

Stress related activities of Sun-ginseng in SD Rats and ICR Mice

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Abstract – The main aim of this study was to investigate stress related activities of Sun-ginseng extract as a candidate for anti-stress-related functional supplement by comparing its effect to those of red ginseng, which is also known to alleviate stress. Normal group was not exposed to stress while the control group was exposed to stress. Rats were orally administered once a day with 200 mg red ginseng (RG) extract, 100 or 200 mg Sun-ginseng (SG) extract/kg body weight. Mice were given water containing 400 mg red ginseng extract, 200 or 400 mg SG/100 mL potable water. Rats were given supplements for 5 days without stress, and 5 days with restraint and electroshock stress. After final stress, stress-related behavioral changes of experimental animals were recorded and levels of blood corticosterone were measured. Mice were given supplements for 5 days through drinking water, and then fatigue related motor activity were recorded. SG-supplementation partially blocked stress effect on locomotion and elevated plus maze test in rats, and also partially blocked stress-induced behavioral changes such as freezing, burrowing, smelling, facewashing, grooming and rearing behavior in rats. SG-supplementation decreased blood corticosterone level which is increased by stress in rats. Effects of SG on them were not influenced by Flumazenil administration in EPM test. The stress related activities of SG may not be modulated by GABAergic nervous system. SG-supplementation prolonged swimming time and staying time on the wire and rotarod wheel in mice. These results suggest that SG partially protects living organisms from stress attack in some cases and thus has the potential to be used as a functional food to alleviate stress response.

Key words □ Sun-ginseng, anti-stress, behavioral activity, corticosterone, GABA

Ginseng, the root of *Panax ginseng* C.A. Meyer (Araliaceae), is one of the most widely used herbal medicine in the Orient. Park *et al.* had developed Sun-ginseng (SG) through the process of steaming ginseng at high temperature (Kim *et al.*, 2000; Park *et al.*, 2002). They have reported that SG exhibited greatly enhanced biological activities such as vasodilatation, kidney protection, neuroprotection, anti-oxidation and cancer chemoprevention (Kim *et al.*, 2000; Keum *et al.*, 2000).

Stress is the plague of modern society as well as an avoidable consequence of life. As Selye noted, “without stress, there would be no life” (Selye, 1993). It is the “wear and tear” our bodies experience as we adjust to the continuously changing environment. Whether it is a good or a bad change does not matter; they are both stress. However, overstress is a life-long problem and can cause physical damage to the gastrointestinal tract,

endocrine system, skin, and cardiovascular system (Hurst *et al.*, 1976; Barsky *et al.*, 1986; Breier *et al.*, 1987; Blazer *et al.*, 1987; Chrousos and Gold, 1992). It can also result in feelings of distrust, rejection, anger, and depression, which, in turn, can lead to health problems such as headaches, stomach ulcer, liver disease, rashes, insomnia, hypertension, heart disease, stroke, diabetes, immune disorders, and sexual disorders (Hurst *et al.*, 1976; Glass, 1977; Breier *et al.*, 1987; Glaser *et al.*, 1990; Dimsdale *et al.*, 2000).

To alleviate depression, anxiety, sleep disorder and stress-related disorders, many functional materials such as Ginseng extract, DHA, Ginkgo biloba and extract of *Eleutherococcus senticosus* cortex were tested (Rai *et al.*, 2003; Takeuchi *et al.*, 2003; Kim *et al.*, 2003; Kaneko and Nakanishi, 2004; Kimura and Sumiyoshi, 2004). Red Ginseng (the roots of *Panax ginseng* C.A. Meyer) is one of the most famous herbal medicines, and has been used for tonic and anti-stress medicine (Kaneko, and Nakanishi, 2004). Its constituents such as ginsenosides are well known to have anti-stress effect (Kim *et al.*, 2003a). In our

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previous report, a method to evaluate anti-stress effect of functional foods was introduced. Experimental animals were overstressed psychologically through electroshock stimulation and restraint (Kim *et al.*, 2003b). In addition, changes in behavior, organ weights, and level of blood corticosterone induced by overstress were examined. Many reports have suggested that levels of blood corticosterone can be used as parameters of stress level, because stress alters the function of adrenal gland and corticosterone secretion (Armario *et al.*, 1985; Djordjevic *et al.*, 2002).

