

Original Article

## The Ergogenic Effects of Red Ginseng and *Paeonia radix* Mixtures

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**Objectives :** This study was designed to identify the ergogenic effects of Red ginseng and *Paeonia radix* mixtures and optimal ratios.

**Materials and Methods :** This study was conducted by administering treatments of Red ginseng and *Paeonia radix* mixtures to rats and by measuring the time to exhaustion by treadmill running.

**Results :** The treatment of Red ginseng and *Paeonia radix* mixtures to the rats increased the time to exhaustion by treadmill running. The most potent inhibition of Red ginseng and *Paeonia radix* mixtures on the 5-HT synthesis and the TPH expression in the dorsal raphe was observed at the dose of 200 mg/kg and the optimal ratio of Red ginseng and *Paeonia radix* for the maximum efficacy was 50:50. Under normal conditions (not exercise), long-term treatment of Red ginseng and *Paeonia radix* mixtures did not affect the 5-HT synthesis and the TPH expression in the dorsal raphe, suggesting that Red ginseng and *Paeonia radix* mixtures does not alter serotonin level in the normal rats. The suppressive effect of Red ginseng and *Paeonia radix* mixtures on the 5-HT synthesis and the TPH expression during exercise is a possible ergogenic mechanism of these mixtures.

**Conclusions :** Red ginseng and *Paeonia radix* mixtures reduce exercise-induced fatigue, and have the effect of acting as ergogenic aids on the time to exhaustion by treadmill exercise and on 5-HT synthesis and TPH expression.

**Key Words:** Ergogenic effects, Red ginseng, *Paeonia radix*, 5-hydroxytryptamine, Tryptophan hydroxylase

### Introduction

It has been reported that multiple neurological factors influence fatigability during prolonged exercise<sup>1)</sup>. The central fatigue hypothesis states that maximal exertion or exhaustion may directly enhance serotonergic activity via locomotor regulation or stimulation of long-

term stress responses, and that the increase of serotonin (5-hydroxytryptamine, 5-HT) concentration in the brain, resulting from the enhanced activity, impairs central nervous system (CNS) functions and brings about deterioration in exercise performance and fatigue, which was supported by several studies<sup>2-5)</sup>.

Red ginseng is produced from Ginseng radix by steaming. Ginseng radix is the root of *Panax Ginseng* C.A. MEYER (Araliaceae; Ginseng radix). The aqueous extract of Ginseng radix has been used to replenish the vital energy and to promote the secretion of body fluids. It has been shown to possess the effects of stimulating the central nervous system, cardiogenic, antifatigue and stimulating the mechanism of blood formation<sup>6-8)</sup>.

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*Paeonia radix* is the root of *Paeonia japonica* MIYABE, which is a perennial plant classified under the family of Paeoniaceae. The aqueous extract of *Paeonia radix* has been used to nourish the blood and to activating circulation<sup>9</sup>.

5-HT is known to participate in the modulation of body temperature, blood pressure, endocrine activity, appetite, sexual behavior, movement, emesis, and pain<sup>10</sup>. 5-HT is one of the central neurotransmitters, which regulate the behavioral functions in the vertebrate brain. The activity of serotonergic neural projections is influenced by extrinsic and intrinsic impulses carrying body information<sup>11</sup>. This 5-HT system plays an important role in neuromodulation of cognition and behavior<sup>12</sup>. Most of the cell bodies of the serotonergic neurons in the brain arise from the dorsal raphe nuclei and these cells send their projections to diverse target regions including the limbic system, hypothalamus, striatum, and cerebral cortex<sup>11</sup>. Tryptophan hydroxylase (TPH) catalyzes the rate-limiting step of serotonin biosynthesis in the serotonergic neurons of the raphe nuclei, and TPH enzyme activity in the brain is known to regulate 5-HT synthesis during normal development<sup>13</sup>.

Red ginseng and *Paeonia radix* was found to inhibit exercise-induced increasing numbers of 5-HT positive and TPH-positive cells in the dorsal raphe respectively in the published reports<sup>2,3</sup>.

