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Anti-stress Effects of *Kimchi*

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Abstract The anti-stress effects of *kimchi* were studied in the Sprague-Dawley rats dosed with *kimchi*. The rats in the stress groups were subjected to immobilization stress for 2 hr/day for 5 days. At the end of the experimental period, daily average food intake and body weight (BW) gain had been reduced in the stress group compared to the control group. Daily average food intake was significantly increased in the stress-*kimchi* diet group compared to the stress-only group. The weights of the thymus and spleen were decreased by immobilization stress, but this reduction was partially suppressed by the addition of *kimchi*. The weights of the adrenal gland and epididymal adipose tissue were increased in the stress group, but ingestion of *kimchi* completely and partially suppressed these stress-induced changes, respectively. Serum levels of total cholesterol and triglyceride, and plasma levels of corticosterone were increased in the stress group, but at control levels in the stress-*kimchi* diet group.

Keywords: *kimchi*, immobilization stress, food intake, blood corticosterone

Introduction

Kimchi has been gaining a higher popularity among consumers worldwide due to its health promoting effects (1). In *kimchi*, there are a lot of natural antioxidants such as phenol, phenolic acid, hydroxy cinnamic acid derivatives, flavonoids, and ascorbic acid, which eradicate reactive oxygen species (ROS) or free radicals. It is also known as a relatively strong antioxidant activator, an action that is influenced by the relative amounts of each of the ingredients and the duration of fermentation (2). Lee *et al.* (3) separated the antioxidant 3-(4'-hydroxyl-3',5'-dimethoxyphenyl) propionic acid from the dichloromethane fraction of cabbage *kimchi*, and reported that it can be used to reduce fat in the human body and to suppress the hardening of the arteries. It has revealed that red pepper powder has blood cholesterol-reducing effects in the animal experiments. The ingredient responsible for this effect is capsaicin and dehydrocapsaicin (4), and Phillips *et al.* (5) reported it has a similar vasodilatory effect to acetylcholine. Lactic acid, which is formed while *kimchi* is stored, is known to suppress increases blood cholesterol levels, and to lower the blood pressure (6). It has been reported that rats fed with *kimchi* have lower plasma levels of triglyceride and total cholesterol, and liver lowers levels of triglyceride and cholesterol (7,8).

Animal experiments have also shown that the anticancer action of general fermented milk product was attributable to the suppression of the formation of cancer-causing ingredients as the result of improvements in intestinal microorganisms, and suppression of cancer cell proliferation by activating intestinal immune function (9). If the lactic acid in *kimchi* that is similar to the one in milk products has anti-cancer effects and promotes activation of the immune system, it may have a high potential as a medicinal food, since it is able to react with the phytochemicals in *kimchi*. Stress causes the secretion of hormones in the body, the degree to which hormones are secreted depending upon the extent of and duration of the stress. Acute stress, like fear and anger, increases the circulating level of catecholamines in the serum by up to 1,000 times, while constant and slowly imposed stress increases the secretion of glucocorticoids (GC)(10,11). According to one report (12), adrenalectomy significantly increases the mortality rate of animals subjected to stress, but if animals are given GC before being subjected to that same stress, the mortality rate is reduced. Hormones like cortisol, the catecholamines, and corticosterone, circulating levels of which are increased by stress, increase energy consumption and sometimes cause aging and degenerative diseases as a result of an increase in ROS (13,14). In particular, they reduce the weight of the thymus and spleen, both of which are involved in immune activities, thus causing immune system disorder in the human body (15, 16). In addition, when exposed to stress, drastic changes in carbohydrate metabolism increase the blood sugar level. In spite of this, in extreme stress, triglyceride decomposition increases in fat tissue, resulting in high circulating levels of fat and blood proteins (16-18).

Recent research reports a close relationship between stress and taste (19). When under stress, the intake of carbohydrate and fatty foods increase (19), and we prefer to eat spicy and sweet foods (20,21); the intake of spicy food is particularly high in women with premenstrual dysphoric disorder. Capsaicin, the spicy taste of pepper, is an ingredient that stimulates the trigeminal, glossopharyngeal, and vagus nerves, so as ally-isothiocyanate and diallyl sulfide, which are the constituents of both onion and mustard that give a pungent, sharp, and hot sensations (22). These ingredients were once hypothesized to stimulate the olfactory nerves to cause endogenous opioid-like endorphin secretion to alleviate the pain (23). The aim of this research was to study the anti-stress effects of *kimchi* using animal experiments, a traditional Korean fermented food whose spicy taste is derived from capsaicin.

