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Anti-stress Effect of *Scutellaria baicalensis* in SD Rats and ICR Mice

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Abstract – The aim of this study is to investigate anti-stress effect of *Scutellaria baicalensis*(SB). The experiments were performed with the use of young (9 weeks of age) male rats of SD strain and the male ICR mice (20-25 g) at the time of first treatment with SB. Animals of the normal group were not exposed to any stress and the control group were exposed to stress. The rats of the Ginseng, Diazepam(BZ) and SB supplementary group were orally administered once a day 100 mg of red ginseng extract, 5 mg of BZ or 100 mg of SB extract/kg body weight and they were exposed to stress. The mice of the Ginseng, BZ and SB supplementary group were given water containing 200 mg of red ginseng extract, 10 mg of BZ or SB extract/100 ml potable water and exposed to stress. Animals were given supplements for 7 days without stress, and then were given supplements for 5 days with restraining and electroshock stress. We recorded stress related behavioral changes of the experimental animals by stressing them using the Etho-vision system and measured levels of blood corticosterone and IL-2. SB-supplementation partially blocked the stress effect on locomotion in the rats and mice, and also partially blocked stress-induced behavioral changes such as freezing, burrowing, grooming, smelling, and rearing behavior in the rats and smelling, grooming, tailing, and rearing in the mice. In elevated plus maze test, the staying time of the stressed rats and mice in the open area decreased while it increased in the closed area. But these changes also partially were blocked by SB-supplementation. SB-supplementation decreased levels of the blood corticosterone which was increased by stress in the rats but did not significantly increase levels of blood interleukin 2 which was decreased by stress in mice.

Key words □ *Scutellaria baicalensis*, Stress, Behavior, Corticosterone, Interleukin-2

INTRODUCTION

Scutellaria baicalensis is one of the most important medicinal herbs in traditional Chinese medicine. It possesses anti-bacterial activity and sedative effects, and is traditionally used for treatment of hepatic disease, G-I disorder, psychological disorder and inflammatory disease(Hui *et al.*, 2002; Zhu, 1998). Wogonin and baicailin, the major chemical constituents of this herb, are flavone derivatives containing a phenylbenzopyrone nucleus(Hui *et al.*, 2002; Lin and Shieh, 1996). The biological and pharmacological properties of flavonoids are broad and include anti-inflammatory actions (Lin and Shieh, 1996; Barnard *et al.*, 1993) the reduction of neuronal oxidative metabolism (Oyama et al., 1994), steroid hormone like effects (Miksicek, 1993), and the inhibition of enzymes including protein kinase C and tyrosine kinase (Ferriola *et al.*, 1993; Cushman *et al.*, 1991). Until recently, their effects on stress was not proven although several groups have reported anxiolytic effects of flavonoids (Paladini *et al.*, 1999; Salgueiro *et al.*, 1997; Wolfman *et al.*, 1994).

Stress is the plague of the modern society as well as an avoidable consequence of life. As Seyle (Selye, 1993) noted, "without stress, there would be no life". Overstress is a life-long problem and can cause physical damage to the gastrointestinal tract, endocrine system, skin or cardiovascular system (Chrousos and Gold, 1992; Breier *et al.*, 1987; Hurst *et al.*, 1976). As a negative influence, it can result in feelings of distrust, rejection, anger, and depression, which in turn can lead to health problems such as headaches, upset and ulcer of stomach, liver disease, rashes, insomnia, hypertension, heart disease, stroke, diabetes, immune disorders, and sexual disorders

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(Dimsdale *et al.*, 2000; Selye, 1993; Breier *et al.*, 1987; Glass, 1977; Hurst *et al.*, 1976).

In order to be free from depression, anxiety, sleep disorder and stress related disorders, many functional foods such as Ginseng extract, DHA, and Gingko biloba extract were introduced and tested (Kim *et al.*, 2003a; Takeuchi *et al.*, 2003; Ward *et al.*, 2002; Yuan *et al.*, 1989). These functional foods has been tried to treat short-term memory loss, lack of attention or vigilance, cerebral and vascular disorders, high blood pressure, and sexual disorders and to alleviate over stress (Ai and Bolling, 2002; Petrowicz *et al.*, 1997). However, it has not been proven whether they could cure stress induced disorders. It was not confirmed to evaluate anti-stress effect of functional food. We tried to introduce a method to evaluate anti-stress effect of functional foods. We especially intended to apply psychological stress into experimental animal and tested anti-stress effect of a functional food. We selected simple methods that could psychologically load overstress to animals and the signs of overstress. Animals were given an electroshock stimulation and a restraint stress. In previous studies, we have investigated on stress loading method (Kim *et al.*, 2003b). We examined the behavioral changes, the changes of organ weights, blood corticosterone and interleukin 2 (IL-2) levels induced by overstress. Many reports suggested that levels of blood corticosterone and IL-2 can be used as a parameter of stress level (Djordjevic *et al.*, 2002; Koh and Lee, 1998; Glaser *et al.*, 1990; Armario *et al.*, 1985).

