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Anti-Stress Effects of Ginseng in Immobilization-Stressed Rats

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Abstract

Stress is a global menace exacerbated by the advancement of industrialization. Failure of stress management is to a breakdown of the psychological and physiological protection mechanisms against stress. The aim of present study was to investigate the anti-stress potential of ginseng against immobilization stress. Male Sprague-Dawley rats (n=24) were divided into three groups; (i) control, (ii) immobilization stress (2 hr daily, for 2 weeks), and (iii) immobilization stress (2 hr daily, for 2 weeks) plus oral administration of ginseng (200 mg/kg BW/d). Immobilization stress resulted in a significant inhibition of body weight gain by 45% and a significant decrease in the tissue weights of thymus and spleen ($p<0.05$). The concentrations of blood GOT and GPT were significantly increased in the immobilization-stressed group compared to the control group ($p<0.05$). There were no differences in the blood cholesterol levels among groups. Ginseng administration in the immobilization-stressed group tended to reverse the lack of body weight gain and food intake, though not significantly. The ginseng-administered group showed a significant reversal in the stress-induced effect on spleen and thymus weight, increasing the tissue weights by 16% and 20%, respectively, compared to immobilization-stressed group ($p<0.05$). The plasma corticosterone level was significantly increased in the stressed group by 39% compared to the control group ($p<0.05$), but ginseng administration significantly reversed the stress-induced increase in plasma corticosterone by 15% compared to the immobilization-stressed group. The present study suggests that the anti-stress effect of ginseng is mediated by normalization of stress-induced changes in the circulating hormones and a reversal of tissue weight loss, thereby returning the body to normal homeostasis.

Key words: ginseng treatment, immobilization stress, body weight, blood corticosterone

INTRODUCTION

Stress is a stimulus which affects the body in a variety of ways, and the body works to suppress changes which occur as a result, and to restore homeostasis. Stress occurs when one has many kinds of diseases, but it also appears commonly in one's everyday life. Excessive stress, above tolerable limits, can have dangerous effects. The nervous system (1), immune system (2), and digestive system (3) of the body respond to every kind of stimulus, which sometimes leads to high blood pressure, indigestion, fatigue, aching, and roughening of the skin. If the symptoms continue chronically, it can result in the general adaptive symptoms which induce several non-specific diseases such as nervous breakdowns and gastric ulcers. This can be thought as the stage in the body's regulation of homeostasis (4). If stress continues for a long time, the stress causes many reactions through the hypothalamic-pituitary-adrenal axis. Corticotrophin releasing hormone (CRH) is secreted at the hypothalamus and the CRH releases adrenocorticotropic hormone (ACTH) at the pituitary gland. The ACTH stimulates the adrenal gland to secrete cortisol or corticosterone, which is characteristic stress reactions.

Because food influence continuously on the metabolic regulators it has the potential to greatly modify every physiological system. Thus, the existence of protective factors among food for the body is very important for promoting public health. Recently, from the new view of food, the distinct bioactive components in food have been found to regulate the protective system of human body through affecting the circulatory, endocrine, and immune systems and nerve function. If a regulatory action or protective function of a food bioactive compound is found, the identification of a biological response modifier may make possible the development of agents with

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therapeutic efficacy. Recently food research has increasingly focused on analyzing functional properties of foods and their bioactive components (5). It has been reported ginseng, *Ocimum sanctum*, *Tinosposa malabarica*, *Cordyceps sinensis*, *Bacopa monniera*, and melatonin have anti-stress effects (6-9). Furthermore, ursodexycholic acid, which is most commonly used in the treatment of liver disease, is reported to also have anti-stress effects (10).

Ginseng has a long history of use as an important traditional medicine. Recent studies have reported that ginseng increases resistance to stress, decreases blood pressure, benefits the nervous system, and improves energy and nucleic acid metabolism (11-16). Research into the anti-stress effects of ginseng has identified ginseng saponins as the active components involved in the alleviation of mental and physical stress and the normalization of work capacity (17). It has been reported that ginseng reversed the stress-induced repression of lymphocyte synthesis in laboratory rats (18). Breckman and Dardymov (19) suggested that ginseng possesses adaptogenic activity in which the body works to maintain normal function by exerting non-specific resistance to stressful situations.

Ginseng has been demonstrated to diminish heat, cold, and X-ray induced stresses (20). It has been reported that ginseng reduces chemical-induced stress, and increases the lethal dose of drugs such as strychnine or picrotoxin (21). Ginseng has also been shown to increase the resistance to a variety of environmental stresses (22).

