Journal of Ethnopharmacology 133 (2011) 213–219

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Bioactivity-guided fractionation for anti-fatigue property of *Acanthopanax senticosus*

Lin-Zhang Huang[a,b], Bao-Kang Huang[a], Qi Ye[a], Lu-Ping Qin[a,*]

[a] Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, 325 Guohe Road, Shanghai 200433, PR China
[b] Department of Pharmacy, Fujian University of Traditional Chinese Medicine, 1 Huatuo Road, Fuzhou 350108, PR China

ARTICLE INFO

Article history:
Received 22 June 2010
Received in revised form 9 September 2010
Accepted 20 September 2010

Keywords:
Acanthopanax senticosus
Eleutherococcus senticosus
Anti-fatigue
Bioassay-guided
Sleep-deprived
Behavioral
Macroporous resin

ABSTRACT

Ethnopharmacological relevance: The root of *Acanthopanax senticosus* (also called *Eleutherococcus senticosus* or Siberian ginseng) has been used extensively in China, Russia and Japan as an adaptogen to fight against stress and fatigue.
Aim of the study: The present study was designed to ascertain the anti-fatigue property of *Acanthopanax senticosus* by load-weighted swimming test, sleep deprivation test, also to isolate and characterize the active constituents.
Materials and methods: Animals were orally administered with the extract of *Acanthopanax senticosus*. The anti-fatigue effects of the four fractions with different polarities from the 80% ethanol extract, and the different eluates collected from D101 macroporous resin chromatography and eleutheroside E, were examined based on the weight-loaded swimming capacity (physical fatigue) and the change of biochemical parameters in ICR mice. Moreover, the active fraction was later submitted to sleep-deprived mice (mental fatigue).
Results: The results shown that the *n*-butanol fraction significant extends the swimming time of mice to exhaustion. Furthermore, the 60% ethanol–water eluate, more purified eleutherosides (including eleutheroside E, E_2 and derivatives), were the exactly active constituents. Two compounds were isolated, which were identified as eleutheroside E, E_2.
Conclusions: The eleutherosides possess the potent abilities to alleviate fatigue both in physical and mental fatigue. Eleutheroside E may be responsible for the pharmacological effect of anti-fatigue. Furthermore, the possible mechanisms were reduced the level of TG by increasing fat utilization, delayed the accumulation of blood urea nitrogen (BUN), and increased the LDH to reduce the accumulation of lactic acid in muscle and then protect the muscle tissue.

1. Introduction

Acanthopanax senticosus (Rupr. & Maxim.) Harms (also called *Eleutherococcus senticosus* or Siberian ginseng), belonging to the genus *Acanthopanax* (Araliaceae), is a hardy shrub, native to the northeastern region of China, Korea, Japan and the far-eastern region of Russia. In China, it has been used for invigorating the liver and kidney, replenishing the vital essence and strengthening bone for a long history time. While the extract of the *Acanthopanax senticosus* (AS), once the substitute drug of panax ginseng, has also been known as a powerful tonic herb with an impressive range of health benefits in Russia. That has been used widely in Asia for the control of blood pressure, for mental and emotional problems, as analeptic or agents to cope with stress (Brekhman and dardymov, 1969; Kimura and Sumiyoshi, 2004).

Fatigue is known to be accompanied by a feeling of extreme physical or mental tiredness, resulting from severe stress and hard physical or mental work. It can be divided into two categories: physical fatigue caused by such things as forced exercise or swimming; mental fatigue caused by sleep deprivation etc. (Akazawa et al., 2010; Chen et al., 2009). In the past few decades, health scholars and athletic physiologist have been looking for natural active products that not only can improve athletic ability, postpone fatigue and accelerate the elimination of fatigue in human beings, but also have few side effects (Kim et al., 2002). The AS taken as an adaptogen is doomed to be the right candidate. Although the anti-fatigue and anti-stress activities of AS have been reported previously (Nishibe et al., 1990; Fujikawa et al., 1996; Jung et al., 2004; Kimura and Sumiyoshi, 2004), the exactly what kinds of components were responsible for, and the systematic study of bioactivity-guided fractionation, were rather limited. Based on the

* Corresponding author. Tel.: +86 21 81871300; fax: +86 21 81871300.
E-mail addresses: lpqin@smmu.edu.cn, qinsmmu@126.com (L.-P. Qin).

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doi:10.1016/j.jep.2010.09.032

previous investigations, we studied the anti-fatigue activity of the petroleum ether, ethyl acetate, *n*-butanol and the water fractions from the aqueous ethanol extract, and found that the *n*-butanol fraction had a powerful anti-fatigue activity in preliminary experiment. The present study was designed to separate the *n*-butanol fraction by D101 macroporous resin, and then further examine the anti-fatigue activity and the possible mechanism as well as elucidating its purification process of the active components and providing a scientific basis for the industry production.

2. Materials and methods

2.1. Drugs and chemicals

The following reagents were used: EtOH (AR), petroleum ether (AR), ethyl acetate (AR), *n*-butanol (AR) (Sinopharm chemical reagent Co. Ltd., China).

D101 macroporous resin was purchased from Chemical Plant of Nankai University (Tianjin, China). The resin was prepared under the instructions, briefly, washed by 95% ethanol, then by distilled water, after that, washed by 5% HCl, 5% NaOH and distilled water. Prior to use, the resin was washed by distilled water thoroughly (Sun et al., 2009).