The main aim of this study was to investigate anti-stress effect of *Scutellaria baicalensis* as a candidate for anti-stress related functional supplements by comparing its effect to those of ginseng and diazepam, which were also known to cure stress.

MATERIALS AND METHODS

Animals and materials

The male Sprague-Dawley(SD) rats (8-10 weeks of age) and the male ICR mice (20-25 g) used in this study were obtained from Hanlim experimental animal Co. We used *Scutellaria baicalensis* which was obtained from herbal suppliers in Seoul and red Ginseng extract (KRG extract) produced by Korean Ginseng Corp. Water extract of *Scutellaria baicalensis* was lyophilyzed and stored at -20°C. It was well known that Ginseng extract or its constituents such as ginsenosides have an anti-stress effect (Kim *et al.*, 2003a; Yuan *et al.*, 1989). We chose Ginseng extract as a positive control. All animals were housed under a constant temperature (22 ± 2°C) and humidity (55 ± 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 experiments. The animals were divided into five groups after stabilizing them for 1 week in our animal room. Animals belonging to the normal group were not exposed to any stress. The rats of control group were orally administered saline 1 mL/100 g body weight and were exposed to stress, and the mice of control group were given normal water and exposed to stress. The rats of the Ginseng, SB, and BZ supplemented group were orally administered once a day 100 mg of Ginseng extract, SB extract/kg body weight, and 5 mg of BZ/kg body weight and exposed to stress. The mice of the Ginseng, SB, and BZ supplemented group were given water containing 200 g of Ginseng extract and SB extract/100 mL drinking water, and 10 mg of BZ/100 mL drinking water and exposed to stress. Animals were given only supplementary materials for 7 days after stabilizing them, and then were supplemented with the materials for 5 days with stress.

Induction of Stress

Animals were given supplementary materials before exposing them to stress. The mice were usually subjected to restraint stress by keeping them in a well-ventilated conical plastic tubes (3 cm in diameter and 7 cm in length) for 30 minutes each day. During the restraining period, the mice did not have any access to food and water. At the end of each restraint stress, the mice were exposed to electroshocks with intensity of 1 mA (1 sec duration; 20 sec intershock interval) for 5 minutes (Kim *et al.*, 2003b). The rats were usually subjected to restraint stress by keeping them in a well ventilated conical polypropylene tubes (6.2 cm in diameter and 16.5 cm in length) for 30 minutes each day. During the restraining period, the rats did not have any access to food and water. At the end of each restraint stress, the rats were exposed to electroshocks with intensity of 3 mA (1 sec duration; 20 sec intershock interval) for 5 minutes (Kim *et al.*, 2003b).

Behavioral apparatus

The equipment was located in the animal room allowing the observer to view and observe the animals through a computer outside the room. After inducing terminal stress (in the manner described above), behavioral changes of animals were monitored automatically using a computerized EthoVision system (Noldus IT b.v., Netherlands). In the locomotor activity and elevated plus-maze tests, the behavioral parameters were ana-

lyzed by an automatic videotracking system.

Locomotor activity

The apparatus consisted of 9 black plastic boxes (47 × 47 cm), and the field was bordered by 42-cm-high side walls. The total moved distance, total movement time and turn angles were monitored for 20 minutes after terminal stress (Kim *et al.*, 2003b; Noldus *et al.*, 2001).

Elevated plus-maze test

The Elevated plus-maze box and arms were made of plastic. 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 in rats and 20 cm in mice. The four arms delimited a central area of 10 × 10 cm. The whole apparatus was placed 50 above the floor. Animals were placed in the central square after measuring stress related activity and allowed to explore the maze freely for 5 minutes. The parameters measured were the times spent in open and closed areas (Kim *et al.*, 2003b; Noldus *et al.*, 2001).

