muscle glycogen of mice are shown in Table 4.

DISCUSSION

American ginseng has become universally popular in recent years. The major active ingredients have been demonstrated to be saponins and the most abundant saponins are neutral ginsenosides, Rb_1, Rb_2, Rc, Rd, Re and Rg_1 (Kim et al., 2007), they contribute to their multiple medicinal properties. The present study demonstrated for the first time that SAG has an anti-athletic fatigue effect.

In the present study, results showed that the increased weights in the treated groups (LS group, MS group and HS group) were of no significant difference compared with the control group (C group) (P > 0.05). So the SAG had no significant effect on body weight.

The swimming exercise was employed in our study to evaluate anti- athletic fatigue activity of SAG on mice. It is commonly accepted that swimming is an experimental exercise model (Orlans, 1987; Lapvetelainen et al., 1997). In the present study, the average swimming time of the LS, MS and HS group was increased by 51.89, 108. 63 and 77.09% respectively. These results indicated that different doses of SAG could significantly lengthen the swimming endurance time and the dosage of 100 mg/kg was more effective, which indicated that SAG could elevate the endurance of the mice.

In order to clarify anti-athletic fatigue mechanism, biochemical parameters were measured in the forced swimming-treated mice. The swimming exercise is known to induce biochemical changes (Moriura et al., 1996). 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 (Yu et al., 2008). Therefore, the blood lactate is one of the important indicators for judging the degree of athletic fatigue. The

Table 4. Effect of the SAG on liver and muscle glycogen of mice.

Group	The levels of glycogen (mg/g)	
	Liver	Muscle
C	7.87 ± 1.23	1.25 ± 0.87
LS	13.91 ± 1.14	1.92 ± 0.65
MS	18.64 ± 2.16	2.29 ± 0.92
HS	16.45 ± 1.35	2.06 ± 1.01

N = 8; (means ± S.E); P < 0.05 as compared with C group.

increase ratios of the blood lactate was the percentage of the increase of blood lactate after swimming and that of before swimming, which can be used as an indicator of the degree of athletic fatigue. The reduce ratios of blood lactate, reflecting the reducing of blood lactate after 20 min rest, represents the condition of recovery. In the present study, it was found that the levels of blood lactate of each group had no significant difference (P > 0.05) before swimming. However, after swimming, the increase ratios of the blood lactate of the LS group (0.77), MS group (0.47) and HS group (0.65) were lower than that of the control group (1.04) (P < 0.05). The reduce ratios of the LS group, MS group and HS group was 0.41, 0.45 and 0.43, respectively, which were higher than the reduce ratio of 0.21 achieved by control group (P < 0.05). These results indicated that SAG could effectively retard and lower the blood lactate produced after swimming, postpone the appearance of athletic fatigue and accelerate the recovering from athletic fatigue and the dosage of 100 mg/kg was more effective. Serum blood urea nitrogen is the other important biochemical parameter related to athletic fatigue. In this experiment, the serum BUN of the LS, MS and HS groups were 9.06 ± 1.04, 8.94 ± 0.82, 9.01 ± 1.07 mmol/L, which were lower than that of the control group (9.83 ± 1.36 mmol/L), but there was not significant difference (P > 0.05).

Our results showed the trend that serum BUN of the LS, MS and HS groups were lower than control group. It was known that endurance capacity of body was markedly decreased if the energy was exhausted. Energy for exercise is derived initially from the breakdown of glycogen and, later, from circulating glucose released by the liver (Suh et al., 1996). So liver and muscle glycogen are sensitive parameters related to athletic fatigue (Ma et al., 2008). In the present study, It was found that the levels of liver glycogen of LS group, MS group and HS group were higher than that of the control group (P < 0.05). These data indicated that SAG could significantly increase the levels of liver and muscle glycogen of mice after swimming and the dosage of 100 mg/kg were more effective. However its detailed mechanism isn't clear. The possible reason is that SAG may increase the content of liver and muscle glycogen of mice post exercise by improving glycolgen reserve, or by reducing the consume of glycogen during exercise, or both (Ma et al., 2008). Further studies are needed to confirm these results.

Conclusion

This study results suggested that SAG had significant anti-athletic fatigue effects on mice and these effects were dose- dependent, and the strongest effect on most biomarkers was seen with 100 mg/kg dose. The detailed mechanism of anti-athletic fatigue properties of SAG might be mediated through regulating central nervous system, antioxygen and improving substance metabolism and aerobic capacity.

ACKNOWLEDGMENTS

This work was funded by Beijing Sport University, China (project number BT20090034) at the same time; we really appreciate Mr Guang Li (Dezhou University, Shandong Province, China) for authenticating plant sample.

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