In this experiment, SG extract was used to treat psychologically stressed animals. Ginseng has been traditionally used for the treatment of immune disorder, stress related disease, and cancer, and the biological activities of SG and its constituents such as radical-scavenging activity, antioxidant activity, anti-tumor promoting activity, relaxation of blood vessel and protection of neuronal degeneration were reported; however, effects of SG on psychological stress were not proven.

The main aim of this study was to investigate stress related activities of SG extracts as a candidate for anti-stress-related functional supplement by comparing its effect to those of red ginseng extract, which is also known to alleviate stress.

MATERIALS AND METHODS

Animals and materials

Male Sprague-Dawley (SD) rats (8-10 weeks of age) and the male ICR mice (20-25 g) were obtained from Hanlim experimental animal Co (Hwasung, Korea). Water extract of Sun-ginseng (SG) was provided by Ginseng science Co. (Seoul, Korea). Red Ginseng (RG) extract (KRG extract; Korean Ginseng Co., Seoul, Korea) was used as a positive control. All animals were housed in a temperature ($22\pm 2^\circ\text{C}$) and humidity ($55\pm 5\%$) controlled animal room on a 12 hr/12 hr light/dark (6 A.M.-6 P.M.) schedule. They had free access to food and water throughout the experimental period. The animals were divided into five groups after stabilization for 1 week in the animal room. Animals belonging to the normal group were not exposed to stress. Prior to stress exposure, rats of the control group were orally administered 2 mL saline/kg body weight, and mice of the control group were given tap water. Rats of the RG200-, SG100 and SG200-supplemented groups were orally administered once a day 200 mg RG extract/kg body weight, 100 and 200 mg SG extracts/kg body weight, and exposed to stress. In order to minimize damage, mice of the RG200-, SG100 and SG200-supplemented groups were given water containing 400

mg RG extract, 200 and 400 mg SG extracts/100 mL drinking water, and exposed to stress. Prior to determination of supplements concentration in drinking water, it was identified that the mean drinking amount a day was about 100 mL/kg body weight. We determined above concentration of supplements considering loss in drinking water and the difference between rat and mouse. In case of restraint stress and foot shock stress, supplementary materials were given for a total of 10 days; 5 days prior to stress exposure and 5 days with stress, and in case of swimming, wire and rotarod test, supplementary materials were given for 5 days prior to stress exposure. Flumazenil (Sigma chemical Co.) 3 mg/kg was intraperitoneally injected 30 min prior to testing. Behavioral activities of animals were monitored between 10 A.M and 3 P.M.

Induction of stress

During 5 days with stress, rats were exposed to stress after oral administration of supplementary materials between 9 A.M and 2 P.M. The rats were subjected to restraint stress by placing them in well ventilated conical polypropylene tubes (6.2 cm in diameter and 16.5 cm in length) for 30 min each day. During the restraining period, they did not have any access to food and water. At the end of each restraint stress period, they were exposed to electroshocks with an intensity of 3 mA (1 s duration; 20 s intershock interval) for 5 min (Kim *et al.*, 2003b).

Apparatus for monitoring behavioral activities

Observation boxes and a camera were located in the animal room, allowing observation of animals through a computer outside the room. After inducing terminal stress, behavioral changes of animals were monitored automatically using a computerized EthoVision system (Noldus IT b.v., Wageningen, Netherlands). In the locomotor activity and elevated plus-maze tests, the behavioral parameters were analyzed by an automatic videotracking system.

Locomotor activity in rats

The observation apparatus consisted of nine black plastic boxes (47×47 cm), and its field was bordered by 42-cm-high side walls. The total distance moved, total movement time, and turn angles were monitored for 20 min after terminal stress (Noldus *et al.*, 2001; Kim *et al.*, 2003b).

Elevated plus-maze test in rats

The Elevated plus-maze box and arms were made of plastic materials. The apparatus consisted of two open arms (50×10

cm in rats; 30×6 cm in mice), alternating at right angles, with two arms enclosed by high walls of 30 cm and 20 cm in rats and mice, respectively. The four arms delimited a central area of 10×10 cm. The whole apparatus was placed 50 cm above the floor. Animals were placed in the central square after measuring stress-related activity and allowed to explore the maze freely for 10 min. The parameters measured were the times spent in open and closed areas (Noldus *et al.*, 2001; Kim *et al.*, 2003).