2.2. Plant material

The stem and root of *Acanthopanax senticosus* (Rupr. et Maxim.) Harms (*Eleutherococcus senticosus*, or *Ciwujia* in Chinese), which were identified by Prof. Lu-Ping Qin, Department of Pharmacognosy, Second Military Medical University, were obtained from Jilin Province, China. A voucher specimen has been deposited in the herbarium of the Department of Pharmacognosy, School of Pharmacy, Second Military Medical University. Red Ginseng pieces were purchased from Shanghai Leiyunshang Drug store.

2.3. Preparation of the ethanol extract and the fractions

Powdered stem and root of AS (20 kg) were immersed in aqueous ethanol for 12 h, and then extracted for 2 h, twice under reflux. The solvent was evaporated under vacuum to afford 1436 g crude extract (yield, 7.18%). This extract was then suspended in water and partitioned successively with petroleum ether, ethyl acetate and aqua-saturated *n*-butanol. Each fraction was evaporated in vacuo to yield the residues of petroleum ether 68 g (4.74%), ethyl acetate 411 g (28.62%), *n*-butanol 407 g (28.34) and aqueous 550 g (38.3%). The extracts were concentrated under reduced pressure at 45–55 °C, lyophilized to obtain powder, and then used as test samples. For pharmacological studies, fractions were suspended in a 1% aqueous solution of Tween-80. The doses employed are expressed as mg of the dried extract per kg body weight.

The residue of *n*-butanol fraction was dissolved into distilled water. Then the solution was instilled into the prepared D101 macroporous resin, at a rate of 3 ml/min, till the sample was absolutely adhered to the resin. After that the resin was washed with distilled water until there was no sugar in the eluate, and then eluted by ethanol solutions of different concentrations (20%, 60% and 100%) successively at 5 ml/min. Each desorption solution was collected and concentrated to dryness under vacuum.

2.4. Animals

Five-week-old male ICR mice (18–22 g) were used and housed in cages (20 cm × 32 cm × 14 cm) under automatically controlled conditions of temperature (25 ± 1) °C with 60% relative humidity and provided with free access to laboratory standard diet and water. The room lights were on for 12 h/day starting at 7:00 h. The care and treatment of experimental animals conformed to the guidelines for the Ethical Treatment of Laboratory Animals.

2.5. Experimental design

2.5.1. Experiment I

After an adaptation period for one week, mice were randomly divided into ten groups, a control group, a positive group (Red ginseng group) and treatment groups, of ten each. The mice in the treatment groups of the four fractions (petroleum ether, ethyl acetate, *n*-butanol, and aqueous fractions from the aqueous ethanol extract of AS), were gavaged with two different doses, 500 mg/kg for the high-dose (HD) group (Nishibe et al., 1990; Fujikawa et al., 1996; Kimura and Sumiyoshi, 2004) and 200 mg/kg for the low-dose (LD) group. The control groups were treated with the similar volume of vehicle.

2.6. Measurement of the weight-loaded swimming capacity

The extracts of AS were administered orally daily (9:00 h) for consecutive 9 days. In order to make the animals to accustom to swim, swimming stress was carried out on day 1, 3, 5 and 7 for 10 min, distinguished from day 9, in which mice were loaded nothing on their tails. During the periods, those who could be not able to learn to swimming were kicked out.

The weight-loaded swimming test was employed in our study to evaluate the effects of the different fractions of AS on exercise durability of mice. The procedure used was described previously (Chen et al., 2005; Zhang et al., 2006; Tang et al., 2008) with some modifications. Briefly, 30 min after the last oral administration, the mice were dropped individually into an acrylic plastic pool (90 cm × 45 cm × 45 cm) filled with fresh water maintained at 30 ± 1 °C, approximately 40 cm deep so that mice could not support themselves by touching the bottom with their tails. A lead block (5% of body weight) was loaded on the tail root of the mice. The swimming time to exhaustion was used as the index of the forced swimming capacity. The mice were assessed to be exhausted when they failed to rise to the surface of water to breathe within a 7-s period.

2.7. Determination of BUN, TG, LDH

Thirty minutes after the last oral administration, mice were individually forced to swim in the acrylic plastic pool (90 cm × 45 cm × 45 cm) containing water at 30 ± 1 °C (40 cm deep). The mice were loaded with a lead block weighting approximately 5% of their body weight attached to the tail. After exhaustion, 0.5 ml of blood was collected in the tube without anticoagulant, by extirpating the left eyeball. Blood sample cooled for about 3.5 h at 4 °C, the serum was prepared by centrifugation at a speed of 1000 × *g*, 4 °C for 20 min and the levels of blood urea nitrogen (BUN), plasma triglyceride (TG), and lactate dehydrogenase (LDH) were determined by automatic biochemical analyser with commercial kits.

2.7.1. Experiment II

In this experiment, mice were randomly divided into eleven groups, a control group and treatment groups, of ten each. The treatment groups of the different eluates collected from D101 macroporous resin were administered with two different doses, 280 mg/kg for the high-dose and 70 mg/kg for the low-dose group. Eleutheroside E was treated with 10 or 50 mg/kg. Those doses selection for the test samples was based on the results of preliminary experiments.

