Scientific Research and Essays Vol. 5(9), pp. 883-886, 4 May, 2010
Available online at http://www.academicjournals.org/SRE
ISSN 1992-2248 © 2010 Academic Journals

*Full Length Research Paper*

# Effect on enhancing physical strength and anti-stress activity of flavonoids from the Chinese medicinal plant *Epimedium koreanum* Nakai

**Xiuling Ou[1]* and Wei Li[2]**

[1]Tianjin Agricultural University, Tianjin City, 300384, People's Republic of China.
[2]Dezhou University, Dezhou City, 253023, People's Republic of China.

Accepted 29 March, 2010

**Flavonoids from the *Epimedium koreanum* Nakai (FEN) were elucidated on enhancing physical strength and anti-stress effects on *in vivo* using mice and rats. Swimming time to exhaustion of mice administered with the FEN (15 and 30 mg/kg body weigh for 7 days) significantly prolonged. When the FEN (15 mg/kg) was given to the rats for 7 days, including the 48 h stress period, the FEN showed a remarkable anti-stress effect on the weight of the adrenal, spleen, thymus, and thyroid. The FEN also significantly inhibited the increase in total cholesterol and the decrease in alkaline phosphatase levels as biochemical parameters of immobilization stress in rats. The results suggested that, FEN had effect on enhancing physical strength and anti-stress activity.**

**Key words:** Flavonoids from the *Epimedium koreanum* Nakai, enhancing physical strength effects, anti-stress activity.

## INTRODUCTION

*Epimedium koreanum* Nakai, belonging to epimedium, is one of the epimedium species which are recorded in the Chinese Pharmacopoeia (2005) as a traditional Chinese medicine (Li et al., 2006). It has been used to treat various kinds of disorders such as hypertension, coronary heart disease, osteoporosis, menopause syndrome, breast lump, rheumatism, arthritis, neurasthenic, bronchitis and hypogonadism (Guo and Xiao, 2003). Pharmacological studies and clinical practice demonstrated that its extract has anti-cancer, anti-AIDS, anti-bacterial, anti-phlogistic, anti-tussive, and expectorant effects (Liu et al., 2005).

The active constituents of the plant are flavonoids (chemical structures shown in Figure 1). To date, no systematic studies have been carried out to evaluate enhancing physical strength effects and anti-stress activity of flavonoids from *E. koreanum* Nakai (FEN). Therefore, in the present study, we aimed to investigate the effect on enhancing physical strength and anti-stress activity of FEN *in vivo* using mice and rats.

*Corresponding author. E-mail: ouxiulingtn@yahoo.com.cn. Fax: +86- 022-23783097.

## MATERIALS AND METHODS

### Plant materials

*E. koreanum* Nakai herb were brought from Tianjin Medical Material Corporation, and identified by Wang, Professor of Plant Biotechnology, Tianjin Agricultural University, China.

### Preparation of flavonoids from *E. koreanum* Nakai

*E. koreanum* Nakai herb was ground to powder (about 30 mesh) by a disintegrator. The powder (500 g) was extracted with 5 L of 70% ethanol for 2 h under reflux. The extraction procedure was repeated three times. The extracts were combined together and were evaporated by using a rotary evaporator (RE-52A, Shanghai Yalong Co, Ltd., Shanghai, China) under vacuum at 60℃ to get the crude extract (Zhao et al., 2007; Zhang et al., 2008). The NKA-9 macro porous resins were chosen to separate and purify the FEN from the crude extract (She et al., 2004; Sheng et al., 2008). The FEN solution was diluted to 1.5 mg/ mL and stored at 4℃ before use.