Stress related activity tests

After terminal stress, animals were placed alone in individual plastic cages (40 × 20 × 18 cm in rats; 26 × 20 × 13 cm in mice). The behavioral activities were measured soon after stress. Smelling, feeding, burrowing, freezing, tailing, face washing and grooming time were recorded for 5 minutes (Kim *et al.*, 2003b; Takeuchi *et al.*, 2003). Rearing frequency was measured using EthoVision system for 20 minutes after the terminal stress (Kim *et al.*, 2003b; Noldus *et al.*, 2001).

Blood sampling and Measurement of Serum Corticosterone and IL-2

After monitoring locomotor activity, 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 then adrenal gland in the rat and spleen in the mouse were dissected and weighed.

The serum corticosterone level was measured by a modified method (Harikai *et al.*, 2003) using HPLC system composed with 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). Corticosterone and dexamethasone (Sigma, St. Louis, MO, U.S.A) were used as the internal standard. We injected the 40 μL of treated sample solution into HPLC column. We used acetonitrile : methanol : sulfuric acid solution (32 : 4 : 64) as the mobile phase with a flow rate of 500 μL/min. We determined corticosterone level as absorbance in wavelength 240 nm using dsCHROM computing program (Shiseido, Tokyo, Japan).

The serum IL-2 level was measured by a modified method using commercial Elisa kit (R&D systems, Minneapolis, MN) (Kim *et al.*, 2003c).

Statistical analysis

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

RESULTS

Fig. 1 shows that stress affected locomotor activity in both rats and mice. Locomotor activities, measured as the total moved time and moved distance, were significantly different between animals exposed to stress and those not exposed to. The stress condition resulted in the significant decrease of total moved time and moved distance in both animals but SB-supplementation partially blocked this stress-induced suppression of locomotion.

The influence of SB-supplementation on the stress behaviors induced by immobilization and electroshock was assessed for 5 or 20 minutes. As shown in Fig. 2, the stress exposure resulted in a significant increase of time spent in freezing, grooming, facewashing, and burrowing behaviors of rats for 5 minutes and a decrease of time spent in smelling behavior for 5 minutes and rearing frequency of rats for 20 minutes but SB-supplementation partially blocked these stress-induced changes of behavior such as freezing, burrowing, grooming, smelling, and rearing. Fig. 3 shows that the changes of stress behavior in mice were similar to that of the rats. The stress condition resulted in a significant increase of time spent in freezing, grooming, facewashing, and tailing behaviors of the mice for 5 minutes and a decrease of time spent in smelling behavior for 5 minutes and rearing frequency in the mice for 20 minutes but SB-supplementation partially blocked this stress-induced changes of behavior such as smelling, grooming, tailing, and rearing. These effects were also similar to Ginseng's effects.

The time spent in the open or closed arm for 5 minutes significantly differed between the animals exposed to stress and the unexposed animals as shown in Fig. 4. The animals exposed to stress spent less time in the open arm than the unexposed animals. Furthermore, the animals exposed to stress