The measurement of the weight-loaded swimming test and biochemical analysis of serum were similar to that of the experiment I. Difference, a lead block (7% of body weight) was loaded on the tail

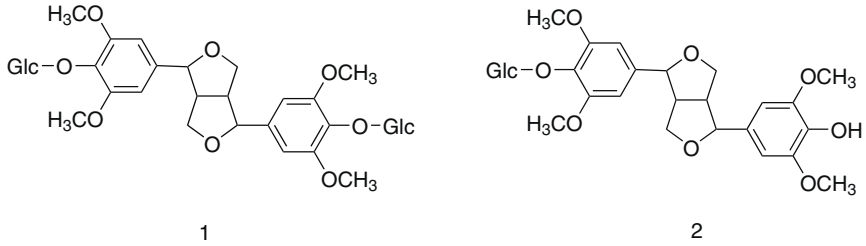

Fig. 1. Chemical structures of two lignan glucosides isolated from *Acanthopanax senticosus*.

root of the mice. The swimming time to exhaustion was used as the index of the forced swimming capacity. The mice were assessed to be exhausted when they failed to rise to the surface of water to breathe within a 7-s period. After that, the levels of BUN, TG, and LDH were determined by the automatic biochemical analyser with commercial kits.

2.7.2. Experiment III

After an adaptation period for one week, animals were divided into four groups, a control group, a sleep deprivation group and treatment groups. They were treated with saline, 70 mg/kg or 280 mg/kg of eleutherosides. During the last 72 h, sleep deprivation test was carried out. The method of sleep deprivation was conducted as previously described by Silva et al. (2004, 2007). Briefly, groups of 5 animals were placed in a filled water tank (41 cm × 34 cm × 17 cm), containing 11 platforms of 3.5 cm in diameter surrounded by water up to 1 cm beneath the surface, for 72 h, with food and water ad lib. In this method, the mice are capable of moving inside the tank, jumping from one platform to the other. The control animals were maintained in cages containing platforms and bedding instead of water.

2.8. Behavioral tests

The method of passive avoidance was used as previously described (de Oliveira et al., 2004; Silva et al., 2004). The apparatus employed was a two-way shuttle-box provided with a guillotine door placed between the modular testing chambers. One box was the safe compartment illuminated by a 40 W bulb, while the other remained in the dark where animals received the shock. In the training session, mice were individually placed in the illuminated chamber for 1-min adapting period, and then received a shock (0.5 mA) as they enter into the dark compartment. In the test sessions, the animals were again placed in the illuminated chamber, but no foot shock was applied. After the 24 h, 48 h and 72 h sleep deprivation, the latency time for entering into the dark compartment was measured, up to a maximum of 5 min.

Locomotor activity was assessed in a digital photoactometer. The total numbers of counts indicating movement of the mice were measured for 30 min and were expressed as total photocell counts of the photoactometer for 30 min per animal. Modification of locomotor function was assessed on day-7, day-8, day-9 and day-10 in control, sleep-deprived (SD), SD with ES 70 and SD with ES 280 groups (Lyle et al., 2009).

2.9. Statistical analysis

All the results were expressed as the mean ± S.E.M. in the tables and are indicated by vertical bars in the figures. Firstly, the data were analyzed by homogeneity test for variance. If the data were homoscedasticity, the significance of the mean difference was determined by one-way ANOVA, followed by a LSD-*t* test for multigroup comparisons. Otherwise, it was determined by Games–Howell test. All statistical analyses were performed using SPSS v13.0 statistical analysis software. Probability values $P < 0.05$ were considered significant.

3. Results and discussion

3.1. Chemical analysis

From the 60% ethanol fraction eluted from macroporous resin, two lignan glucosides (Fig. 1), were obtained and identified as eleutheroside E (1) and eleutheroside E_2 (2) (Lami et al., 1991; Li et al., 2001), both of which have been previously reported and isolated from the root of AS. The structures were elucidated unambiguously by spectroscopic methods including 1D and 2D NMR analysis and also by comparing experimental data with literature data.

Compound 1: White amorphous powder (1200 mg); ^{1}H and ^{13}C NMR (Lami et al., 1991). Compound 2: White amorphous powder (200 mg); ^{1}H and ^{13}C NMR (Li et al., 2001).

3.2. Effects on body weight change

The change of body weight in experiments I and II were shown in Tables 1 and 2, respectively. The one-way ANOVA results suggested that there were no significant differences in the body weight of the mice in the treatment groups, compared with the control group during initial and terminal stages in the experiments I and II. However, in the experiment III, significant weight loss was observed in sleep deprivation and after eleutherosides treatment ($P < 0.05$, Fig. 2). The combination of eleutherosides treatment and sleep deprivation suggested a tendency for reversal of weight loss compared with that of sleep deprivation.

Table 1
Effects of the fractions of *Acanthopanax senticosus* and Red ginseng on body weights in mice.

Groups	Body weights (g)		
	Initial	Final	Increased
Control	24.3 ± 0.6	28.2 ± 0.6	4.2 ± 0.5
Red ginseng	24.1 ± 0.5	28.0 ± 0.5	3.9 ± 0.7
A	24.0 ± 0.4	27.8 ± 0.3	4.0 ± 0.5
B	24.0 ± 0.5	27.3 ± 0.7	3.1 ± 0.7
C	25.0 ± 0.5	29.1 ± 0.6	4.0 ± 0.8
D	23.0 ± 0.4	28.3 ± 0.7	5.6 ± 0.8
E	24.6 ± 0.5	28.9 ± 0.6	4.7 ± 0.7
F	24.5 ± 0.4	28.5 ± 0.5	3.8 ± 0.6
G	23.9 ± 0.4	28.1 ± 0.5	4.5 ± 0.8
H	23.5 ± 0.5	27.6 ± 0.8	3.7 ± 0.9

A: low dose of petroleum ether fraction, B: high dose of petroleum ether fraction, C: low dose of ethyl acetate fraction, D: high dose of ethyl acetate fraction, E: low dose of *n*-butanol fraction, F: high dose of *n*-butanol fraction, G: low dose of water reside, H: high dose of water reside. Low dose means 200 mg/kg, high dose means 500 mg/kg for each mouse. Data are expressed as mean ± S.E.M. There are no significant differences between the control group and each treatment group by Student's *t*-test.