Stress related activities in rats

After terminal stress, animals were placed in individual plastic cages (40×20×18 cm), alternating at right angles, with two arms enclosed by high walls of 30 cm. The behavioral activities were measured soon after loading stress. Smelling, feeding, burrowing, freezing, face washing, and grooming time were recorded for 10 min (Noldus *et al.*, 2001; Kim *et al.*, 2003b). Rearing frequency was measured using EthoVision system for 20 min after the terminal stress (Noldus *et al.*, 2001; Kim *et al.*, 2003b).

Measurement of swimming time, Rotarod evaluation and Horizontal Wire test in mice

The water extracts of SG and RG were supplied for consecutive 5 days. Mice were placed in a swimming pool. They were allowed to swim in cold water (6–8°C). The swimming time until exhaustion were measured (Kimura and Sumiyoshi 2004). Mice were allowed to grasp a horizontally strung wire (4 mm diameter, 150 cm long, and 80 cm height) with their forepaws and tail (Kim *et al.*, 2004). The staying time on the wire until exhaustion were measured. The mouse was allowed to walk on a rotating rod (60 rpm) of fixed diameter (3.5 cm) until it can no longer maintain its position on the Rotarod (UGO BASILE, Italy). The latency to fall were recorded with a stopwatch.

Blood sampling and measurement of serum corticosterone in rats

After monitoring stress related activities, blood samples (rat 4 mL; mouse 1.5 mL in heparinized tubes) were taken through heart puncture between 10:00 A.M.–2:00 P.M., and rat adrenal gland were dissected and weighed.

The serum corticosterone level was measured by a modified method (Harikai *et al.*, 2003) using HPLC system composed of SI-2 3001 pump, SI-2 3002 UV-Visible detector, SI-2 3004 column oven, separation (Shiseido, Tokyo, Japan), and column Capacell Pak C18 MG 120 (5 µm, 3×250 mm). Dexametha-

sone (Sigma, St. Louis, MO, U.S.A) was used as the internal standard. Forty microliters of treated sample solution was injected into HPLC column using acetonitrile : methanol :sulfuric acid solution (32:4:64) as the mobile phase at 500 µL/min. Corticosterone level was determined as the absorbance at 240 nm using dsCHROM-computing program (Shiseido, Tokyo, Japan).

Statistical analysis

Data are expressed as means±S.E.M.. ANOVA was used to compare the scores among the groups for one variable, followed by post hoc comparisons using the Newman-Keuls test.

RESULTS

Effects of SG on locomotor activity test in SD rats

Fig. 1 shows changes of locomotor activities in rats. The stress condition resulted in significant decreases of total moved time and moved distance; however, SG-supplementation blocked this stress-induced suppression of locomotion but differences