Therefore, we tried to make the Red ginseng and *Paeonia radix* mixture and to investigate the synergic effect and optimal ratios of Red ginseng and *Paeonia radix* on the time to exhaustion by treadmill exercise and on 5-HT synthesis and TPH expression in the dorsal raphe via immunohistochemistry.

## Materials and Methods

### 1. Animals and preparation of herbs

Male Sprague-Dawley rats weighing  $180 \pm 10$ g (5 weeks old) were used for the experiment. Each animal was housed under controlled temperatures ( $20 \pm 2$  °C) and maintained under light-dark cycles, each consisting of 12 h of light and 12 h of darkness (lighting from 08:00 h to 20:00 h) with food and water made available ad libitum.

Red ginseng and *Paeonia radix* used in this experiment were obtained from Kyung Won University (Seongnam, Korea). After washing, Red ginseng and *Paeonia radix* were immersed in cold water for 12 h. To obtain aqueous extract of Red ginseng and *Paeonia radix*, 200 g of Red ginseng and *Paeonia radix* were added to distilled water, heat-extracted at 80 °C, concentrated using a rotary evaporator, and lyophilized. The resulting powders, weight 21 g for Red ginseng and 25 g for *Paeonia radix*, were diluted with saline after filtering through a 0.45 µm syringe filter.

### 2. The effects of ratios of Red ginseng and *Paeonia radix* on the time to exhaustion by treadmill exercise

In order to determine the optimal ratio of the Red ginseng and *Paeonia radix* on the maximum effect of the mixture, the dosage applied to the rats was set as 200 mg/kg. The animals were divided into eight groups (n= 8 in each group): the control group, the exercise group, the exercise and 200 mg/kg Red Ginseng-treated group, the exercise and 150 mg/kg Red ginseng and 50 mg/kg *Paeonia radix*-treated group, the exercise and 100 mg/kg Red ginseng and 100 mg/kg *Paeonia radix*-treated group, the exercise and 50 mg/kg Red ginseng and 150 mg/kg *Paeonia radix*-treated group, the exercise and 200 mg/kg *Paeonia radix*-treated group, and the exercise and 50 mg/kg caffeine-treated group.

The Red ginseng and *Paeonia radix* were administrated to the rats at 1 hour before the starting of treadmill exercise at the respective dosage orally for 8

days. The rats of the caffeine-treated group received 50 mg/kg caffeine orally once a day for 8 days and those of the control group and the exercise group received equivalent amounts of water once a day for the same duration of time.

### 3. The effects of dosages of Red ginseng and *Paeonia radix* on the time to exhaustion by treadmill exercise

In order to determine the optimal dosage for the maximum effect of this mixture, the ratio of Red ginseng and *Paeonia radix* was set as 50:50. The animals were divided into eight groups (n = 8 in each group): the control group, the exercise group, the exercise and 25 mg/kg, the exercise and 50 mg/kg, the exercise and 100 mg/kg, the exercise and 200 mg/kg, the exercise and 400 mg/kg, the exercise and 800 mg/kg of Red ginseng and *Paeonia radix* mixture-treated group.

The mixture of Red ginseng and *Paeonia radix* was administrated to the rats once a day at 1 hour before the starting of treadmill exercise at the respective dosage orally for 8 days, and those of the control group and exercise group received equivalent amounts of water once a day for the same duration of time.

### 4. The effects of long-term treatment of Red ginseng and *Paeonia radix* mixtures on the time to exhaustion by treadmill exercise

We investigated the effect of long-term (4 weeks) application of this Red ginseng and *Paeonia radix* mixtures on the time to exhaustion and on the 5-HT synthesis and the TPH expression in the dorsal raphe.

The animals were divided into nine groups (n = 8 in each group): the control group, the 50 mg/kg mixtures-treated group, the 200 mg/kg mixtures-treated group, the 800 mg/kg mixtures-treated group, the exercise group, the exercise and 50 mg/kg mixtures -treated

group, the exercise and 200 mg/kg mixtures-treated group, the exercise and 800 mg/kg mixtures-treated group, and the exercise and 50 mg/kg caffeine-treated group.