Table 2
Effects of the fractions of *n*-butanol and eleutheroside E on body weights in mice.

Group	Body weight (g)		
	Initial	Final	Increased
Control	23.2 ± 0.31	28.2 ± 0.31	5.3 ± 0.22
A	23.7 ± 0.29	28.0 ± 0.39	4.4 ± 0.96
B	23.3 ± 0.33	27.9 ± 0.51	4.6 ± 0.69
C	23.8 ± 0.33	28.3 ± 0.74	4.0 ± 0.26
D	24.3 ± 0.33	28.8 ± 0.73	4.7 ± 0.85
E	23.3 ± 0.43	27.9 ± 0.50	4.6 ± 0.76
F	23.5 ± 0.33	28.7 ± 0.71	5.0 ± 0.74
G	23.4 ± 0.42	27.4 ± 0.50	3.9 ± 0.61
H	23.4 ± 0.34	28.2 ± 0.52	4.6 ± 0.72
EE 10	22.9 ± 0.21	27.2 ± 0.15	4.2 ± 0.58
EE 50	23.5 ± 0.35	28.7 ± 0.34	5.2 ± 0.64

A: low dose of water eluate, B: high dose of water eluate; C: low dose of 20% ethanol eluate, D: high dose of 20% ethanol eluate, E: low dose of 60% ethanol eluate, F: high dose of 60% ethanol eluate, G: low dose of 100% ethanol eluate, H: high dose of 100% ethanol eluate. Data are expressed as mean ± S.E.M. There are no significant differences between the control group and each treatment group by Student's *t*-test.

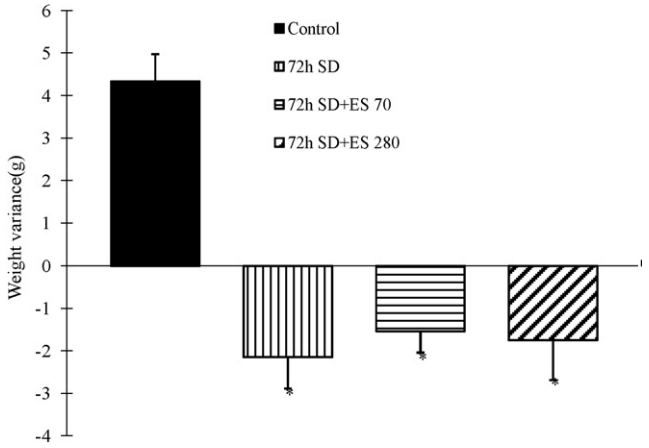

Fig. 2. Effect of eleutherosides (ES) on the body weight of sleep-deprived mice. Animals were treated with eleutherosides, ES (70 and 280 mg/kg, i.g.), during 9 days, and, on the seventh day of treatment, were submitted to 72 h of sleep deprivation (SD) or maintained in home cages as control. "72 h SD" means that animals were submitted to 72 h sleep deprivation. "72 h SD + ES 70/280" mean that mice were treated with different doses of eleutherosides (70 or 280 mg/kg) and then submitted to 72 h sleep deprivation. Values represent mean ± S.E.M. of the weight variance between the 7th and 10th days of treatment. *$P < 0.05$ vs. control.

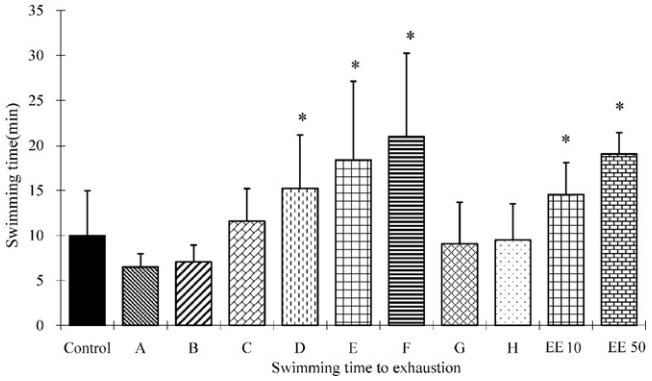

Fig. 4. Effects of the fractions of *n*-butanol on the swimming time to exhaustion of weight-loaded mice. A: low dose of water eluate, B: high dose of water eluate, C: low dose of 20% ethanol eluate, D: high dose of 20% ethanol eluate, E: low dose of 60% ethanol eluate, F: high dose of 60% ethanol eluate, G: low dose of 100% ethanol eluate, H: high dose of 100% ethanol eluate. Those fractions were collected from D101 macroporous resin. Low dose means 70 mg/kg, high dose means 280 mg/kg for every mouse. EE 10 and EE 50 mean that animals were treated with eleutheroside E 10 mg/kg and 50 mg/kg. Values are means ± S.E.M. of mice per group. (*) Significantly different from control group ($P < 0.05$).

3.3. Effects on weight-loaded swimming test

The results were shown in Figs. 3 and 4. The swimming time to exhaustion of the high-dose *n*-butanol fraction was significantly longer than the control group ($P < 0.01$), and the positive group (Red ginseng) was also significantly prolonged (Fig. 3, $P < 0.05$). Although the high-dose of petroleum ether fraction possessed the capacity to prolong the swimming time ($P < 0.05$), GC/MS study revealed that most of the components were fatty acids (data not shown). Fig. 4 revealed that the high-dose of 20% ethanol, 60% ethanol fractions and eleutheroside E treated groups significantly improved the mice swimming time to exhaustion ($P < 0.05$).