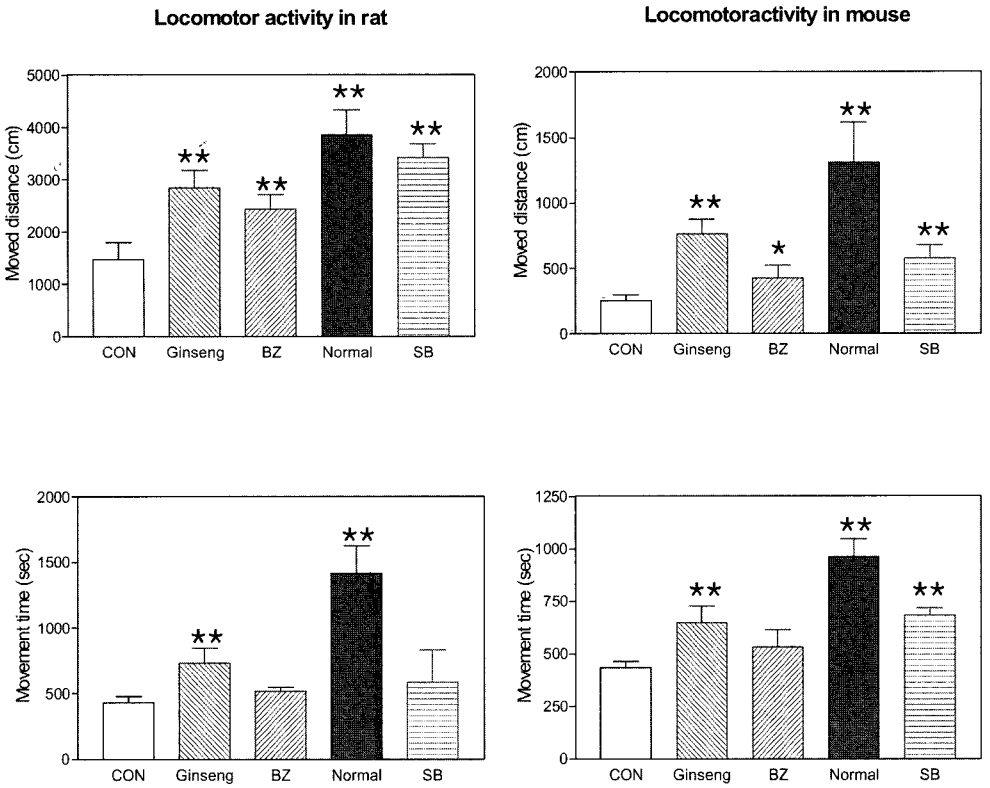

Fig. 1. Effects of *Scutellaria baicalensis* on locomotor activity test in SD rats (n=7) and ICR mice (n=8). Each bar represents mean ± SEM of total moved times (right) and distances (left) for 20 minutes after loading stress. The normal group was not exposed to any stress and the control group was exposed to stress. The others were supplemented BZ, Ginseng or SB and exposed to stress. Normal, BZ (Diazepam), Ginseng or SB (*Scutellaria baicalensis*) versus Control, **p<0.01; *p<0.05.

spent more time in the closed arm than the unexposed animals. Stress exposure decrease the total turned degree of the animals. SB-supplementation significantly reversed this stress-induced response in Elevated plus maze test. These effects were also similar to Ginseng's effects.

As shown in Fig. 5, the stress condition resulted in a significant increase in wet weight of adrenal gland. The stressed animals have higher corticosterone levels than the stress free animals. SB-supplementation partially blocked this stress-induced increase of adrenal gland size and blood corticosterone level. This effect was also similar to Ginseng's effect.

Fig. 6 shows that stress affected spleen shrinkage and blood IL-2 levels in the mice. The spleen size and IL-2 level in the mice were reduced by over-stress. They were significantly recovered by Ginseng or SB-supplementation but IL-2 levels were not done by SB-supplementation.

DISCUSSION

Stress affected locomotor activity in both rats and mice, but SB-supplementation blocked this stress-induced suppression of locomotion. This effect was similar to that of Ginseng's effect. We can see that general activities of the animals shrink due to stress but SB and Ginseng can help animals to overcome it. The influence of SB-supplementation on the stress behaviors induced by immobilization and electroshock was assessed for 5 minutes and 20 minutes. As shown in Fig. 2, 3 and 4, the stress exposure resulted in changes of stress-related behaviors in the rats and the mice. But SB-supplementation partially blocked these changes of behaviors and the time spent in the open or closed arm. These effects were also similar to Ginseng's effects. In the previous study, we also applied this method to induce stress response in rats and mice and the results are similar to the results from the other stress condition (Takeuchi *et al.*, 2003; Kim *et al.*, 2003b; Beck and Fibiger, 1995; Glass, 1977). We chose the Ginseng extract and diazepam as a positive control in testing anti-stress effect of functional foods. It was well known that Ginseng extract or its constituents such as ginsenosides have anti-stress activity on animals subjected to stressful stimuli such as footshock, cold and heat (Choi *et al.*,