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Materials and Methods

***Kimchi* preparation** The *kimchi* used in experiments was cabbage *kimchi* (ChongGahJip *kimchi*; Dusan, Seoul, Korea), the ingredients of the entire preparation being cabbages, radish, pepper powder, green onion, garlic, fermented shrimp sauce, and anchovy sauce. This *kimchi* was manufactured in 1.6% salt and vacuum packed with high blocking (PET/Al-foil/PE) film in 500-g packs, and stored for 14 days in a refrigerator before use (Fig.1). The pH of the *kimchi* preparation after 14 days of storage was 4.29 and the total acidity was 0.72 g/L. After the 14 days of storage the *kimchi* was freeze-dried, ground in a blender (MC-880W; Samsung Electronics, Seoul, Korea), and filtered through a 60-mm mesh sieve. The ingredients of *kimchi* after being freeze-dried and ground are shown in Table 1.

Animals and treatment Male Sprague Dawley rats (initial weight, 185±11 g, Charles River, Korea, n=21) were housed individually in a temperature (22±1°C), relative humidity (45±5%), and light-controlled (lights on, 600-1,800 hr) room. They were fed a non-purified diet (Rodent Laboratory Chow, Ralston Purina, St. Louis, MO, USA) for a 7-day stabilization period. The animals were then divided into 3 groups (n=7 each): a control group (C), an immobilization-stressed group (S), and an immobilization-stressed plus *kimchi* group (S+K). The C and S groups were given AIN 93-G diet. The S+K group was given the AIN 93-G diet containing 5%(w/w) powdered *kimchi*. All of the rats were given free access to food and water for 2 weeks. The planning chart for test diet feeding and stress load is shown in Fig. 2.

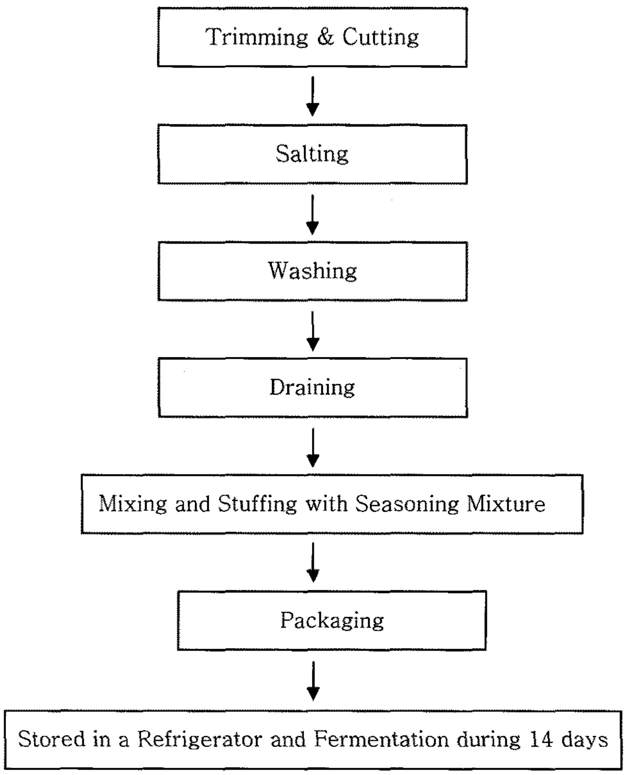

Fig. 1. Preparation of *kimchi*.

Table 1. Composition, dietary fiber, and capsaicin content of the freeze-dried and ground *kimchi* powder

Composition of *kimchi*		Contents[1)]
Moisture (%)		16.03±0.02
Carbohydrate (%)		55.63±0.05
Protein[2)] (%)		1.67±0.01
Crude fat (%)		1.04±0.01
Dietary fiber (%)		23.14±0.01
Organic acid contents (g/g)	Malic	1.617±0.038
	Lactic	21.174±0.488
	Acetic	5.239±0.288
	Citric	2.775±0.066
	Succinic	13.620±0.710
	Fumaric	0.008±0.002
Capsaicin content (g/g)	Capsaicin	34.01±0.02[3)]
	Dihydrocapsaicin	30.12±0.02[3)]

[1)]Mean of 3 replications; Values represent mean±SE.
[2)]N×6.25.
[3)]Mean of 4 replications; Values represent mean±SE.