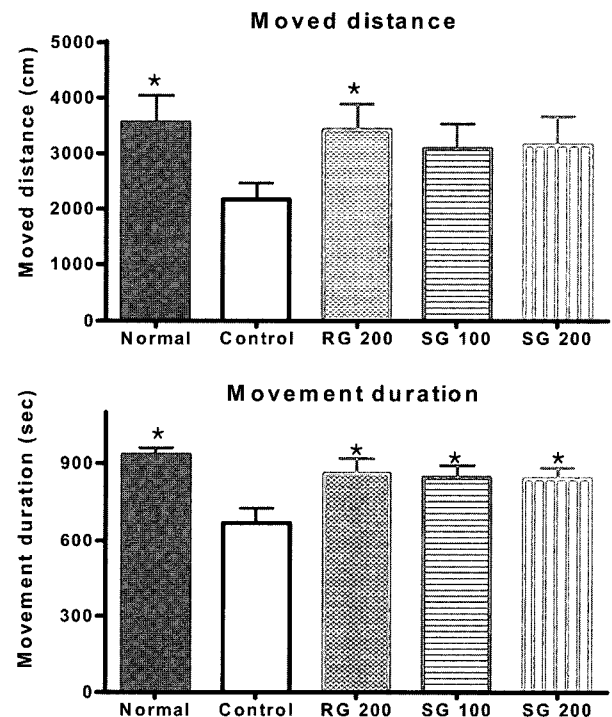


Fig. 1. Effects of SG on Locomotor activity test in SD rats (n=9). Each bar represents mean ± SEM of total moved times (below) and distances (above) for 20 minutes after loading stress. The normal group were not exposed to any stress and the control group were exposed to stress. The others were supplemented RG 200 (Red Ginseng 200 mg/kg, p.o.), SG-100 (Sun ginseng 100 mg/kg, p.o.), SG-200 (Sun ginseng 200 mg/kg, p.o.), and exposed to stress. Normal, RG, or SG versus Control, **p<0.01; *p<0.05.

between doses were not. Effects of SG were similar to the effect of RG 200. Stress induced suppression in activities of general behavior, but SG and RG-supplementation recover the changes.

Effects of SG on Stress related activity test in SD rats

The influences of SG-supplementation on the stress behaviors induced by immobilization and electroshock were assessed for 10 minutes or 20 minutes. Results revealed stress exposure

significantly increased times spent on freezing, grooming, face-washing, and burrowing and decreased time spent on smelling in rats assessed for 10 minutes, and decreased rearing of rats assessed for 20 min (Fig. 2). On the other hand, SG-supplementation partially blocked these stress-induced behavioral changes. They were similar to the effects of RG although they were not the same as RG's effects.