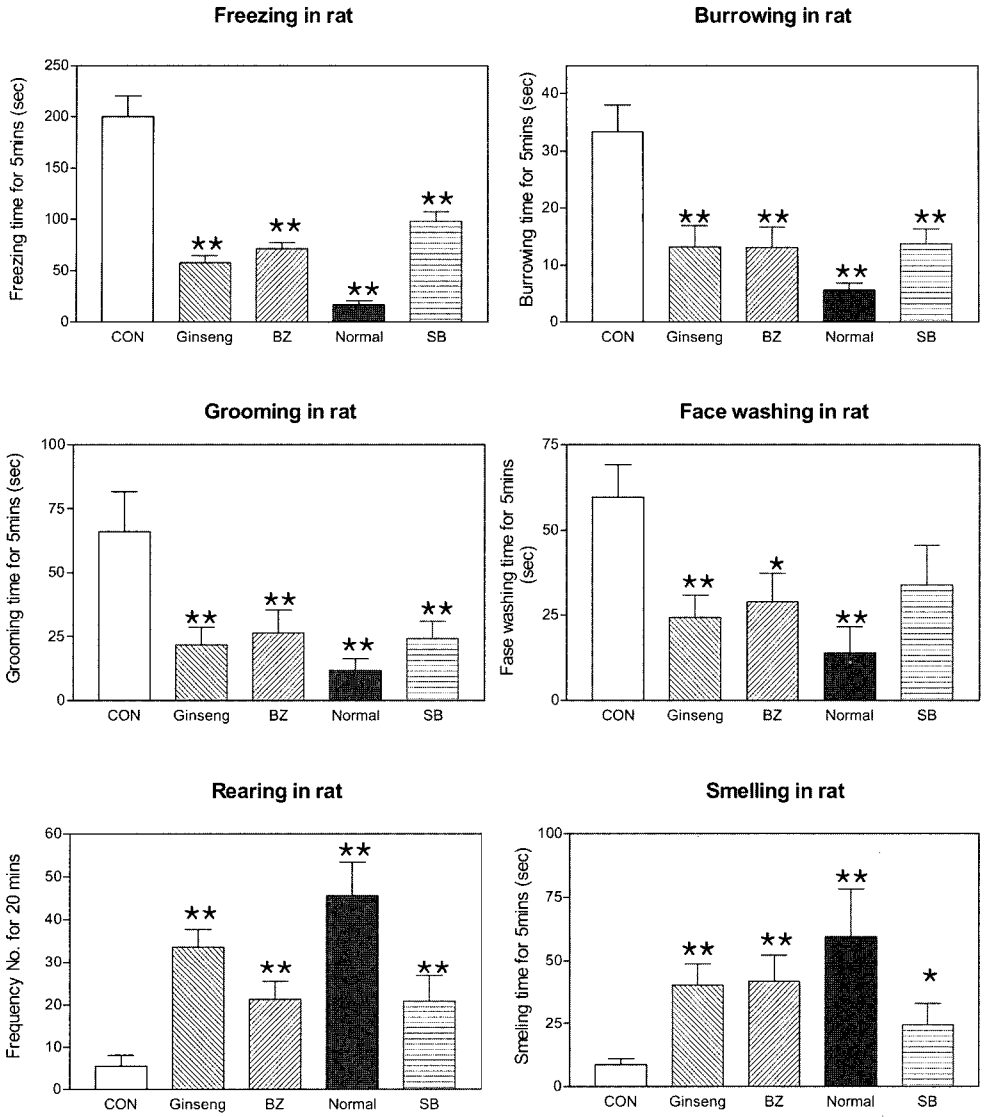

Fig. 2. Effects of *Scutellaria baicalensis* on stress related activity test in SD rats (n=7). Each bar represents mean ± SEM of total activity times for 5 minutes or frequency numbers for 20 minutes after loading stress. The normal group was not exposed to any stress and the control group was exposed to stress. The others were supplemented BZ, Ginseng or SB and exposed to stress. Normal, BZ (Diazepam), Ginseng or SB (*Scutellaria baicalensis*) versus Control, **p<0.01; *p<0.05.

2003; Kim *et al.*, 2003d; Kaneko *et al.*, 1996; Yuan *et al.*, 1989) and diazepam has also an anti-stress activity on acute and chronic stress through GABAnergic stimulation (Beck and Fibiger, 1995; Ida *et al.*, 1985; Finlay *et al.*, 1985).

Changes in locomotor behavior, stress behavior, plus maze test, plasma corticosterone level, and organ weight induced by stress condition which we established in this study and the previous study were similar to the results of the other studies (Takeuchi *et al.*, 2003; Djordjevic *et al.*, 2002; Fanselow, 1980). In order to test anti-stress effect of SB, behavioral activity was assessed using three parameters and six sub-parameters in rats and mice. These reflect properly the levels of psychological stress in rats and mice. SB-supplementation blocked stress-induced suppression of locomotion, and also partially blocked stress-induced behavioral changes. SB-supplementation significantly reversed stress-induced response in elevated plus maze test. These effects were also similar to Ginseng's effects. This result indicates that SB can partially protect from psychological stress. Therefore, the method of stress exposure applied in this study was established properly in testing anti-stress effect of functional foods and Ginseng was properly used as a positive control.