Food intake was monitored 3 times a week and body weight (BW) gain was monitored once a week. At the end of the experiment, rats were deprived of food for 12 hr and then anesthetized using diethyl ether. The animals that had been subjected to stress were sacrificed 20 min after the final stress (2). A central longitudinal incision was made into the abdominal wall of each of the rats, and blood samples were collected by cardiac puncture. Blood samples were centrifuged at 4°C for 30 min at 1,500×g, and the serum was separated and stored at 20°C for 30 min. The epididymal adipose tissue, thymus, adrenal gland, and spleen were dissected, rinsed with saline, and weighed.

Stress protocol The stress load for the test animals (S and S+K groups) was carried out using a modification of the method of Pitman *et al.* (24). Immobilization stress was used as the stress model. The stress effect was enhanced by administering the stress loads during the night, when rats are active. The stress load involved hanging the S and S+K animals upside down in a stress cage for 2 hr and rats were unable to move in these boxes, thus making them stressed. This protocol was implemented for 5 days (Fig. 2).

Blood analysis Serum levels of triglycerides and total cholesterol were measured enzymatically using a commercially available kit (Shinyang Pharm, Seoul, Korea). Serum

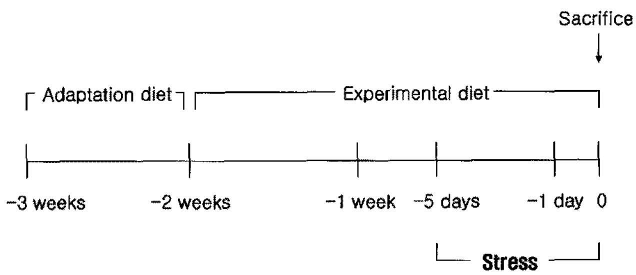

Fig. 2. Experimental design.

glutamic oxaloacetic transaminase (GOT, also known as aspartate aminotransferase) and glutamic pyruvic transaminase (GPT, also known as alanine aminotransferase) levels were determined using commercially available kits. Plasma corticosterone levels were measured using a commercially available ^{125}I rat corticosterone radioimmunoassay kit (Coat-A-Count® TKRC1; Diagnostic Products Corporation, Los Angelis, CA, USA).

Statistics Data are expressed as the mean±standard error (SE). Differences between the control (C) and experimental (S and S+K) data were analyzed by one-way ananlysis of variance (ANOVA). The level of statistical significance was set at $p<0.05$. Where ANOVA revealed a significant difference, differences among groups were evaluated by Duncan's multiple-range tests.

Table 2. Effects of *kimchi* on food intake and body weight (BW) gain of rats with or without immobilization stress

Group[1)]	Food intake (g/day)	BW gain (g/ 2 weeks)	Food efficiency[2)]
C	28.23±1.27[a3)]	94.14±8.26[NS4)]	5.93±0.38[NS]
S	23.76±0.52[b]	74.43±7.07	5.60±0.54
S+K	26.55±0.67[a]	81.86±1.56	5.52±0.14

[1)]C, control; S, immobilization-stress; S+K, immobilization-stress plus *kimchi*.
[2)]Weight gain (g)/food intake (100 g).
[3)]Mean±SE (n=7 rats/group); Values with different letters within a column are significantly different at $p<0.05$ (as assessed by Duncan's multiple-range test).
[4)]Values are not significantly different among the groups at $p<0.05$ (as assessed by Duncan's multiple-range test).

Results and Discussion

Body weight (BW) gain, food intake, and food efficiency Immobilization stress significantly decreased food intake ($p<0.05$). The BW gain of rats in the S group was decreased by 21%, with only a 16% decrease in food intake, resulting in an almost 6% decrease in the food efficiency ratio. The intake of *kimchi* by stressed rats (S+K group) partially and significantly reversed the decrease in food intake ($p<0.05$). *Kimchi* had no effect on BW gain or the food efficiency ratio in immobilization-stressed (S) rats (Table 2).

The findings of the present study regarding the effects of immobilization stress on food intake and BW gain concur with those of Marti *et al.* (25). The effects of stress on GC and catecholamine levels result in a faster rate of lipolysis from adipose tissue (26). Stress in rats increases the secretion of GC from the adrenal gland, which in turn stimulates the catabolism of protein and the degradation of triglyceride in adipose tissue (27). It is assumed, therefore, that the stress-induced weight decrease is attributable to both a reduction in food intake and an increase in catabolism by GC and catecholamines. It has been reported that humans prefer spicy foods when stressed (20,21). Likewise, it is thought that rats prefer spicy foods when under stress, hence the increase in the intake of *kimchi* in the S+K diet group demonstrated here.