### Determination of flavonoids

The flavonoid content was determined according to the method given in the Chinese medicine pharmacopoeia 2005 edition. The standard solution contains 25 ug/mL of icarrin (She et al., 2004). The flavonoids content was calculated using the following linear

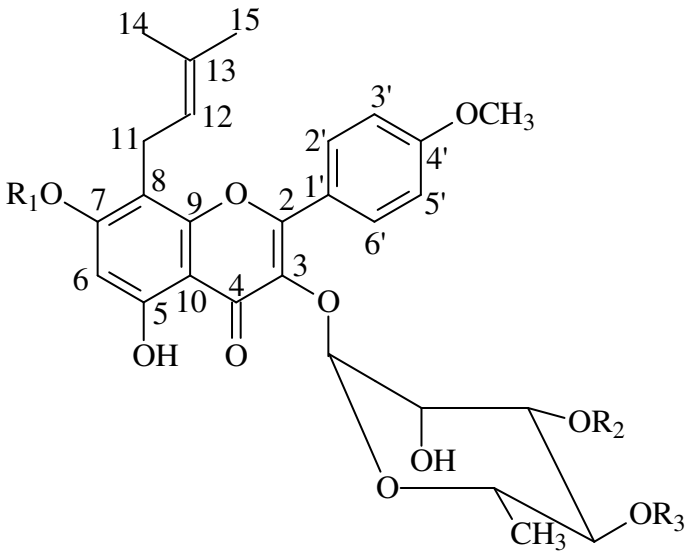


**Figure 1.** Chemical structures of flavonoids from *Epimedium koreanum* Nakai.
$R_1$, $R_2$ , $R_3$ , Epimedokoreanoside I, Glc, β-(6-Ac)Glc, Ac, Icariin, Glc, H, H, Icariside II, H, H, H.

**Table 1.** Composition of standard diets (g/100 g).

| Ingredients | Standard diets | Ingredients | Standard diets |
|---|---|---|---|
| β-corn starch | 38.00 | Soy bean oil | 6.00 |
| Vitamin-free casein | 25.00 | Mineral mixture | 6.00 |
| α-potato starch | 10.00 | Granulated sugar | 5.00 |
| Cellulose powder | 8.00 | Vitamin mixture | 2.00 |

equation based on the calibration curve that was prepared by icarrin:

A = -0.0083+0.0557C. r = 0.9994

Where, A is the absorbance,
C is the flavonoids content in μg / mL.

### Experimental animals

Male ICR mice (weighing 20 ± 2 g) and male Sprague-Dawley (SD) rats (weighing 275 ± 25 g), were purchased from the Institute of Experimental Animals, Academy of Medical Sciences (Tianjin, China) and were acclimatized for 1 week before experiment. The animals were housed in individual cages with free access to water and regular *ad libitum*, in a controlled environment maintaining a 12 h light - 12 h dark cycle, the temperature of 24 ± 1℃, and the humidity of 55 ± 10%. Standard diets were prepared in pellet form by Eekang Co. (Tianjin, China), with the components of each presented in Table 1. The experimental protocol was approved by the Animal Studies Committee of Tianjin Agricultural University.

### Testing for effect on enhancing physical strength

To determine the effect on enhancing physical strength, 40 male ICR mice were randomly divided into 4 groups (n = 10 per group) when experiments began: control group (CG), low dose of administration groups (LAG), middle dose of administration groups (MAG) and high dose of administration groups (HAG). The mice of administration groups were given FEN in doses of 7.5, 15 and 30 mg/kg body weight by stomach intubation at 10:00 for 7 d. The mice were submitted to daily swimming exercise supporting constant loads (lead fish sinkers, attached to the tail) corresponding to 4% of their body weight (Fushiki et al., 1995; Jung et al., 2004; Yu et al., 2008).

The swimming exercise was carried out in a tank (30 × 50 × 30 cm), filled with water to 26 cm depth and maintained at a temperature of 30 ± 1℃. 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 (Jung et al., 2004; Ishola et al., 2008).