As shown above, there are numerous reports of the anti-stress effects of ginseng, but the mechanism action has yet to be elucidated. In the present study, we investigated the anti-stress effects of ginseng against immobilization stress.

MATERIALS AND METHODS

Ginseng pretreatment

Root hair from four years-old ginseng was dried, pulverized, and filtered through a 60 mesh sieve. The composition of ginseng powder was: carbohydrate, 18.6%; protein, 16%; fat, 1.6%; fiber, 16.1%; ash, 7.3%; and water, 2.3%. The ginseng sample plus 1% (w/w) citric acid was pressed out with a Bhuler DNDL-44 extruding machine using the following mechanical conditions: 23 kg ginseng/hour; rotation speed, 315 rpm; temperature, 170°C pressure, 10 bar; and specific mechanical energy consumption, 340 kJ/kg.

Animals and treatment

Sprague-Dawley rats (initial weights, 150 ± 5 g, Charles River, Korea) were housed individually in a temperature (22 ± 2°C), relative humidity (55 ± 5%), and light (dark, 06:00~18:00 h) controlled room. Rats were fed a non-purified diet (Rodent Laboratory Chow, Ralston Purina, St. Louis, MO) for a 7 d stabilization period. Male Sprague-Dawley rats (n=24) were divided into three groups; (i) control group (C), (ii) immobilization-stressed group (S), and (iii) immobilization-stressed plus ginseng group (S+GS). The immobilization-stress was induced via immobilization of animals for 2 hr everyday for 2 weeks. The immobilization-stressed plus ginseng group was given ginseng (200 mg/kg BW) dissolved in water along with immobilization for 2 weeks. Rats were given free access to food and water for 2 weeks, and food intake and body weight gain were monitored twice per week. At the end of the experiment, rats were deprived of food for 12 h and then anesthetized using diethyl ether. A central longitudinal incision was made into the abdominal wall, and blood samples were collected by cardiac puncture. Blood samples were centrifuged at 4°C for 20 min at $1500\times g$, and the serum was separated and stored at 20°C until analyzed. The epididymal adipose tissue, thymus, adrenal, and spleen were dissected, rinsed with saline, and weighed. Care and treatment of experimental animals were in accordance with the guide for the care and use of laboratory animals of Ewha Womans University.

Serum analysis

The plasma total cholesterol level was measured enzymatically using a commercially available kit (Wako, Japan). The plasma GOT and GPT levels were determined using commercially available kit (Yeongdong Pharm. Corp., Korea). The plasma corticosterone level was measured using commercially available RIA kit (Coat-A-Count® TKRC1, Diagnostic Products Corporation, USA).

Statistics

Data are expressed mean±SE. Data for the control, Stress, Stress plus ginseng groups were analyzed by one-way ANOVA; $p\geq0.05$ was taken as indicating no significant difference. Differences among groups were evaluated by Duncan's multiple range test.

RESULTS

Body weight gain, food intake and food efficiency

Random assignment of rats to the three experimental groups resulted in initial body weights that were not different (Table 1). Immobilization stress significantly decreased body weight gain and food intake ($p<0.05$) (Fig. 1). Body weight gain of rats with immobilization

Table 1. Body weight gain, food intake, and food efficiency of immobilization stressed rats fed with or without ginseng

Group[1)]	Initial body weight (g)	Body weight gain (g/2 weeks)	Food intake (g/2 weeks)	Food efficiency[5)]
C	212±10.9[2)NS3)]	93.2±20.4[a4)]	294±22.9[a]	0.32±0.02[a]
S	211±8.2	51.3±18.0[b]	258±29.0[b]	0.20±0.03[b]
S+GS	212±7.2	56.7±11.4[b]	278±19.6[ab]	0.20±0.02[b]

1)C: control, S: immobilization stress, S+GS: immobilization stress plus ginseng.
2)Values are expressed as mean±SE, N=5.
3)NS: Not significant.
4)Values in a column with different superscripts are significantly different, $p<0.05$.
5)Weight gain (g)/Food intake (g).