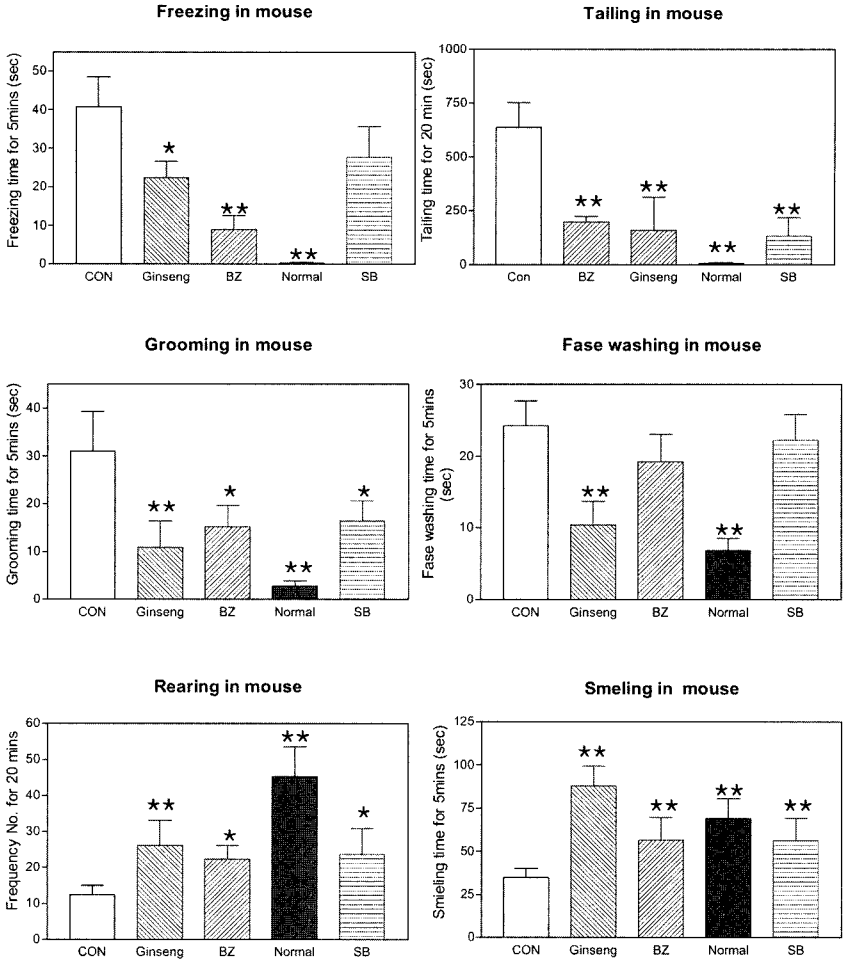

Fig. 3. Effects of *Scutellaria baicalensis* on stress related activity test in ICR mice (n=8). Each bar represents mean ± SEM of total activity times for 5 minutes or frequency numbers for 20 minutes after loading stress. The others were supplemented BZ, Ginseng or SB and exposed to stress. The normal group was not exposed to any stress and the control group was exposed to stress. Normal, BZ (Diazepam), Ginseng or SB (*Scutellaria baicalensis*) versus Control, **p< 0.01; *p<0.05.

As shown in Fig. 5, the stress condition resulted in a significant increase in secretion and size of adrenal gland. This effect was also similar to Ginseng's effect. Stress was thought to be a non-specific response to stressors always including the activation of adrenal glucocorticoid and catecholamine release (Djordjevic *et al.*, 2002; Beck and Fibiger, 1995; Ida *et al.*, 1985; Finlay *et al.*, 1985). The hypothalamic-pituitary-adrenal (HPA) axis is one of the hormonal systems mediating the stress response (Kim *et al.*, 2003a). The main regulation of stress-related activity of the HPA axis occurs at the level of parvicellular 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 then stimulates corticosterone synthesis and release from the adrenal cortex 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. These results are similar to them from the other studies. It was well known that stress induces activation of adrenal gland resulting to the enlargement of adrenal gland and increase of corticosterone secretion (Takeuchi *et al.*, 2003; Djordjevic *et al.*, 2002; Park *et al.*, 1996). SB-supplementation partially blocked this stress-induced adrenal secretion, and the effect was similar to Ginseng's effect.