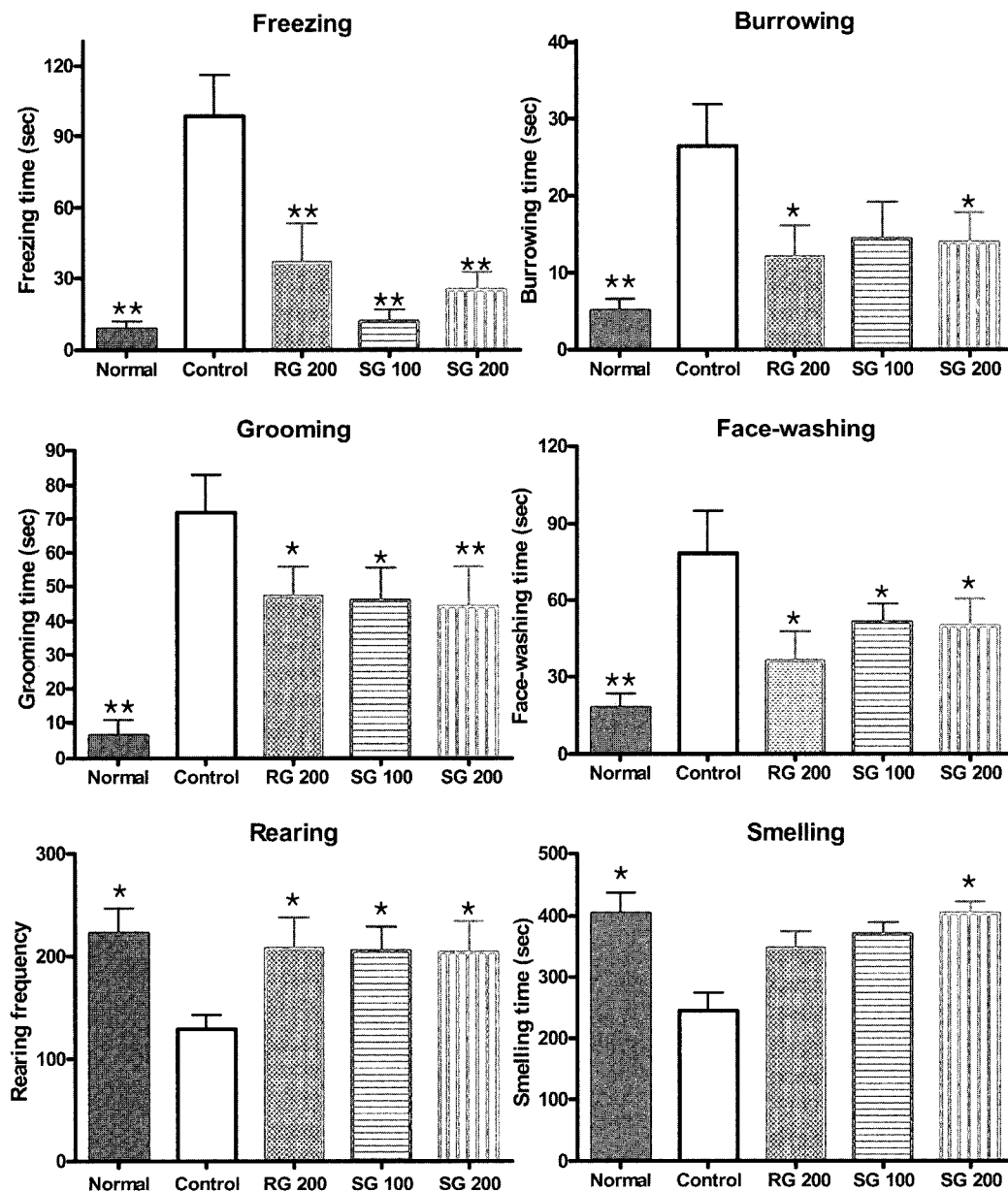


Fig. 2. Effects of SG on Stress related activity test in SD rats (n=10). Each bar represents mean \pm SEM of total activity times for 10 minutes or frequency numbers for 20 minutes after loading stress. Normal, RG-200(Red Ginseng 200 mg/kg), SG-100(Sun ginseng 100 mg/kg), SG-200(Sun ginseng 200 mg/kg) versus Control, ** $p < 0.01$; * $p < 0.05$.

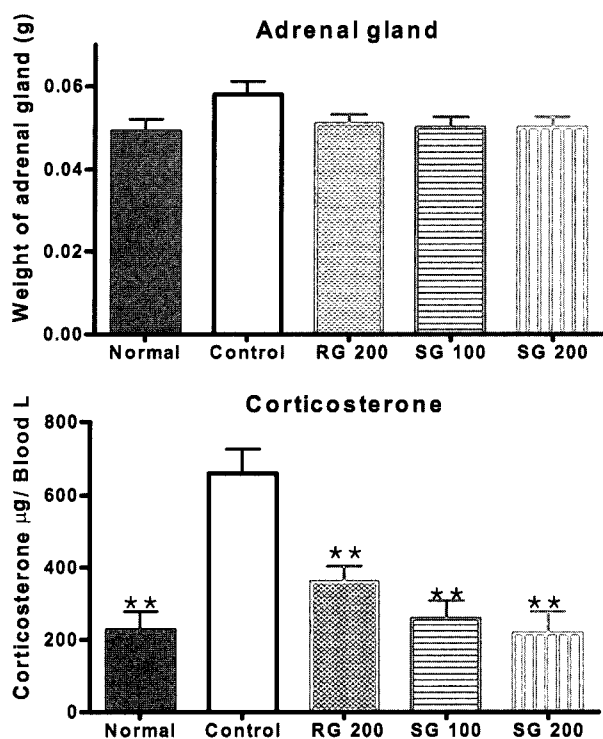


Fig. 3. Effects of SG on serum level of corticosterone and wet weight of adrenal gland in SD rats ($n=10$). Each bar represents mean \pm SEM of wet weights of adrenal glands and serum corticosterone levels after loading immobilization and foot-shock stress. Normal, RG 200 (Red Ginseng 200 mg/kg), SG-100 (Sun ginseng 100 mg/kg), SG-200 (Sun ginseng 200 mg/kg) versus Control, ** $p<0.01$; * $p<0.05$.

Effects of SG on serum level of corticosterone and weight of adrenal gland in SD rats

Stress has been thought to be a non-specific response to stressors accompanied by the activation of adrenal gland. Stress condition resulted in a significant increase in the wet weight of adrenal gland (Fig. 3). Stressed animals have higher corticosterone levels than the stress-free ones. SG-supplementation partially blocked this stress-induced increase of adrenal gland size and blood corticosterone level, similar to the effects of RG.