The animals of mixture-treated groups received mixtures at the respective dosages orally once a day at 1 hour before the starting of treadmill exercise for 4 consecutive weeks. The rats of the caffeine-treated group received 50 mg/kg caffeine orally once a day for 4 weeks and those of the control group and exercise group received equivalent amounts of saline once a day for the same duration of time.

### 5. Treadmill exercise protocols

The physical exercise load applied in the present study took the form of treadmill running on a motor-driven treadmill. The rats of the exercise groups were made to run on treadmills for 30 min once a day for 8 days in the first and second experiments, and three times a week for 4 weeks in the third experiment. Those of the non-exercise groups, including the control group, were left on the treadmill without running for 30 min. The exercise load consisted of running at a speed of 10 m/min for 10 min, at 16 m/min for another 10 min, and at 21 m/min for the last 10 min, with 0 degree of inclination.

On the 8th day in the first and second experiments, and on the 28th day of the third experiment, time to exhaustion for treadmill running was determined for the exercise groups. Time to exhaustion is defined as the time between the commencement of exercise and the first occurrence of the experimental animal failing to keep up with the treadmill machine for a period of 3 min or more. The speed used for measurement of the time to exhaustion was 10 m/min for 5 min, 16 m/min for 5 min, 18 m/min for 5 min, 21 m/min for 5 min, 24 m/min for 5 min, 26 m/min for 5 min, 29 m/min for 5 min, 32 m/min for 5 min, 34 m/min for 5 min, 37

m/min for 5 min and then 40 m/min until exhaustion, the presumed equilibrium speed of running for rats. Immediately after determination of the time to exhaustion, the rats were sacrificed.

## 6. Tissue preparation

For preparation of the brain tissue, the animals were first fully anesthetized with Zoletil 50® (10 mg/kg, i.p.; Vibac Laboratories, Carros, France), transcardially perfused with 50 mM phosphate-buffered saline (PBS), and then fixed with a freshly prepared solution consisting of 4% paraformaldehyde (PFA) in 100 mM phosphate buffer (PB, pH 7.4). The brains were then removed, postfixed in the same fixative overnight, and next transferred into a 30% sucrose solution for cryoprotection. Coronal sections of 40  $\mu$ m thickness were made using a freezing microtome (Leica, Nussloch, Germany).

## 7. Immunohistochemistry for 5-HT synthesis and TPH expression

For the detection of 5-HT-positive and TPH-positive cells in the dorsal raphe, immunohistochemistry was performed as a previously described method<sup>9</sup>. Briefly, an average of 10 sections was selected from the Bregma region -7.30 mm to -8.00 mm. The sections were then incubated in PBS for 10 min and then washed three times using PBS. The sections were next incubated in 1% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) for 30 min. After this, the sections were incubated overnight with rabbit anti-5-HT antibody (Oncogene Reserch Product, Cambridge, UK) at a dilution of 1:500 for visualization of 5-HT synthesis or with mouse monoclonal anti-TPH antibody (Oncogene Reserch Product) at a dilution of 1:1000 for visualization of TPH expression. The sections were next incubated for 1 hour with biotinylated anti-rabbit secondary antibody (Vector Laboratories, Burlingame, CA, USA) or with anti-

mouse secondary antibody (Vector Laboratories). The sections were subsequently incubated with a avidin-biotin-peroxidase complex (Vector Laboratories) for 1 hour at room temperature. The immunoreactivity was visualized by incubating the sections in a solution consisting of 0.05% 3,3'-diaminobenzidine (DAB) and 0.01% H<sub>2</sub>O<sub>2</sub> in 50 mM Tris-buffer (pH 7.6) for approximately 3 min. The sections were then mounted on gelatin-coated glass slides. The slides were allowed to air dry overnight at room temperature, and the coverslips were mounted using Permount®. The number of 5-HT-positive or TPH-positive cells in the dorsal raphe was counted using a light microscope (Olympus, Tokyo, Japan).