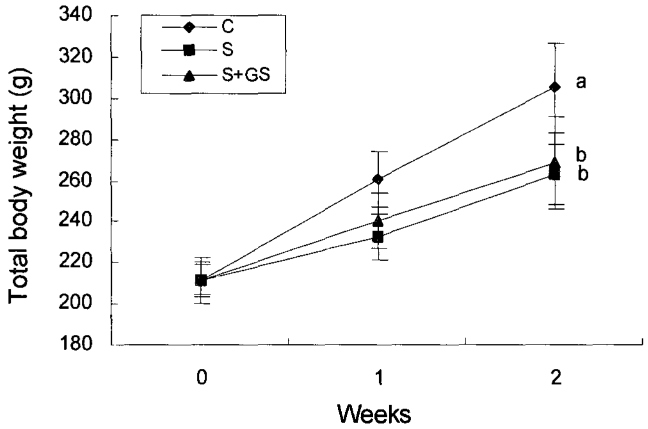

Fig. 1. Effect of ginseng on the body weight of rats with or without immobilization stress.
Each bar represents the mean±SE in each group; control (C), immobilization stress (S), immobilization stress plus ginseng (S+GS). Different superscripts are significantly different at that time point, $p<0.05$.

stress was decreased by 45% with only a 12% decrease in food intake resulting in almost a 38% decrease in the food efficiency ratio. Ginseng administration to immobilization-stressed rats partially revered the decrease in body weight gain and food intake, but not significantly. There was no effect of ginseng administration on food efficiency ratio of immobilization-stressed rats (Table 1).

Tissue weights

The tissue weights of spleen and thymus were significantly lower in the stress group compared to control group by 20% and 21%, respectively ($p<0.05$) (Table 2). The weight of epididymal adipose tissue was significantly decreased in stress group compared to control group by 18% ($p<0.05$). Ginseng administration to immobilization-stressed animals resulted in no significant difference in the epididymal adipose tissue. The ginseng-administered group had a significant reversal in the stress-induced decrease in spleen and thymus weight by 16% and 20%, respectively, compared to immobilization-stressed group ($p<0.05$). The weights of adrenal glands tended to increase in the stress group and to be normalized by ginseng administration, though not significantly (Table 2).

Blood analysis

Rats subjected to immobilization stress for 2 weeks had significantly higher plasma GOT and GPT concentrations by 97% and 79%, respectively ($p<0.05$) (Table 3) than the control group. The plasma GOT and GPT concentrations tended to be lower in animals administered ginseng, though not significantly. There were no differences in blood cholesterol concentrations among the groups (Table 3). The plasma corticosterone concentration was significantly higher in the stressed group by 39% compared to control group ($p<0.05$) (Fig. 2). The ginseng-administered group exhibited a significant reversal in the stress-induced plasma corticosterone concentration, decreasing it by 15% compared to immobilization-stressed group ($p<0.05$).

DISCUSSION

Yan et al. (23), Tache et al. (24) and Lawson et al. (25)

Table 2. Effect of ginseng on the tissue weights of rats with or without immobilization stress

Group[1)]	Spleen	Thymus	Adrenal gland	Epididymal adipose tissue
	(mg/100 g of body weight)			
C	208±25.3[2)a3)]	175±12.4[a]	17.7±4.0[NS4)]	1285±94.9[a]
S	166±17.1[b]	138±16.4[b]	19.5±4.7	1050±122.4[b]
S+GS	192±17.0[a]	167±26.4[a]	18.9±4.0	1053±137.0[b]

1)See the legend of Table 1.
2)Values are expressed as mean±SE, N=5.
3)Values in a column with different superscripts are significantly different, $p<0.05$.
4)NS: Not significant.

Table 3. Effect of ginseng on blood biochemical parameters of rats with or without immobilization stress

Group[1)]	GOT (unit/mL)	GPT (unit/mL)	Cholesterol (mg/dL)
C	66.1±34.9[2)b3)]	14.3±4.2[b]	61.6±12.8[NS4)]
S	129.9±36.1[a]	25.0±5.5[a]	53.5±7.9
S+GS	116.9±31.1[a]	23.6±4.3[a]	57.5±9.8

[1)]See the legend of Table 1.
[2)]Values are expressed as mean±SE, N=5.
[3)]Values in a column with different superscripts are significantly different, $p<0.05$.
[4)]NS: Not significant.