3.4. Effects on BUN, TG, LDH

The results were shown in Tables 3 and 4, respectively. The concentration of BUN was significantly different almost in all the groups in the experiment I ($P < 0.05$, $P < 0.01$), while the results were not that so in the experiment II. Evidence showed that free fatty

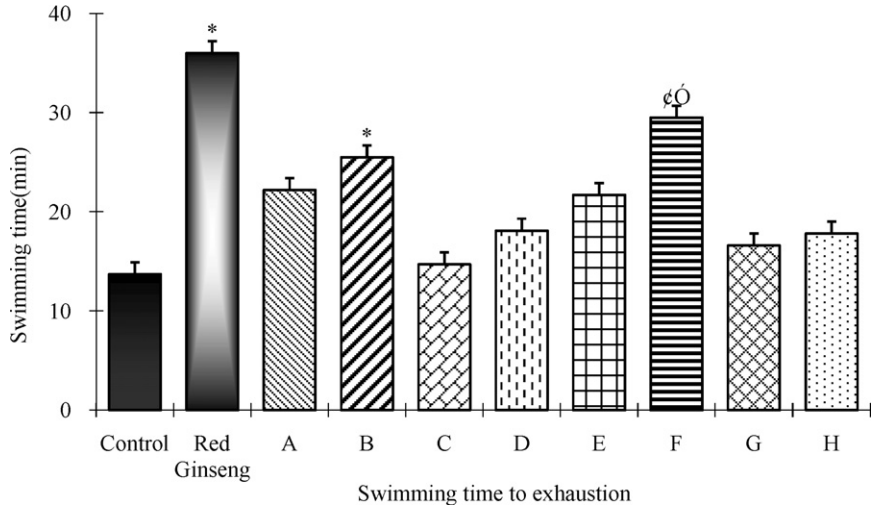

Fig. 3. Effects of the fractions of *Acanthopanax senticosus* and Red ginseng on the swimming time to exhaustion of weight-loaded mice. A: low dose of petroleum ether fraction, B: high dose of petroleum ether fraction, C: low dose of ethyl acetate fraction, D: high dose of ethyl acetate fraction, E: low dose of *n*-butanol fraction, F: high dose of *n*-butanol fraction, G: low dose of water reside, H: high dose of water reside. Low dose means 200 mg/kg, high dose means 500 mg/kg for each mouse. Values are means ± S.E.M. of mice per group. (*) Significantly different from control group ($P < 0.05$). (¢Ó) Significantly different from control group ($P < 0.01$).

Table 3
Effects of the fractions of *Acanthopanax senticosus* and Red ginseng on BUN, TG, LDH. A: low dose of petroleum ether fraction, B: high dose of petroleum ether fraction, C: low dose of ethyl acetate fraction, D: high dose of ethyl acetate fraction, E: low dose of *n*-butanol fraction, F: high dose of *n*-butanol fraction, G: low dose of water reside, H: high dose of water reside. Low dose means 200 mg/kg, high dose means 500 mg/kg for each mouse. Data are means ± S.E.M. of mice per group. (¢Ó) Significantly different from control group ($P < 0.01$).

Groups	BUN (mmol/l)	TG (mmol/l)	LDH (U/l)
Control	10.4 ± 0.32	2.862 ± 0.145	585 ± 30.0
Red ginseng	9.3 ± 0.34*	2.690 ± 0.041*	551 ± 39.2
A	9.8 ± 0.36	2.970 ± 0.158	562 ± 14.3
B	8.8 ± 0.14¢Ó	2.612 ± 0.086*	535 ± 24.8
C	9.7 ± 0.32	3.235 ± 0.137	652 ± 16.6
D	9.2 ± 0.19*	2.854 ± 0.170*	596 ± 23.2
E	8.8 ± 0.69¢Ó	3.171 ± 0.095	565 ± 44.3
F	8.3 ± 0.32¢Ó	2.810 ± 0.074¢Ó	538 ± 23.0
G	9.1 ± 0.24*	3.269 ± 0.117	709 ± 33.1
H	8.4 ± 0.16*	2.826 ± 0.216	667 ± 30.1

* Significantly different from control group ($P < 0.05$).

acids and triglyceride fatty acids could provide energy for muscular contraction (Jones and Havel, 1967). In this study, plasma triglyceride (TG) levels in the Red ginseng high-dose of petroleum ether and ethyl acetate groups were lower than in the control group ($P < 0.05$), while the high-dose *n*-butanol group was more significant ($P < 0.01$). As to the experiment II, the TG levels were also lower in the high-dose of 20%, 60% ethanol fractions and eleutheroside E groups compared to that of control group ($P < 0.05$).

It was shown that the LDH change was not significantly different for all groups in the experiment I. However, in the experiment II, of the high-dose of 20% ethanol fraction, both doses of the 60% ethanol fractions and eleutheroside E, the LDH change was significant in comparison with the untreatment group ($P < 0.05$).

3.5. Effects of eleutherosides on behavioral tests

In the experiment III, no differences were found in latency to enter the dark chamber in any of the groups in the training session. However, significantly differences were observed in groups of sleep deprivation and eleutherosides treatment (70 and 280 mg/kg), compared with that of 24 h, 48 h and 72 h sleep-deprived group ($P < 0.05$, Fig. 5). We observed a memory retention deficit on the sleep-deprived group when compared with the control group, especially after 48-h sleep deprivation. In other words, eleutherosides treatment groups improved the memory retention deficit in parallel with the sleep-deprived mice and the control group.