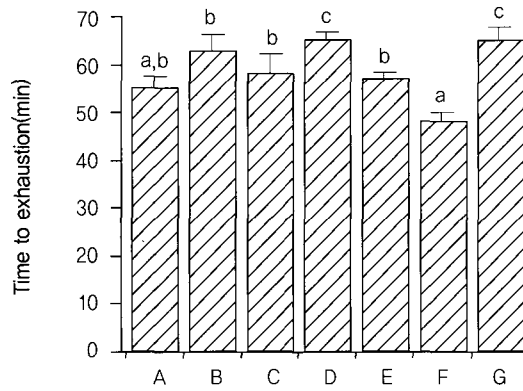
## 8. Data analysis

The data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's Post-hoc test using SPSS®11.0 for windows. The results are expressed as the mean standard error mean (S.E.M.). The difference was considered significant at  $p < 0.05$ .

## Results

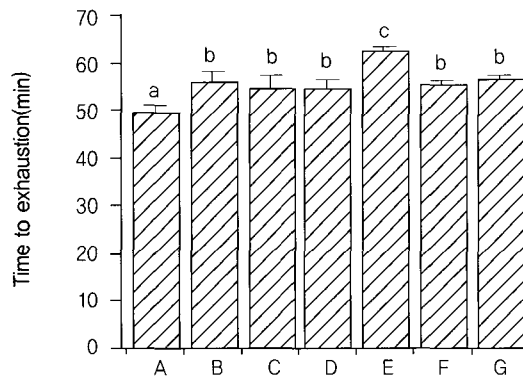
### 1. The effects of ratios of Red ginseng and *Paeonia radix* on the time to exhaustion in treadmill running

The time to exhaustion of the rats in each exercise group is presented in Fig. 1. The mean time to exhaustion for forced treadmill running was  $55.23 \pm 2.03$  min in the exercise group,  $62.83 \pm 3.68$  min in the exercise and 200 mg/kg Red ginseng-treated group,  $58.05 \pm 3.93$  min in the exercise and 150 mg/kg Red ginseng and 50 mg/kg *Paeonia radix*-treated group,  $65.18 \pm 1.50$  min in the exercise and 100 mg/kg Red ginseng and 100 mg/kg *Paeonia radix*-treated group,  $57.25 \pm 1.17$  min in the exercise and 50 mg/kg Red ginseng and 150 mg/kg *Paeonia radix*-treated group,



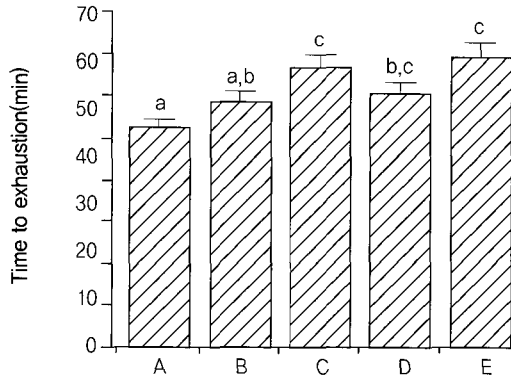
**Fig. 1. The effects of ratios of Red ginseng and *Paenia radix* on the exhaustion time on the treadmill.**

- A. Exercise group
- B. Exercise and 200 mg/kg Red ginseng-treated group
- C. Exercise and 150 mg/kg Red ginseng and 50 mg/kg *Paenia radix*-treated group
- D. Exercise and 100 mg/kg Red ginseng and 100 mg/kg *Paenia radix*-treated group
- E. Exercise and 50 mg/kg Red ginseng and 150 mg/kg *Paenia radix*-treated group
- F. Exercise and 200 mg/kg *Paenia radix*-treated group
- G. Exercise and 50 mg/kg caffeine-treated group



