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Screening of Anti-stress Activities in Extracts from Korean Medicinal Herbs

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ABSTRACT : When we carried out an anti-stress study using the extracts of *A. senticosus*, *R. coreanus*, *F. japonica* root, and *A. fruticosa* fruit grown in Korea, the results showed that blood cell counts returned to normal levels fastest with *R. coreanus* after stress application. The total WBC count was decreased in mice due to stress, whereas the number of lymphocytes was increased. The serum glucose level was higher in the control group compared with the comparative stress group. The weight of each organ to returning to normal level was significantly faster in those mice treated with the extracts compared with those in control groups. Especially, it even return to that of normal level with *R. coreanus* extract, suggesting that the administration of the plant extracts in this study would affect hormonal activities in the body to increase immune activities. *F. japonica* root that showed the highest anti-stress activities maybe effective for lipid and carbohydrate metabolism. These results would provide basic data to examine anti-stress effects of natural plants. Further in-depth studies could contribute in the development of functional plant materials with anti-stress activities.

Key words : Medicinal herbs, cold water swimming stress, cold stress, heat stress

INTRODUCTION

Stress is being recognized as the cause of all maladies and many studies are conducted actively on this subject in relation to cardiovascular disease, diabetes and cancer. It is known that psychological stress and physical aspect affect the relationship between stress and immunological functions. This relationship has been revealed through complex interaction among the neurological, endocrine and immological systems. Lee *et al.* (1998) reported that homeostasis and activation of the sympathetic nerve system, and increased blood sugar level, pulse, breathing, blood pressure and blood flow to muscles are the first line of defense to a sudden adverse emotional stimulus such as anger and fear. Being a cause of decreased immune function to cause different diseases, Cohen *et al.* (1996) reported that psychological stress would decrease immune functions when external stress factors affect the CNS and hormonal system or change behaviors. Among those studies that evaluated immune functions using blood samples after measuring psychological stress, Dobbin *et al.* (1991) and Stone *et al.* (1994) reported that activities of immunoglobulins, NK cell, and T cell would differ depending on the intensity and type of psychological stress. Cho *et al.* (2002) reported that chronic psychological stress would increase the number of NK cells and affect T-cell and mitogen. Shephard *et al.* (1995) found deceased function of T-cell and decreased production of interlukine-2 due to decreased immune functions, and increased number of NK cells in depression patients are due to stress. Brenner *et al.* (1997) examined severe to moderate repetitive exercise among different factors causing stress and found increased serum concentrations of catabolic hormones including cortisol, epinephrine, and norepinephrine. Collines *et al.* (1968) reported that this type of exercise could affect the sympathetic nerve system and hypothalamic-pituitary- adreno-cortical axis (HPA axis). Lee *et al.* (1998) found that sudden intense exercise is a bigger factor than mental stress in changing the levels of human T- and B-cell and hormonal concentrations. Furthermore, Demmiere *et al.* (1989) and Piazza *et al.* (1989) discovered that active mice were more resistant to stress since they show arousal to amphetamine-related drugs compared with inactive mice in a voluntary exercise test. As stress factors, researchers measured serum cholesterol and glucose and ALP (alkaline phosphatase) levels (Flegg *et al.*, 1973; Bowers *et al.*, 1966). When corpuscles are exposed to external environment, the total number of lymphocytes is increased, whereas that of neutrophils is decreased (Manser,

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1993). This result suggests that their levels are sensitive indices of stress (Chae *et al.*, 1996). Thus, we measured these levels in the group without stress by analyzing corpuscle in serum. Dallman *et al.* (1977) reported that the adrenal gland affected easily by hormone levels would enlarge with stress but the liver and spleen are also well known as the factors related with stress.