Locomotor activity test showed that the groups of sleep deprivation and treatment with eleutherosides, 280 mg/kg, significantly

Table 4
Effects of the fractions of *n*-butanol and eleutheroside E on BUN, TG, LDH.

	BUN	TG	LDH
Control	9.07 ± 0.30	1.4822 ± 0.11	1068.3 ± 45.08
A	10.05 ± 0.30	1.9862 ± 0.12	1233.7 ± 85.01
B	9.95 ± 0.18	1.6610 ± 0.27	1278.3 ± 134.68
C	8.52 ± 0.32	1.8920 ± 0.24	1391.5 ± 113.24
D	8.97 ± 0.46	1.4108 ± 0.06*	1492.5 ± 108.25*
E	9.47 ± 0.32	1.5902 ± 0.14	1439.2 ± 149.18*
F	8.95 ± 0.27	1.2700 ± 0.12*	1647.5 ± 166.52*
G	8.68 ± 0.27	1.5475 ± 0.13	1597.0 ± 107.91
H	8.95 ± 0.39	1.8845 ± 0.09	1049.7 ± 59.41
EE 10	8.64 ± 0.34	1.4801 ± 0.11	1411.2 ± 121.08*
EE 50	8.14 ± 0.25	1.2834 ± 0.09*	1509.4 ± 141.31*

A: low dose of water eluate, B: high dose of water eluate; C: low dose of 20% ethanol eluate, D: high dose of 20% ethanol eluate, E: low dose of 60% ethanol eluate, F: high dose of 60% ethanol eluate, G: low dose of 100% ethanol eluate, H: high dose of 100% ethanol eluate. Data are means ± S.E.M. of mice per group.
* Significantly different from control group ($P < 0.05$).

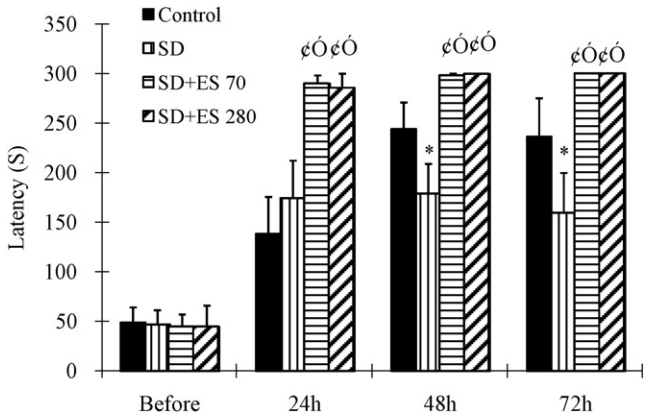

Fig. 5. Latency (s) to enter the dark chamber of a passive avoidance apparatus (mean ± S.E.M.) presented by control or sleep-deprived (SD) mice repeatedly treated with vehicle or 70 or 280 mg/kg eleutherosides (ES) in training (before) and test. *$P < 0.05$ represent the difference between SD and control group. ¢Ó$P < 0.01$ represent the difference between SD and ES treated group.

increased the number of movements in the three sessions ($P < 0.05$, $P < 0.01$); while the low dose of 70 mg/kg, obviously increased the number of movement just after 48 h and 72 h sleep deprivation ($P < 0.05$, $P < 0.01$, Fig. 6).

4. Discussion

It is well accepted that the most important physiological effect of fatigue is on the energy metabolism of muscular activity (Belluardo et al., 2001). The improvement of exercise endurance is the most powerful macro representation of anti-fatigue enhancement. In the present study, we selected a weight-loaded forced swimming test for evaluation of the extent of physical fatigue. The length of the swimming time to exhaustion indicates the degree of fatigue (Tanaka et al., 2003). As to the mental fatigue, 72 h sleep-deprived animal model and behavioral such as passive avoidance and locomotor activity tests were adapted (Mead et al., 1995; Lyle et al., 2009; Singh et al., 2009). Our results suggested that the eleutherosides could relieve mental fatigue by improving memory retention deficit and spontaneously activity.

Blood urea nitrogen (BUN), which is the metabolism outcome of protein and amino acid, is a sensitive index to evaluate the bear-

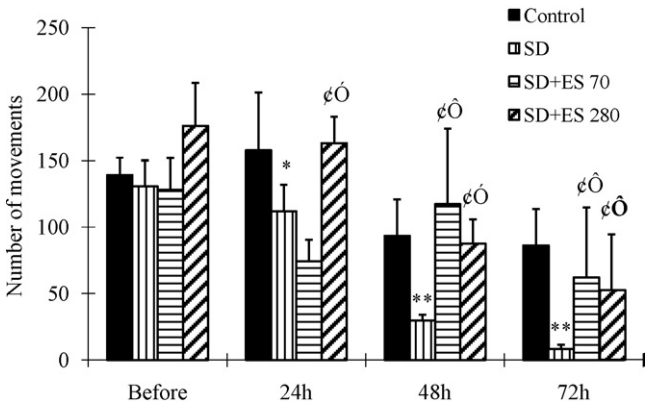

Fig. 6. The effect of eleutherosides on locomotor activity before and after sleep deprivation (SD) in mice. Mice were treated with eleutherosides, ES (70 and 280 mg/kg, i.g.), during 9 days, and, on the seventh day of treatment, were submitted to 72 h of sleep deprivation or maintained in home cages as control. Values are expressed as mean ± S.E.M. *$P < 0.05$, **$P < 0.01$ represent the difference between SD and control. ¢Ó$P < 0.05$, ¢Ô$P < 0.01$ represent the difference between ES and SD group.

ing capability when human bodies suffer from a physical load. Wu (1999) pointed out that the BUN in the blood rises significantly for a long-run athlete after exercise. In other words, the worse the body is adapted for exercise tolerance, the more significantly the BUN level increases (Tsopanakis and Tsopanakis, 1998).