Effects of SG on the elevated plus maze test in SD rats

Time spent in the open and closed arms as assessed for 10 minutes significantly differed between animals exposed to stress and the unexposed ones (Fig. 4). Animals exposed to stress spent less time in the open arm and more time in the closed arm than the unexposed ones. Stress exposure decreases the total turned degree of the animals; SG-supplementation significantly reversed this stress-induced response in elevated plus maze test. These effects were also similar to those of RG. Fig. 5

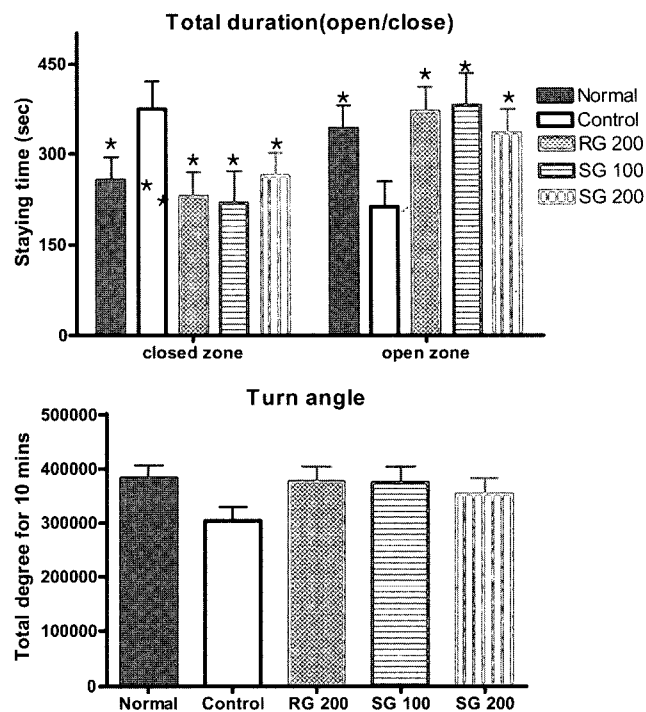


Fig. 4. Effects of SG on the Elevated plus maze test in SD rats ($n=10$). Each bar represents mean \pm SEM of times spent in open or closed area and total turn angles for 10 minutes after loading stress. Normal, RG-200 (Red Ginseng 200 mg/kg), SG-100 (Sun ginseng 100 mg/kg) or SG-200 (Sun ginseng 200 mg/kg) versus Control, ** $p<0.01$; * $p<0.05$.

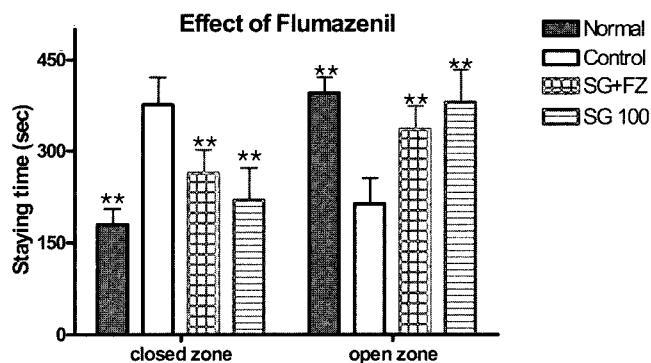


Fig. 5. Interference of Flumazenil on SG's effects in Elevated Plus Maze test of SD rats ($n=10$). Each bar represents mean \pm SEM of times spent in open or closed area and total turn angles for 10 min after loading stress. SG-100 (Sun-ginseng 100 mg/g), FZ (Flumazenil 3 mg/kg, I.p.).

shows interference of Flumazenil on SG's effects in the Elevated Plus Maze test of SD rats. Effects of SG on them were not influenced by Flumazenil administration.

Effects of SG on physical stress-related activities in ICR mice

Horizontal wire test, Rotarod evaluation and swimming test were measured to evaluate motor coordination and endurance