**Fig. 2. The effects of dosages of Red ginseng and *Paeonia radix* on the time to exhaustion on the tread-mill**

- A. Exercise group
- B. Exercise and 25 mg/kg Red ginseng and *Paeonia radix*-treated group
- C. Exercise and 50 mg/kg Red ginseng and *Paeonia radix*-treated group
- D. Exercise and 100 mg/kg Red ginseng and *Paeonia radix*-treated group
- E. Exercise and 200 mg/kg Red ginseng and *Paeonia radix*-treated group
- F. Exercise and 400 mg/kg Red ginseng and *Paeonia radix*-treated group
- G. Exercise and 800 mg/kg Red ginseng and *Paeonia radix*-treated group



**Fig. 3. The effects of Red ginseng and *Paeonia radix* mixtures application for 4 weeks on the time to exhaustion on the treadmill**

- A. Exercise group
- B. Exercise and 50 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group
- C. Exercise and 200 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group
- D. Exercise and 800 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group
- E. Exercise and 50 mg/kg caffeine-treated group

48.27 ± 1.90 min in the exercise and 200 mg/kg *Paeonia radix*-treated group, and 65.12 ± 2.77 min in the exercise and 50 mg/kg caffeine-treated group.

The present results show that 100 mg/kg Red ginseng and 100 mg/kg *Paeonia radix*-treated group increased the time to exhaustion most potently.

### 2. The effects of dosages of Red ginseng and *Paeonia radix* on the time to exhaustion by treadmill exercise

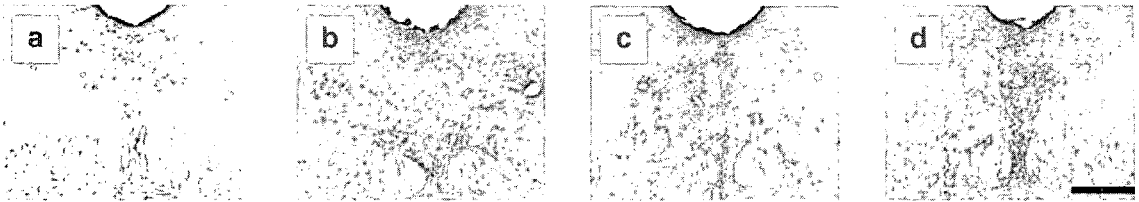
From the present experiment, the ration of Red ginseng and *Paeonia radix* was set as 50:50. The time to exhaustion of the rats in each exercise group is presented in Fig. 2. The mean time to exhaustion for forced treadmill running was 47.72 ± 1.45 min in the exercise group, 56.27 ± 2.03 min in the exercise and 25 mg/kg Red ginseng and *Paeonia radix*-treated group, 54.65 ± 2.63 min in the exercise and 50 mg/kg Red ginseng and *Paeonia radix*-treated group, 54.72 ± 1.78 min in the exercise and 100 mg/kg Red ginseng and

*Paeonia radix*-treated group, 62.53 ± 0.88 min in the exercise and 200 mg/kg Red ginseng and *Paeonia radix*-treated group, 55.60 ± 0.87 min in the exercise and mixed 400 mg/kg Red ginseng and *Paeonia radix*-treated group, 56.53 ± 1.02 min in the exercise and 800 mg/kg Red ginseng and *Paeonia radix*-treated group.

The present results show that the 200 mg/kg Red ginseng and *Paeonia radix*-treated group increased the time to exhaustion most potently.

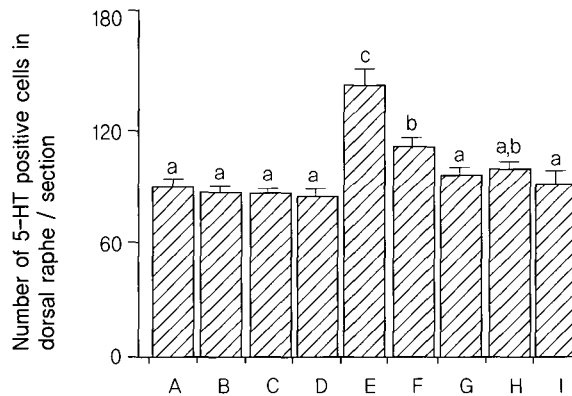
### 3. The effects of long-term treatment of Red ginseng and *Paeonia radix* mixtures on the time to exhaustion by treadmill exercise