Muscular work, performed aerobically in the post-absorptive state, depends mainly on the utilization of fat. There is evidence that triglyceride fatty acids can provide energy for muscular contraction, and reported increased clearance of plasma triglyceride (TG) by skeletal muscle in normal rat during prolonged exercise (Jones and Havel, 1967).

Meanwhile, the muscle produces plenty of lactic acid when it obtains enough energy from anaerobic glycolysis almost at the same time when doing high-intensity exercise. Therefore, the increased level of lactic acid will bring about a reduction of pH in muscle tissue and blood, and also induce many side effects of various biochemical and physiological processes, that were harmful to the body performance. So, rapid removal of lactic is beneficial to attenuate fatigue. Serum LDH is known to be accurate indicators of muscle damage, which function to catalyze lactic acid into pyruvate, thereby reduce the accumulation of lactic acid in muscle (Tang et al., 2008).

The results of swimming test in the experiment I, showed that compared with the control group, the administration of AS or Red ginseng had no significant difference in body weights of mice. But the Red ginseng, petroleum ether and *n*-butanol fractions in the high-dose groups extended the weight-loaded swimming time in mice ($P < 0.05$, $P < 0.05$ and $P < 0.01$, respectively), and the BUN level was reduced significantly in those three groups ($P < 0.05$, $P < 0.01$ and $P < 0.01$). In addition, the content of TG in Red ginseng, high-dose ethyl ether and high-dose of *n*-butanol was also decreased, especially in the high-dose *n*-butanol fraction group ($P < 0.01$). However, the effects on the LDH level had no significant differences in all groups. That's to say, the high-dose *n*-butanol fraction possessed the capacity to enhance the swimming time by lessening of fatigue and the possible mechanism may be related to the reduction of level of BUN and TG.

In order to clarify which kinds of compounds in it were responsible for, separation and enrichment measures were conducted through D101 macroporous resin. In the eluting process, compounds with different structures and polarities were gradually separated from each other along with the flowing of eluents, and eventually eluted from the resin column in sequence due to their different adsorption and desorption capabilities on resin and different solubilities in eluents (Sun et al., 2009). We found that eleutherosides (Davydov and Krikorian, 2000; Deyama et al., 2001) were the mainly components contained in the 20% and 60% ethanol eluate. Eleutheroside B accounted for 40.0%, eleutheroside E and derivatives for 4.6% in 20% ethanol eluate; while in the 60% ethanol eluate, the eleutheroside E and E_2 accounted for 69.3% (data not shown). Moreover, the experiment II showed that the 60% eluate could more effectively extend the swimming time in comparison with the control group ($P < 0.05$). The levels of TG had been significantly reduced in the groups of high-dose 20% and 60% ethanol eluate ($P < 0.05$). The concentration of LDH had also significantly increased in groups of high-dose 20% and low, high-dose 60% fractions ($P < 0.05$), although not significant difference on the level of BUN. Furthermore, the experiment III preliminary suggested that the eleutherosides could improve the memory retention deficit of sleep-deprived mice.

In conclusion, the anti-fatigue active components of AS were determined to be eleutherosides, in which eleutheroside E, E_2 were isolated. (Nishibe et al., 1990). Differences, our research showed that the eleutherosides can not only alleviate physical fatigue, but also mental fatigue. Eleutheroside E may be partly responsible for the pharmacological effects. Moreover, the possible anti-fatigue mechanisms were reduced the level of TG by increasing fat utilization, delayed the accumulation of BUN, and increased the LDH to reduce the accumulation of lactic acid in muscle and then protect the muscle tissue. However, further study is needed to elucidate the mechanism of eleutherosides or eleutheroside E on mental fatigue.

References

Akazawa, K.H., Cui, Y., Tanaka, M., Kataoka, Y., Yoneda, Y., Watanabe, Y., 2010. Mapping of regional brain activation in response to fatigue-load and recovery in rats with c-Fos immunohistochemistry. Neuroscience Research 66, 372–379.

Belluardo, N., Westerblad, H., Mudo, G., Casabona, A., Bruton, J., Caniglia, G., Pastoris, O., Grassi, F., Ibanez, C.F., 2001. Neuromuscular junction disassembly and muscle fatigue in mice lacking neurotrophin-4. Molecular and Cellular Neurosciences 18, 56–67.

Brekhman, I.I., dardymov, I.V., 1969. New substances of plant origin which increase nonspecific resistance. Annual Review of Pharmacology 9, 419–430.

Chen, J.R., Wang, T.J., Huang, H.Y., Chen, L.J., Huang, Y.S., Wang, Y.J., Tseng, G.F., 2009. Fatigue reversibly reduced cortical and hippocampal dendritic spines concurrent with compromise of motor endurance and spatial memory. Neuroscience 161, 1104–1113.

Chen, Y., Kong, L.D., Xia, X., Kung, H.F., Zhang, L., 2005. Behavioral and biochemical studies of total furocoumarins from seeds of Psoralea corylifolia in the forced swimming test in mice. Journal of Ethnopharmacology 96, 451–459.