The spleen size and IL-2 level in the mice were reduced by over-stress but they were recovered by SB-supplementation. The effect was also similar to Ginseng's effect. The changes of IL-2 levels and spleen size have been provided as an evidence on immune function. An immune stimulation induces proliferation of immune cells in spleen and production of IL-2. IL-2 is

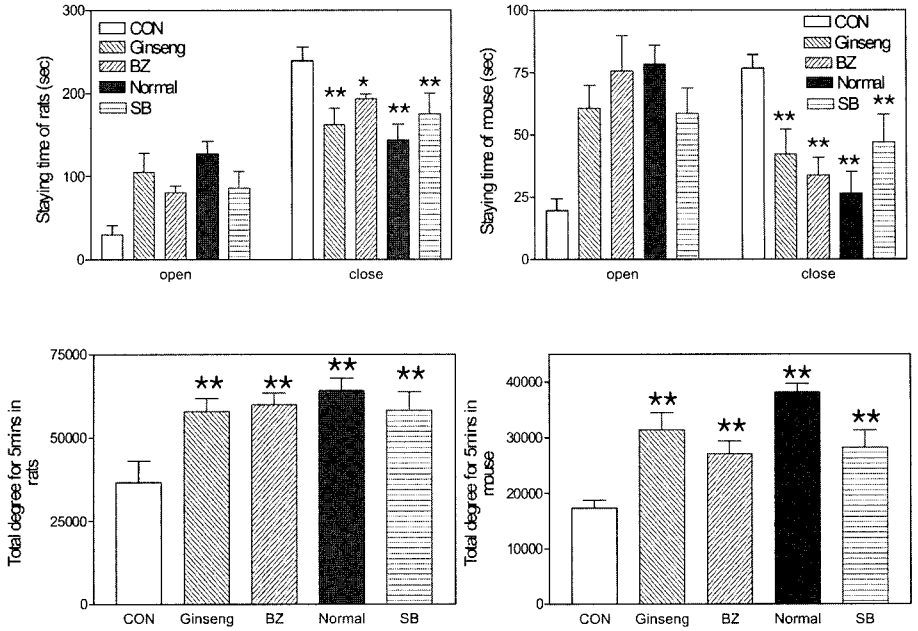

Fig. 4. Effects of *Scutellaria baicalensis* on elevated plus maze test in SD rats (n=7) and ICR mice (n=8). Each bar represents mean ± SEM of times spent in open or closed area and total turn angles for 5 minutes after loading stress. The normal group were not exposed to any stress and the control group was exposed to stress. The others were supplemented BZ, Ginseng or SB and exposed to stress. Normal, BZ (Diazepam), Ginseng or SB (*Scutellaria baicalensis*) versus Control, **p<0.01; *p<0.05.

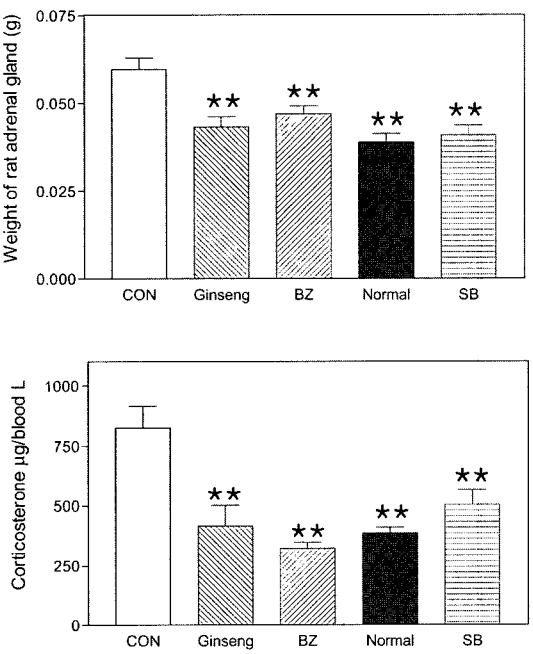

Fig. 5. Effects of *Scutellaria baicalensis* on function of adrenal cortex in SD rats (n=7). Each bar represents mean ± SEM of wet weights of adrenal glands and blood corticosterone levels after loading stress. The normal group was not exposed to any stress and the control group were exposed to stress. The others were supplemented BZ, Ginseng or SB and exposed to stress. Normal, BZ (Diazepam), Ginseng or SB (*Scutellaria baicalensis*) versus Control, **p<0.01; *p<0.05.