The ratio of Red ginseng and *Paeonia radix* was set as 50:50. The time to exhaustion of the rats in each exercise group is presented in Fig. 3. The mean time to exhaustion for forced treadmill running was 52.50 ± 1.73 min in the exercise and 5 water-treated group, 58.95 ± 1.70 min in the exercise and 50 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group,



**Fig. 4-1. Photomicrographs of 5-HT-positive cells in the dorsal raphe nuclei**

- a. Control group
  - b. Exercise group
  - c. Exercise and 200 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group
  - d. Exercise and 50 mg/kg caffeine-treated group
- The scale bar represents 250  $\mu$ m.



**Fig. 4-2. The effects of long-term treatment of Red ginseng and *Paeonia radix* mixtures on the 5-hydroxytryptamine (5-HT) synthesis in the dorsal raphe**

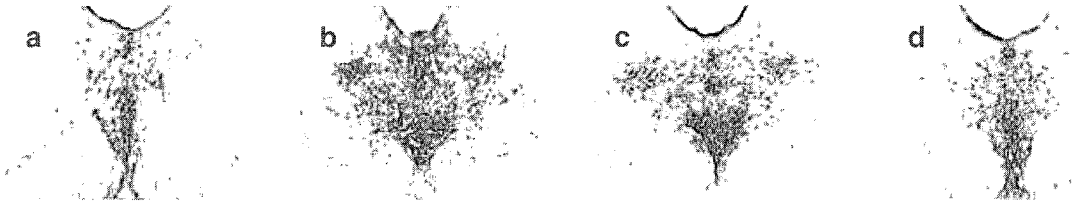
- A. Control group
- B. 50 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group
- C. 200 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group
- D. 800 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group
- E. Exercise group
- F. Exercise and 50 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group
- G. Exercise and 200 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group
- H. Exercise and 800 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group
- I. Exercise and 50 mg/kg caffeine-treated group

Number of 5-HT-positive cells in the dorsal raphe. Values are represented as the mean  $\pm$  S.E.M.

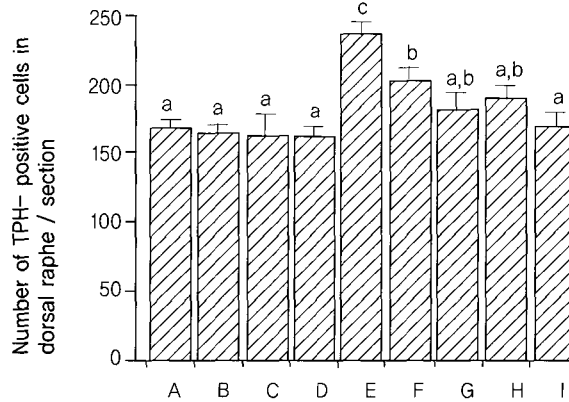
67.05  $\pm$  2.48 min in the exercise and 200 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group, 60.58  $\pm$  2.42 min in the exercise and 800 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group, and

69.33  $\pm$  3.15 min in the exercise and 50 mg/kg caffeine-treated group.

The present results show that long-term application of 200 mg/kg eRed ginseng and *Paeonia radix* mixtures



**Fig. 5-1. Photomicrographs of TPH-positive cells in the dorsal raphe nuclei**  
 a. Control group  
 b. Exercise group  
 c. Exercise and 200 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group  
 d. Exercise and 50 mg/kg caffeine-treated group  
 The scale bar represents 250  $\mu\text{m}$ .