Tissue weight The weights of the spleen and thymus were significantly lower in the S group compared to the C group by 13 and 32%, respectively ($p<0.05$; Table 3). The weights of the adrenal gland and epididymal adipose tissue were significantly increased in the S group compared to the C group, by 30 and 27%, respectively ($p<0.05$). The weight of the spleen in the S+K group was 11% higher than that of the S group ($p<0.05$). Although the weight of the thymus in the S+K group was significantly lower than that of the C group, it was also significantly higher than that of the S group, indicating that *kimchi* intake had, at least to some degree, suppressed the effects of stress on this gland. The intake of *kimchi* also appeared to suppress the stress-induced increase in the weight of the adrenal gland, it being 17% lower in the S+K group than in the S group ($p<0.05$) and not significantly different from that in the C group. Similar to the spleen, intake of *kimchi* appeared to reduce, but not completely suppress, the stress-induced increase in the weight of epididymal adipose tissue (Table 3).

The thymus and spleen, utilized here as representative immune organs, shrank in response to stress, in agreement with the findings of Blecha *et al.* (28). The fact that this stress-induced response was suppressed by the intake of *kimchi* suggests a role in immunity for *kimchi* (8,29,30). It has been shown that the weight of the spleen and the number of CD3e+/CD4+-, CD3e+/CD8+-, and B220+-activating cells in the spleen increases in response to the administration of fermented *kimchi* to rats. Chae *et al.* (31) reported that when rats are fed lactic acid (by mouth) for 2 weeks, there is an increase in the intestinal secretion of antibody-producing cells, resulting in an increase in the circulating levels of interleukin (IL)-2, tumor necrosis factor (TNF) α, and cytokines. It has been demonstrated, therefore, that lactic acid activates the immune system. That same research also showed that abdominal administration of the same lactic acid preparation does not exert this effect, and that the antibodies secreted into the intestines have a heightened ability to bond with intestinal microorganisms as well as lactic acid. On the other hand, the work of Kim and Lee (8) demonstrated an increase in B-cell-related immunity that they believed was due to the alterations in the ingredients of *kimchi* affected by ripening. However, in

Table 3. Effects of *kimchi* on the weights of various tissues from rats with or without immobilization stress

Group	Spleen	Thymus	Adrenal gland	Epididymal adipose tissue
	(mg/100 g of BW)			
C	0.1819±0.0039[a1)]	0.0051±0.0007[a]	0.0069±0.0007[b]	1.27±0.07[b]
S	0.1586±0.0068[b]	0.0035±0.0003[b]	0.0090±0.0003[a]	1.61±0.16[a]
S+K	0.1787±0.0081[a]	0.0042±0.0004[ab]	0.0075±0.0002[b]	1.48±0.05[ab]

[1)]Values with different letters within a column are significantly different at $p<0.05$ (as assessed by Duncan's multiple-range test).

Table 4. Effects of *kimchi* on various blood biochemical parameters of rats with or without immobilization stress

Group	GOT (IU/L)	GPT (IU/L)	Total cholesterol (mg/dL)	Triglycerides (mg/dL)
C	94.00±4.96[b1)]	27.14±1.55[NS2)]	43.86±3.94[b]	109.43±15.34[a]
S	121.86±11.60[a]	28.29±4.05	65.14±4.79[a]	62.29±12.60[b]
S+K	99.71±13.77[b]	30.14±2.93	58.14±3.58[a]	76.00±11.73[ab]

[1)]Values with different letters within a column are significantly different at $p<0.05$ (as assessed by Duncan's multiple-range test).
[2)]Values are not significantly different among the groups at $p<0.05$ (as assessed by Duncan's multiple-range test).

the *kimchi* powder used in the experiments reported here, the lactic acid had mostly perished, and so the reported changes in the weights of the thymus and spleen of the test animals, and the putative increased immune ability, are likely to be a result of the metabolic products of lactic acid as well as of lactic acid itself.

In this study it has confirmed the stress-induced increase in the weight of the adrenal gland that has been demonstrated previously (18,32,33). It is known that this hypertrophy in response to immobilization stress stops at the commencement of the stress load, at which point it is influenced by hormones (34).

Kawada *et al.* (35) measured the weights of various tissues from rats administered a high-fat diet containing capsaicin, using perirenal and epididymal adipose tissue as standards, and reported that although the weight of the epididymal adipose tissue was not affected, that of the perirenal adipose tissue decreased. The work of Choo and Shin (36) showed that the administration of capsaicin completely suppresses the 54% increase in body fat that occurs as a result of ingesting a high-fat diet. Sim and Han (37) reported that red pepper's lipolytic effect in fat cells has been observed, and lipolytic activity increases with the degree of piquant taste, implicating that capsaicin is the compound responsible for this activity. In the present study, it was found that the weight of the epididymal adipose tissue in the S+K group was lower than that of the S group; this is thought to be the effect of the capsaicin present in *kimchi*.