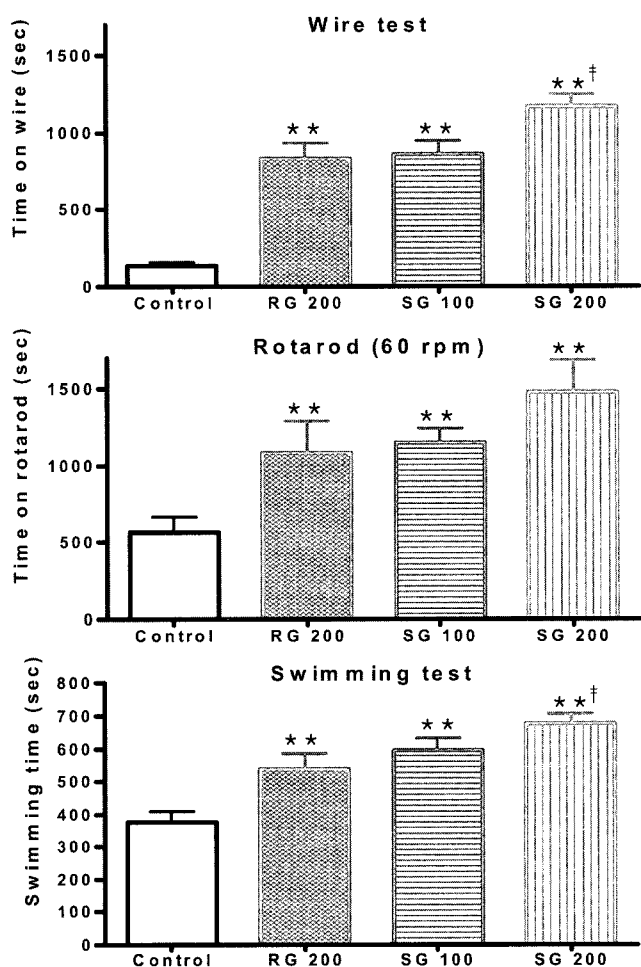


Fig. 6. Effects of SG on Physical stress-related activities in ICR mice ($n=10$). Each bar represents mean \pm SEM of endurance time. Mice were given water containing RG (Red Ginseng 400 mg/100 ml), SG-100 (Sun ginseng 100 mg/100 ml), SG-200 (Sun ginseng 400 mg/100 ml). Supplemented groups versus Control, ** $p<0.01$; * $p<0.05$. SG groups versus RG group, † $p<0.01$; ‡ $p<0.05$.

to physical stress. As shown Fig. 6, the swimming time in mice until exhaustion were increased in dose dependent manner by SG-supplementation. Staying time on a horizontally strung wire and rotating rod were also increased dose dependently by SG. Effects of SG were also more potent than them of RG in physical stress.

DISCUSSION

Stress was found to affect locomotor activity in rats. Locomotor activities, measured as the total moved time and moved distance, were significantly different between stress-exposed and un-exposed animals. In general, activities of animals

decreased due to stress, which was alleviate by the addition of SG and RG. In a previous study, this method was applied to induce stress response in rats and mice, and stress responses are similar to the results of the other stress condition (Beck and Fibiger, 1995; Takeuchi *et al.*, 2003; Kim *et al.*, 2003b). Ginseng extract and its constituents such as ginsenosides have anti-stress activity on animals subjected to stressful stimuli such as footshock, cold, and heat (Kaneko *et al.*, 1996; Kim *et al.*, 2003a; Rai *et al.*, 2003; Kaneko and Nakanishi, 2004). Changes in locomotor behavior, stress behavior, plus maze test, plasma corticosterone level, and organ weight induced by stress condition established in this study were similar to the results of the previous other studies (Morimoto *et al.*, 1993; Djordjevic *et al.*, 2002; Takeuchi *et al.*, 2003; Kim *et al.*, 2003). To test the anti-stress effect of SG, behavioral reactivity was assessed using four parameters and six sub-parameters in rats and mice. Foot shock stress reflect levels of psychological stress in rats. SG-supplementation blocked stress-induced suppression of locomotion in rats, and also partially blocked stress-induced behavioral changes such as freezing, grooming, burrowing, smelling, facewashing, and rearing behavior in rats. There were some differences in freezing and smelling although most effects were also similar to those of RG.

Effects of SG were also more potent than RG in terms of endurance to physical stress in horizontal wire test. Therefore SG may differ from RG in stress related activities. Kim *et al.* reported that SG contains several ginsenosides such as F₄, Rg₅, Rg₆, Rk₁, Rk₂, Rk₃, Rs₃, Rk₄, and Rk₅ which are not present in RG and exhibits some different biological activities from RG (Kim *et al.*, 2000; Keum *et al.*, 2001; Park *et al.*, 2002). We applied two kinds of stress inducing methods in this experiment. We are going to identify the detail differences in stress related activities as we will use various stress inducing methods in the next study.