**Fig. 5-2. The effects of long-term treatment of Red ginseng and *Paeonia radix* mixtures on the 5-hydroxytryptamine (5-HT) synthesis in the dorsal raphe**  
 A. Control group  
 B. 50 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group  
 C. 200 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group  
 D. 800 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group  
 E. Exercise group  
 F. Exercise and 50 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group  
 G. Exercise and 200 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group  
 H. Exercise and 800 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group  
 I. Exercise and 50 mg/kg caffeine-treated group  
 Number of 5-HT-positive cells in the dorsal raphe. Values are represented as the mean  $\pm$  S.E.M.

increased the time to exhaustion most potently.

4. The effects of long-term treatment of Red ginseng and *Paeonia radix* mixtures on the 5-HT synthesis in the dorsal raphe

Photomicrographs of 5-HT-positive cells in the dorsal raphe are presented in Fig. 4. The number of 5-HT-positive cells in the dorsal raphe was  $90.30 \pm 2.95/\text{section}$  in the control group,  $86.90 \pm 2.52/\text{section}$  in the 50 mg/kg Red ginseng and *Paeonia radix*



mixtures-treated group,  $86.12 \pm 2.49$ /section in the 200 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group,  $85.66 \pm 3.04$ /section in the 800 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group,  $142.87 \pm 7.74$ /section in the exercise group,  $110.60 \pm 4.63$ /section in the exercise and 50 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group,  $97.44 \pm 1.79$ /section in the exercise and 200 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group,  $99.50 \pm 3.72$ /section in the exercise and 800 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group, and  $91.10 \pm 6.80$ /section in the exercise and 50 mg/kg caffeine-treated group (Fig. 4).

The present results show that treadmill exercise enhanced the 5-HT synthesis in the dorsal raphe and that Red ginseng and *Paeonia radix* mixtures treatment suppressed exercise-induced increase of 5-HT synthesis in the dorsal raphe. The most potent suppressing effect on the 5-HT synthesis was observed in the 200 mg/kg of Red ginseng and *Paeonia radix* mixtures treatment. Under normal conditions (not exercise), long-term treatment of Red ginseng and *Paeonia radix* mixtures did not affect the 5-HT synthesis in the dorsal raphe.

#### 5. The effects of long-term treatment of Red ginseng and *Paeonia radix* mixtures on the TPH expression in the dorsal raphe

Photomicrographs of TPH-positive cells in the dorsal raphe are presented in Fig. 5. The number of TPH-positive cells in the dorsal raphe was  $168.40 \pm 5.80$ /section in the control group,  $164.60 \pm 6.27$ /section in the 50 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group,  $163.00 \pm 15.35$ /section in the 200 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group,  $163.00 \pm 6.20$ /section in the 800 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group,  $236.40 \pm 8.10$ /section in the exercise and water-treated group,  $203.40 \pm 8.39$ /section in the exercise and 50

mg/kg Red ginseng and *Paeonia radix* mixtures-treated group,  $182.57 \pm 11.12$ /section in the exercise and 200 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group,  $189.33 \pm 9.95$ /section in the exercise and 800 mg/kg Red ginseng and *Paeonia radix* mixtures-treated group, and  $170.14 \pm 9.78$ /section in the exercise and 50 mg/kg caffeine-treated group (Fig. 5).

The present results show that treadmill exercise enhanced the TPH expression in the dorsal raphe and that Red ginseng and *Paeonia radix* mixtures treatment suppressed exercise-induced increase of TPH expression in the dorsal raphe. The most potent suppressing effect on the TPH expression was observed in the 200 mg/kg of Red ginseng and *Paeonia radix* mixtures treatment. Under normal conditions (not exercise), long-term treatment of Red ginseng and *Paeonia radix* mixtures did not affect the TPH expression in the dorsal raphe.

## Discussion

In Oriental medicine, Red ginseng is produced from Ginseng radix by steaming, and the main component of Ginseng radix is saponin, which it contains several triterpene glycosides referred as Ginsenosides. Ginsenosides has known to have an anti-stress effect and attenuate neuronal cell damage induced by glutamate and kainic acid: ginsenosides Rb1 and Rg3 protect neurons against excitotoxicity and oxidative stress<sup>18</sup>). *Paeonia radix* traditionally has been used for stress, pain, and a tonic state, and used for lessening fatigue<sup>2-9</sup>). It is known to refresh and nourish the blood<sup>9</sup>).