a lymphokine that is produced by activated T lymphocytes (Cantrell and Smith, 1984). It is known that glucocorticoids have immunoregulatory activities (Munck *et al.*, 1984). In addition, there is evidence to suggest that there is actually hard wiring between the central nervous system and the immune system as demonstrated by the observation that nerve endings are in direct contact with T lymphocytes in the spleens of rats (Felton and Olschowka, 1987). Therefore we can suggest two mechanisms on spleen shrinkage and decrease of blood IL-2 levels. The first one is a hormone related mechanism namely the elevation of glucocorticoids due to over-stress. The second one is a direct mechanism. The central nervous system which is influenced by over-stress directly inhibits immune function of spleen through neuronal innervation.

Scutellaria baicalensis and its constituents have some biological and pharmacological properties such as anti-inflammatory actions (Lin and Shieh, 1996; Barnard *et al.*, 1993) the reduction of neuronal oxidative metabolism (Oyama *et al.*, 1994), steroid hormone like effects (Miksicek, 1993), sedative and anxiolytic effects (Hui *et al.*, 2002). The major chemical constituents of this herb are flavone derivatives such as wogonin and baicailin containing a phenylbenzopyrone nucleus(Hui *et al.*, 2002; Lin and Shieh, 1996). Wogonin exerts its anxiolytic effect through positive allosteric modulation of the

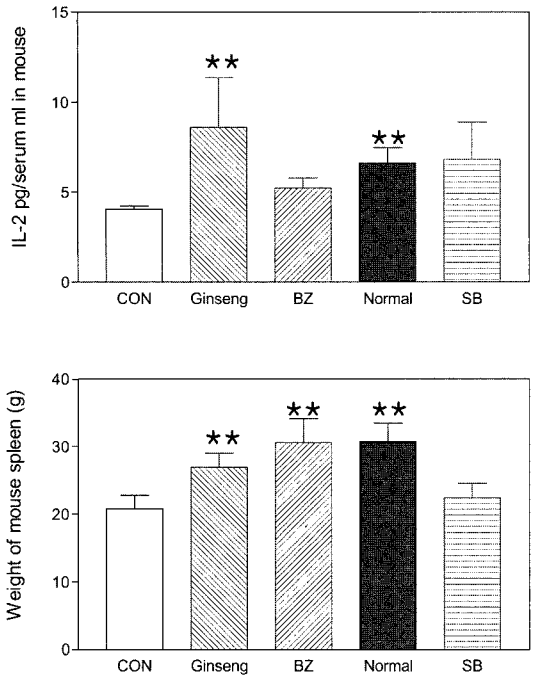

Fig. 6. Effects of *Scutellaria baicalensis* on immune activity in ICR mice (n=6-8). Each bar represents mean ± SEM of wet weight of spleen and blood IL-2 levels after loading stress. The normal group was not exposed to any stress and the control group was exposed to stress. The others were supplemented BZ, Ginseng or SB and exposed to stress. Normal, BZ (Diazepam), Ginseng or SB (*Scutellaria baicalensis*) versus Control, **p<0.01; *p<0.05.

GABA receptor complex via interaction at the benzodiazepine-S (Hui *et al.*, 2002). Its anxiolytic effect was not accompanied by sedative and myorelaxant side-effects typical of benzodiazepines (Hui *et al.*, 2002). Modification of side chains on the flavone molecule has been shown to be selective for a number of receptor systems including the opiate receptor and the benzodiazepine-S (Hui *et al.*, 2002; Hui *et al.*, 2000; Thirugnanasambantham *et al.*, 1992). Therefore, we can consider that the anti-stress effect of SB was due to flavone molecules in it and mediated by GABA receptor. An investigation about anti-stress activity of flavonoids will be the subject of further studies.

In conclusion, stress suppressed locomotor activity and altered stress related behaviors such as freezing, burrowing, smelling, grooming, tailing and rearing in animals but SB-supplementation partially blocked the stress effect. The staying time of stressed animals in the open area decreased but it also partially were blocked by SB-supplementation. SB-supplementation decreased the levels of blood corticosterone, which was increased by stress in rats, and partially blocked the suppression of immune function in mice.

These results suggest that *Scutellaria baicalensis* protects partially the living organism from stress attack in some cases and it has the potential to be used as a functional food to alleviate stress response.

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