The animals exposed to stress spent less time in the open arm and more time in the closed arm than the unexposed ones. SG-supplementation significantly reversed stress-induced response in elevated plus maze test. These effects were also similar to those of RG. These results revealed that SG can partially protect from psychological stress, thus suggesting the method of stress exposure applied in this study was properly established for testing anti-stress effect of functional food candidates. Enhancement of GABAergic activity can alleviate stress responses and diazepam has also an anti-stress activity on acute and chronic stresses through GABAergic stimulation (Ida *et al.*, 1985; Finlay *et al.*, 1995; Beck and Fibiger, 1995). Ginse-

nosides such as Rb1, Rb2, Rc, Rd, Re, Rf, Rg1 and Rg2, enhanced the GABA-induced inward current, and the levels of GABA R₁ binding were strongly elevated in almost all regions of the frontal cortex by the treatment of ginsenoside Rc (Kimura *et al.*, 1994; Kim *et al.*, 2001; Choi *et al.*, 2003). We administered flumazenil with SG to investigate whether the anti-stress activity of SG is exerted via the GABAergic nervous system. We could not find out a significant difference between SG group and SG+flumazenil group. This result suggests that the anti-stress activity of SG may not be modulated by GABAergic nervous system that was considered as modulating anti-stress action of RG.

Stress condition resulted in a significant increase in the wet weight of adrenal gland (Fig. 3). Stressed animals have higher corticosterone levels than the stress-free ones. SG-supplementation partially blocked this stress-induced increase of adrenal gland size and decreased blood corticosterone level more than RG. Stress has been thought to be a non-specific response to stressors accompanied by the activation of adrenal glucocorticoid and catecholamine release (Munck *et al.*, 1984; Ida *et al.*, 1985; Finlay *et al.*, 1995; Djordjevic *et al.*, 2002). The hypothalamic-pituitary-adrenal (HPA) axis is one of the hormonal systems mediating the stress response (Kim *et al.*, 2003). The main regulation of stress-related activity of the HPA axis occurs at the level of parvocellular subdivision of the hypothalamic paraventricular nuclei, and the majority of these neurons secrete corticotropin-releasing hormone and vasopressin, which synergistically stimulate adrenocorticotropin (ACTH) secretion by the pituitary corticotropic cells (Djordjevic *et al.*, 2002). ACTH enters the systemic circulation, and stimulates synthesis and release of corticosterone and enlargement of adrenal gland (Djordjevic *et al.*, 2002). The stress condition resulted in the enlargement of adrenal gland and increase of adrenal secretion in this study, as similarly observed in other studies. Stress induces activation of adrenal gland, resulting in the enlargement of adrenal gland and increase of corticosterone secretion (Schwartz *et al.*, 1988; Morimoto *et al.*, 1993; Djordjevic *et al.*, 2002; Kim *et al.*, 2003).

In screening anti-stress effect, SG was chosen among many candidates. SG contains various ginsenosides. Although some ginsenosides such as were presumed as active materials, the exact active material is yet unknown.

In conclusion, stress suppressed locomotor activity in animals; however, SG-supplementation partially blocked the stress effect on locomotion in rats and mice, as well as stress-induced behavioral changes such as freezing, burrowing, grooming, smelling, facewashing, and rearing in rats. SG-supplementation

decreased the levels of blood corticosterone, which was increased by stress in rats. There were some differences in freezing and smelling although most effects were also similar to those of RG. Effects of SG were also more potent than RG in horizontal wire test, Rotarod evaluation and swimming test to evaluate enduring ability to physical stress in mice. The staying time of stressed rats and mice decreased in the open area, whereas increased in the closed area as shown in the elevated plus maze test. However, these changes were also partially blocked by SG-supplementation. The stress related activities of SG may not be modulated by GABAergic nervous system that was considered as modulating anti-stress action of RG.

These results suggest that SG partially protects living organisms from stress attack in some cases and the protecting mechanism of SG may be different to that of RG. RG may be used as a functional food to alleviate stress response.

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