In the pilot studies<sup>2-3</sup>), *Red ginseng* and *Paeonia radix* treatment in each were shown to inhibit exercise-induced increase in the numbers of 5-HT and TPH-positive cells in the dorsal raphe, and the most potent suppression was observed at 100 mg/kg Red ginseng treated group and 50 mg/kg *Paeonia radix* treated

group .

Increased 5-HT concentration is known to induce lethargy, loss of motivation, and decrease power during sustained exercise. It has been suggested that the increase of 5-HT concentration in the brain and the overall serotonergic activity taking place during endurance exercise are relevant to the increase in the level of physical fatigue and perhaps of mental fatigue as well<sup>14)</sup>. Newsholme et al.<sup>4)</sup> proposed the “central fatigue hypothesis”, which states that maximal exertion or exhaustion may directly affect serotonergic activity via locomotor regulation or stimulation of longer-term stress responsiveness. The increase in the level of serotonin during endurance exercise coincides with the onset of fatigue, and this raises the possibility that differences in serotonin receptor sensitivity may be an important determinant of relative endurance<sup>1,5,15)</sup>. In the present study, treadmill exercise increased 5-HT synthesis in the dorsal raphe, and Red ginseng and *Paeonia radix* mixtures developed in this study suppressed the exercise-induced increase of 5-HT synthesis in the dorsal raphe. The most potent inhibition of Red ginseng and *Paeonia radix* mixtures on the 5-HT synthesis was observed at the dose of 200 mg/kg.

Reduction in TPH activity has been shown to lead to rapid decrease in 5-HT release<sup>16)</sup>, indicating that change in the TPH level can profoundly influence the synaptic 5-HT activity. The increase of TPH mRNA expression heightened TPH activity and 5-HT metabolism, but the extent of the elevation for TPH mRNA level was much larger than the change in 5-HT turnover<sup>17)</sup>.

Red ginseng and *Paeonia radix* mixtures marked in this study inhibited an exercise-induced increase of TPH expression in the dorsal raphe. We investigated the optimal ratios of Red ginseng and *Paeonia radix* mixtures for ergogenic effects, and the most potent effects were observed at a 50:50 mixture (treatment of 100 mg/kg Red ginseng and 100 mg/kg *Paeonia radix*).

Similar to the results we achieved for 5-HT, the most potent suppression of TPH expression was observed at the dose of 200 mg/kg. And we compared Red ginseng and *Paeonia radix* mixtures with caffeine, which is well known ergogenic aid<sup>15)</sup> to investigate the ergogenic effects of Red ginseng and *Paeonia radix* mixtures. Red ginseng and *Paeonia radix* mixtures at 200 mg/kg showed similar efficacy as caffeine at 50 mg/kg on the exhaustion-time by treadmill running and on the 5-HT synthesis and the TPH expression in the dorsal raphe.

Under normal conditions (not exercise), long-term treatment of Red ginseng and *Paeonia radix* mixtures did not affect the 5-HT synthesis and TPH expression in the dorsal raphe, suggesting that Red ginseng and *Paeonia radix* mixtures do not alter normal 5-HT level.

In the present study, we tried to investigate the synergic effects of Red ginseng and *Paeonia radix*, and to make Red ginseng and *Paeonia radix* mixtures, which have the suppressive effects on exercise-induced increase of 5-HT synthesis and TPH expression in the dorsal raphe of rats. These results suggest that Red ginseng and *Paeonia radix* mixtures reduce exercise-induced fatigue, and have the effect of ergogenic aids on the time to exhaustion by treadmill exercise and on 5-HT synthesis and TPH expression.

Further study is needed to identify the main active components of Red ginseng and *Paeonia radix* mixtures that are responsible for the reducing of fatigue, and acts ergogenic aids for endurance exercise.

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