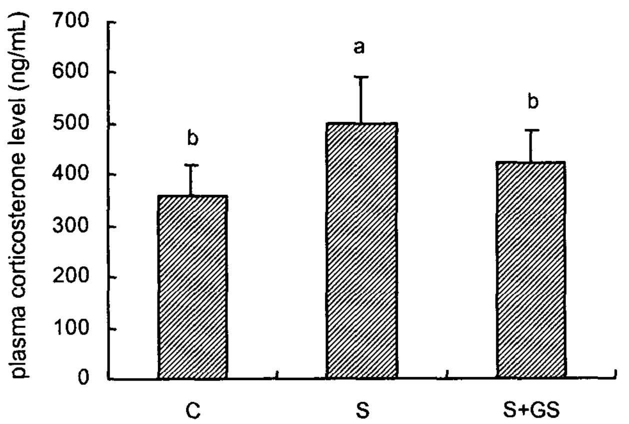

Fig. 2. Effect of ginseng on blood corticosterone levels in rats with or without immobilization stress.
The plasma corticosterone levels were measured as described in Materials and Methods. Each bar represents the mean±SE in each group; control (C), Immobilization stress (S), immobilization stress plus ginseng (S+GS). Values for columns with different superscripts are significantly different, $p<0.05$.

showed that body weight gain and food intake are decreased compared to the control with continuously induced immobilization stress, which was similar to the results of our experiment. The glucocorticoid and catecholamine responses to stress speed up lipolysis from adipose tissue (26). Stress in rats increases the secretion of glucocorticoid from the adrenal gland. The glucocorticoid stimulates the catabolism of protein and degradation of triglyceride from adipose tissue (27). Therefore, the stress- induced weight decrease has been assumed to be due to the decrease in food intake and the increase in catabolism by glucocorticoid and catecholamine. The body weight of white rats treated with the water-soluble extracts of ginseng for four weeks in their feed was apparently higher compared to the weight of the controls. The body weight of white rats treated with the methanol-extracted ginseng started distinctly higher compared to their weight of the control after 30 days (28). Kim (18) reported that the rats fed ginseng had less weight loss compared to the control group, when rats were exposed to the external stress and their recovery rate was much faster than the control group. These results showed a similar tendency to our experimental results. However, treatment with ginseng was not effective in reversing the stress-induced body weight loss.

External stress affects the limbic system which in turn affects the secretion of ACTH from the pituitary gland. ACTH stimulates the adrenal cortex to increase the use of ascorbic acid and synthesis of corticosterone. Corticosterone enlarges the size of the adrenal gland, and also inhibits immune function via the atrophy of thymus and spleen (29). Ricart-Jane et al. (30) reported that the weight of the adrenal gland was increased with chronic stress in the rats. Marti et al. (31) also reported that chronic stress increases ACTH secretion from the pituitary gland, which stimulates the adrenal gland. In this experiment, spleen and thymus weights, which are involved in immune function, were significantly decreased due to stress, but administration of ginseng partially prevented the effect.

The glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) exist commonly in the liver or heart (32). Usually the levels of GOT and GPT are low in the blood, but are increased in case of diseases such as acute hepatitis or myocardial infarction. Those enzymes are released from cells into the blood due to the increased membrane permeability (33). From our results, we can see that the levels of GOT and GPT were increased in the immobilization-stressed group, suggesting that stress may be harmful for the liver function. Although the stress-induced increases of plasma GOT and GPT concentrations were only slightly, and non-significantly, reduced by ginseng administration, a slight normalizing tendency could be observed and it can be postulated that ginseng has a positive effect on the recovery from stress-induced liver damage.

The increased blood corticosterone due to stress influences the fat metabolism and increases blood cholesterol concentration (3). Ricart-Jane et al. (30) reported that when rats were exposed to the chronic stress the blood cholesterol concentration was increased compared to control. However, when acute stress was applied, there was no particular difference. Yan et al. (23) reported that when stress was continuously applied for two months, the blood cholesterol concentration was not changed particularly. Our experiment showed no significant changes in the blood cholesterol levels after immobilization stress exposure.

The function of corticosterone is to increase blood glucose which supplies the energy needed for adaptation against external stress and blood pressure. However, the immunity of the body rendered weak by decreased lymphocytes released from thymus and lymphoid, when the corticosterone is continuously secreted. When stress is over, the body can return to normal. When the stress is loaded chronically, however, then the body loses homeostasis and becomes sick (22-24,33). In our experiment, ginseng administration significantly normalized stress-induced plasma corticosterone increases.

In this study, ginseng administration in the immobilization stress group tended to reverse the body weight loss and decreased food intake, though not significantly.

Ginseng administration resulted in a significant decrease in the spleen and thymus weight in comparison to the immobilization stress alone. The ginseng administration showed a significant reversal in the stress-induced plasma corticosterone level, decreasing it by 15% compared to immobilization stress group. In conclusion, the extracts of ginseng have potent anti-stress activity that is mediated by normalization of stress-induced changes in blood hormones and tissue weight losses, and helps restore homeostasis of the body.

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