Davydov, M., Krikorian, A.D., 2000. Eleutherococcus senticosus (Rupr. & Maxim.) Maxim. (Araliaceae) as an adaptogen: a closer look. Journal of Ethnopharmacology 72, 345–393.

de Oliveira, R.A., Cunha, G.M., Borges, K.D., de Bruin, G.S., dos Santos-Filho, E.A., Viana, G.S., de Bruin, V.M., 2004. The effect of venlafaxine on behaviour, body weight and striatal monoamine levels on sleep-deprived female rats. **Pharmacology, Biochemistry, and Behavior** 79, 499–506.

Deyama, T., Nishibe, S., Nakazawa, Y., 2001. Constituents and pharmacological effects of Eucommia and Siberian ginseng. Acta Pharmacologica Sinica 22, 1057–1070.

Fujikawa, T., Yamaguchi, A., Morita, I., Takeda, H., Nishibe, S., 1996. Protective effects of Acanthopanax senticosus Harms from Hokkaido and its components on gastric ulcer in restrained cold water stressed rats. Biological & Pharmaceutical Bulletin 19, 1227–1230.

Jones, N.L., Havel, R.J., 1967. Metabolism of free fatty acids and chylomicron triglycerides during exercise in rats. American Journal of Physiology 213, 824–828.

Jung, K., Kim, I.-H., Han, D., 2004. Effect of medicinal plant extracts on forced swimming capacity in mice. Journal of Ethnopharmacology 93, 75–81.

Kim, K.M., Yu, K.W., Kang, D.H., Suh, H.J., 2002. Anti-stress and anti-fatigue effect of fermented rice bran. Phytotherapy Research 16, 700–702.

Kimura, Y., Sumiyoshi, M., 2004. Effects of various Eleutherococcus senticosus cortex on swimming time, natural killer activity and corticosterone level in forced swimming stressed mice. Journal of Ethnopharmacology 95, 447–453.

Lami, N., Kadota, S., Kikuchi, T., Momose, Y., 1991. Constituents of the roots of Boerhaavia diffusa L. III. Identification of Ca^{2+} channel antagonistic compound from the methanol extract. Chemical & Pharmaceutical Bulletin (Tokyo) 39, 1551–1555.

Li, X.C., Barnes, D.L., Khan, I.A., 2001. A new lignan glycoside from Eleutherococcus senticosus. Planta Medica 67, 776–778.

Lyle, N., Gomes, A., Sur, T., Munshi, S., Paul, S., Chatterjee, S., Bhattacharyya, D., 2009. The role of antioxidant properties of Nardostachys jatamansi in alleviation of the symptoms of the chronic fatigue syndrome. Behavioural Brain Research 202, 285–290.

Mead, L.A., Hargreaves, E.L., Ossenkopp, K.-P., Kavaliers, M., 1995. A multivariate assessment of spontaneous locomotor activity in the Mongolian gerbil (Meriones unguiculatus): influences of age and sex. Physiology & Behavior 57, 893–899.

Nishibe, S., Kinoshita, H., Takeda, H., Okano, G., 1990. Phenolic compounds from stem bark of Acanthopanax senticosus and their pharmacological effect in chronic swimming stressed rats. Chemical & Pharmaceutical Bulletin (Tokyo) 38, 1763–1765.

Silva, R.H., Abílio, V.C., Kameda, S.R., Takatsu-Coleman, A.L., Carvalho, R.C., Ribeiro, R.d.A., Tufik, S., Frussa-Filho, R., 2007. Effects of 3-nitropropionic acid administration on memory and hippocampal lipid peroxidation in sleep-deprived mice. Progress in Neuro-Psychopharmacology and Biological Psychiatry 31, 65–70.

Silva, R.H., Abílio, V.C., Takatsu, A.L., Kameda, S.R., Grassl, C., Chehin, A.B., Medrano, W.A., Calzavara, M.B., Registro, S., Andersen, M.L., Machado, R.B., Carvalho, R.C., Ribeiro, R.d.A., Tufik, S., Frussa-Filho, R., 2004. Role of hippocampal oxidative stress in memory deficits induced by sleep deprivation in mice. Neuropharmacology 46, 895–903.

Singh, M., Zimmerman, M.B., Beltz, T.G., Johnson, A.K., 2009. Affect-related behaviors in mice misexpressing the RNA editing enzyme ADAR2. Physiology & Behavior 97, 446–454.

Sun, R., Fu, K., Fu, Y., Zu, Y., Wang, Y., Luo, M., Li, S., Luo, H., Li, Z., 2009. Preparative separation and enrichment of four taxoids from Taxus chinensis needles extracts by macroporous resin column chromatography. **Journal of Separation Science** 32, 1284–1293.

Tanaka, M., Nakamura, F., Mizokawa, S., Matsumura, A., Nozaki, S., Watanabe, Y., 2003. Establishment and assessment of a rat model of fatigue. Neuroscience Letters 352, 159–162.

Tang, W., Zhang, Y., Gao, J., Ding, X., Gao, S., 2008. The anti-fatigue effect of 20(R)-ginsenoside Rg3 in mice by intranasally administration. Biological & Pharmaceutical Bulletin 31, 2024–2027.

Tsopanakis, C., Tsopanakis, A., 1998. Stress hormonal factors, fatigue, and antioxidant responses to prolonged speed driving. **Pharmacology, Biochemistry, and Behavior** 60, 747–751.

Wu, I.T., 1999. The effects of serum biochemical value with different beverage to replenish and intermittent exercise in high intensity. Tahan Junior College Engineering Business Journal 13, 387–400.

Zhang, Y., Yao, X., Bao, B., 2006. Anti-fatigue activity of a triterpenoid-rich extract from Chinese bamboo shavings (Caulis bamfusae in taeniam). Phytotherapy Research 20, 872–876.