### Testing for anti-stress activity

To investigate the anti-stress activity, 24 male Sprague-Dawley (SD) rats were randomly divided into 3 groups (n=8 per group) when experiments began: non-stress control group (NSCG), stress control group (SCG) and stress administration group (SAG). The rats were given FEN in doses of 15 mg/kg body weight by stomach incubation at 10:00 for 7 days. The immobilized-stress technique was carried out (Brekhman and Dardymov, 1969; Watanabe and Ayugase,

**Table 2.** Effect of FEN on enhancing physical strength in mice.

| Group | Swimming time to exhaustion | |
|---|---|---|
| | After 60 min | After 7 days |
| CG | 121.47 ± 12.29 | 123.89 ± 13.27 |
| LAG (7.5 mg/kg) | 124.54 ± 11.46 | 134.83 ± 14.82 |
| MAG (15 mg/kg) | 131.29 ± 10.48 | 152.42 ± 11.35* |
| HAG (30 mg/kg) | 132.48 ± 14.52 | 157.61 ± 12.83* |

$p < 0.05$ compared with the CG

**Table 3.** Effect of FEN on some indices of immobilization stress in rats.

| Group | Adrenal | Spleen | Thymus | Thyroid |
|---|---|---|---|---|
| | (mg/100 g body weight) | | | |
| NSCG | 14.87 ± 0.94 | 281.56 ± 18.48 | 236.15 ± 36.51 | 5.93 ± 0.73 |
| SCG | 21.28 ± 1.13* | 164.32 ± 9.47* | 146.31 ± 24.26* | 4.12 ± 0.26* |
| SAG | 16.45 ± 2.68# | 212.69 ± 14.18# | 195.39 ± 19.44# | 5.84 ± 0.48# |

$p < 0.05$ compared with the NSCG;
# $p < 0.05$ compared with the SCG.

2008). The immobilized stress was given for the last 48 h of the experiment. Then, each organ was weighed and the whole blood was obtained by cardiac puncture after anesthetization with ether.

To analyzed biochemical parameters, blood serum was prepared by centrifugation at 3000 rpm and 4℃ for 10 min. The internal organs (adrenal, spleen, thymus and thyroid) were immediately rinsed with ice-cold 0.9% NaCl solution, dried with paper towels, and weighed. Blood serum was prepared by centrifugation at 250× g and 4℃ for 20 min. LDH, ALP, AST, ALT and total cholesterol levels of blood serum induced by stress were measured as stress indicators (Kim et al., 2001; Koh et al., 2003) .

#### Statistical analysis

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

## RESULTS AND DISCUSSION

### Effect on enhancing physical strength

The exhausted swimming capacities on mice are shown in Table 2. There was increase of the swimming time on mice of all administration groups. Some of them had significant differences in the swimming time to exhaustion in comparison with the control group ($p < 0.05$). Such as FEN at dose 15 and 30 mg/kg was after 7 days. It was suggested that, the effect of the FEN on the recovery from exhaustion might be related to the resistance to stress induced intensive exercise and enhanced immune system. According to these results, FEN had significant effect on enhancing physical strength.

### Anti-stress activity

Stress represents the reaction of the body to stimuli that disturb its normal physiological equilibrium or homeostasis, often with detrimental effects. As a result, the weight of the spleen, thymus, and thyroid in the immune system can be decreased by immobilized stress (Khansari et al., 1990; Koh et al., 2003). As shown in Table 3, the weight of the spleen, thymus, and thyroid decreased significantly, but that of the adrenal were increased significantly by immobilization stress ($p < 0.05$). Therefore, it was assumed that the immobilized stress method used in this study was suitable. When the FEN (15 mg/kg) was given to the rats for 7 days, including the 48 h stress period, the FEN showed a remarkable anti-stress effect on the weight of the adrenal, spleen ,thymus, and thyroid in comparison with the stress control group ($p < 0.05$).

An analysis of the serum total cholesterol, LDH, ALP, AST, and ALT level as stress indicators was carried out and the results are shown in Table 4. Immobilization stress induced a marked increase in serum LDH, AST and total cholesterol levels, but the levels of total cholesterol and ALP recovered to those in the non-stress control group with the stress administration ($p < 0.05$). According to these results, FEN had anti-stress activity.