Blood analysis Rats subject to immobilization stress for 2 weeks (the S group) had significantly higher serum GOT and total cholesterol concentrations than that of the control group (by 30 and 49%, respectively; $p<0.05$; Table 4). Serum GOT concentrations were significantly decreased in S+K animals compared to S animals. There were no differences in serum GPT concentrations among the groups. Blood cholesterol concentrations tended to be lower in S+K animals compared to S animals, although not significantly so, and were significantly higher than those of the C animals. Blood triglyceride concentrations in the S group were 43% lower than that of the C group. Although intake of *kimchi* reduced this stress-induced reduction, triglyceride levels in the S+K group did not reach control levels.

Plasma corticosterone concentration was 68% higher in the S group compared to the C group ($p<0.05$; Fig. 3). Intake of *kimchi* completely suppressed this stress-induced increase, K+S corticosterone levels being 30% lower than in the S group ($p<0.05$).

Deepak *et al.* (16,33) reported that the secretion of GC increases in response to chronic stress, and that triglyceride-

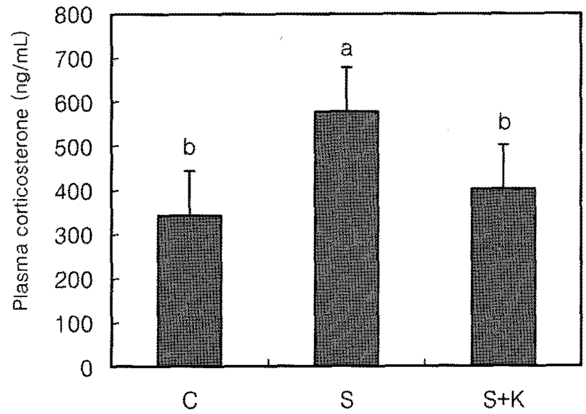

Fig. 3. Effects of *kimchi* on plasma corticosterone concentrations in rats with or without immobilization stress. C, control; S, immobilization-stress; S+K, immobilization-stress plus *kimchi*. Values are means±SE; n=7 for all groups. Bars with different letters are significantly different at $p<0.05$.

decomposing enzymes are activated to reduce the serum triglyceride levels; It has also demonstrated a reduction in the concentration of triglyceride in blood in response to stress. However, Kissebah (38) and Gittleman *et al.* (39) reported that serum triglyceride levels increased in response to short-term exposure to stress. It appears, therefore, that the effect of stress on serum triglyceride levels differs according to the stress model used.

Total cholesterol concentrations increased significantly in response to stress, in agreement with the findings of Kim *et al.* (32) and Deepak *et al.* (33), who showed that total cholesterol concentrations are increased by stress. In addition, it was shown that ingestion of *kimchi* completely suppresses this stress-induced increase. It is likely that the effect of pepper powder, which is a secondary ingredient of *kimchi*, is significant given the finding of Sambiah and Satyanarayna (4) that it has the effect of reducing blood cholesterol levels. In addition, it seems that the capsaicin present in pepper and ginger lowers cholesterol levels by increasing the secretion of mutant bile (40).

GOT and GPT exist commonly in the liver or heart (41). Their blood levels are usually low, but they increase in case of diseases such as acute hepatitis or myocardial infarction. These enzymes are released from cells into the blood as a result of increased membrane permeability (42). The stress-induced increase in plasma GOT activation has been reported previously (32,43). In the present research, the plasma concentration of GOT was increased in response to stress, and administration of *kimchi* seems to have suppressed this increase.

The stress-induced increase in plasma corticosterone concentration concurs with the findings of Inoue *et al.* (44) and Marquez *et al.* (18). The administration of *kimchi* exerted a positive effect, maintaining corticosterone concentrations at controlled levels. In addition, the present research, similar to that of Park *et al.* (45), shows that the increase in plasma corticosterone affects an increase in fat metabolism, resulting in increased blood cholesterol levels.

In conclusion, the intake of *kimchi* suppresses some of the stress-induced changes in parts of the intestines, various glandular tissues, organs, and blood stellate cells in rats. In particular, stress-induced alterations in the weights of the spleen and thymus, and in serum GOT and plasma corticosterone levels, were either partly or completely suppressed by the administration of *kimchi* via the diet.

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