**Table 4.** Effect of FEN on the blood biochemical parameters of immobilization stress in rats.

| Group | TC (mg/dl) | LDH (units) | ALP (units) | AST (Karmen unit) | ALT (Karmen unit) |
|---|---|---|---|---|---|
| NSCG | 81.27 ± 4.85 | 967.58 ± 223.69 | 49.69 ± 1.26 | 141.19 ± 12.38 | 51.32 ± 3.81 |
| SCG | 142.33 ± 11.62* | 1267.25 ± 287.81* | 17.32 ± 0.91* | 185.67 ± 16.93* | 52.64 ± 4.11 |
| SAG | 86.26 ± 10.28# | 1197.61 ± 354.62 | 27.54 ± 1.37# | 193.56 ± 15.24 | 49.23 ± 5.16 |

*$p < 0.05$ compared with the n NSCG;
#$P < 0.05$ compared with the SCG.

## Conclusion

Our results suggested that FEN had effect on enhancing physical strength and anti-stress activity. To evaluate the physiological or pharmaceutical effects of the FEN *in vivo*, we need a more detailed understanding of the factors that enable the FEN to exert effect on enhancing physical strength and anti-stress activity. Therefore, more research on the characteristics of the FEN will be carried out.

### REFERENCES


Brekhman II, Dardymov IV (1969). Pharmacological investigation of glycosides from Ginseng and Eleutherococcus. Lloydia. 32: 46-51.

Fushiki T, Matsumoto K, Inoue K, Kawada T, Sugimoto A (1995). Swimming Endurance Capacity of Mice is increased by Chronic Consumption of Medium-Chain Triglycerides. J. Nutr. 125: 531-539.

Guo BL, Xiao PG (2003). Comment on Main Species of Herba Epimedii. China J. Chinese Materia. 28: 303-304.

Ishola IO, Ashorobi RB, Adeoluwa O (2008). Evaluation of antistress potential and phytochemical constituents of aqueous root extract of Alchornea cordifolia. Asian J. Sci. Res. 1: 476-480.

Jung K, Kim IH, Han D (2004). Effect of medicinal plant extracts on forced swimming capacity in mice. J. Ethnopharmacol. 93: 75-81.

Khansari DN, Murgo AJ, Faith RE (1990). Effects of stress in the immune systeme. Immunol. Today. 11: 170-175.

Kim KM, Yu KW, Kang DH, Koh JH, Hong BS, Sun HJ (2001). Antifatigue and Antistress Effect of Fermented Rice Bran. Biosci. Biotechnol. Biochem. 65: 2294–2296.

Koh JH, Kim KM, Kim JM, Song JC, Suh HJ (2003). Antifatigue and Antistress Effect of the Hot-Water Fraction from Mycelia of Cordyceps sinensis. Biol. Pharm. Bull. 26: 691-694.

Li N, Song SJ, Luo F (2006). Studies of the Chemical Constituents of *Epimedium koreanum* Nakai. J. Traditional Med., 1: 1-4.

Liu RM, Li AF ,Cui JC, Kong LY (2005). Preparative isolation and purification of three flavonoids from the Chinese medicinal plant Epimedium koreamum Nakai by high-speed. J. Chromatography A. 1064: 53-57.

She BR, Qin DN, She YC (2004). Method for extracting and purifying total flavones from Herba Epimedii. China J. Modern Med., 16: 27-29.

Sheng MY, Yang QX, Chen QF (2008). Studies on Extraction Technology of Epimedium acuminatum Flavones. Lishizhen Med. Material Med. Res., 19: 1899–1901.

Watanabe M, Ayugase J (2008). Anti-stress Effects of Flavonoids from Buckwheat Sprouts in Mice Subjected to Restraint Stress. Food Sci. Technol. Res., 14: 253-260.

Yu B, Lu ZX, Bie XM, Lu FX, Huang XQ (2008). Scavenging and anti-fatigue activity of fermented defatted soybean peptides. Eur. Food Res. Technol., 226: 415-421.

Zhang DW, Cheng Y, Wang NL, Zhang JC, Yang MS, Yao XS (2008). Effects of total flavonoids and flavonol glycosides from *Epimedium koreanum* Nakai on the proliferation and differentiation of primary osteoblasts. Phytomedicine. 15: 55-61.

Zhao YY, Xu BM, Jiang B, Liu K (2007). Effects of Different Extraction Methods on Total Flavonoids and Icariin Contents of Herba Epimedii. Journal of Yantai Universiyty (Natural Science and Engineering Edition). 20: 22-25.