As a dicotyledoneae belonging to the ginseng family (Araliaceae), *Acanthopanax senticosus* is a perennial tree originating from Siberia that grows mainly in Korea (north of Jiri Mt.), Japan, Sakhalin, northeastern China and Ussuri River. It was shown to be effective for dropsy, beriberi, bruise and swelling (Tagasugi *et al.*, 1985; Medon *et al.*, 1984; Todorov *et al.*, 1984). It is also known to be effective for activating functions of different organs (Talbert *et al.*, 1991), promoting metabolism (Rossiter *et al.*, 1996), and delaying fatigue (Asano *et al.*, 1986). Belonging to the rose family, *Rubus coreanus* is grown in wild throughout China, Japan and southern parts of Korea including Jeju Island and mid-part of Korea in the elevations higher than 50~1000 m. It is used as food or herbal medicine. Its fruit is used especially in beverages and as a source of sweet (Jeong *et al.*, 1996). Belongs to the Ginseng family (Araliaceae) like *A. senticosus*, *Fatsia japonica* is a evergreen tree that grows in colonies in wild centering around in valleys in beaches of Guje Island and Namhae Island in Bijin Island and Nae Island of Tongyoung city (Lee *et al.*, 1991). It is usually grown as a pet plant and a alternative medicine for joint disease, cough and phlegm (Tadashi *et al.*, 1976). Originally from North American region, *Amorpha fruticosa* was first introduced to Korea around the 1930's. It has small leaves and grows up to 3 m. It has strong germinating force and is strong against environment and salt (Lee *et al.*, 2003).

Nonetheless, there have been not many studies on these 3 herbal plants related to their anti-stress activities. Thus, we screened the conditions of imposing stress and stress indices to compare anti-stress activities of these plants by comparing *A. senticosus* and *R. coreanus*, which are known to have health-promoting effects, with *F. japonica* and *A. fruticosa*, in which their physical activities are not much studied.

MATERIALS AND METHODS

Materials

A. senticosus and *R. coreanus* used in this study were purchased from Kyungdong Market in Seoul, Korea. *F. japonica* root was collected from the naturally growing site in Tongyoung city, South Kyungsang Province. *A. fruticosa* fruit was collected from road side of Namhae Highway in Hadong-Kung, South Kyungsang Province.

In a distillation flask with a vertical tube for reflux condensation attached, distillation was done twice for 12 h each time using distilled water (*A. senticosus*, *R. coreanus*) and ethanol (*F. japonica* root) as the distillation solvent at a volume 10 times as the volume of the sample at 85°C (distilled water) and 65°C (ethanol). For *A. fruticosa* (*Amorpha fruticosa*), extraction was carried out by precipitating the sample in methanol at room temperature for 72 h 3 times. Each extract was pressure-

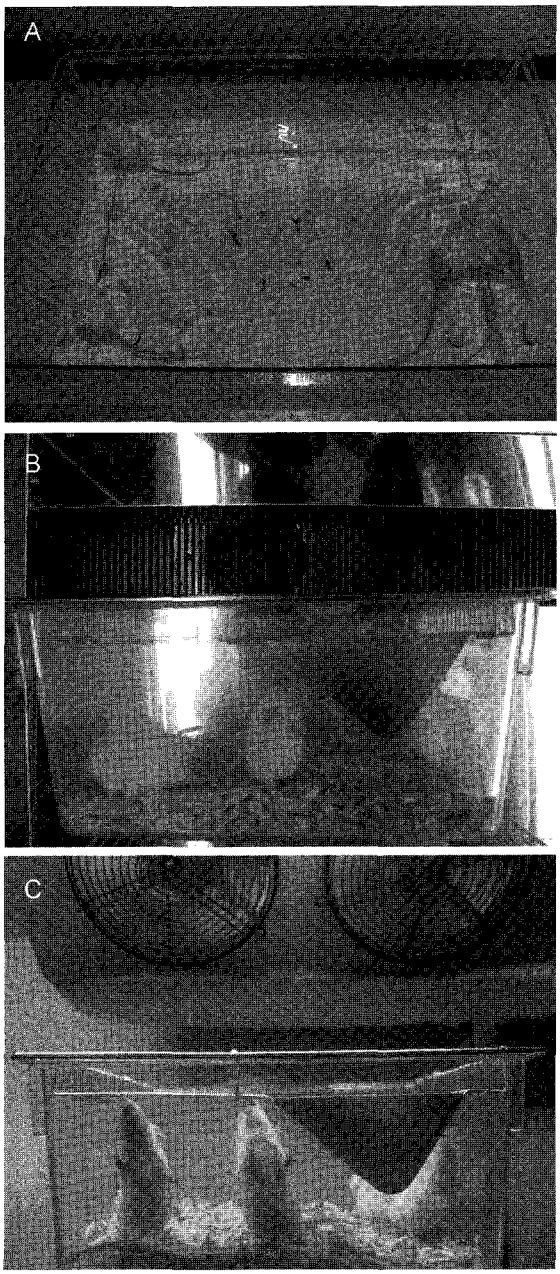

Fig. 1. Apparatus for stress exposure to ICR mice. A: Cold water swimming stress, B: Heat stress, C: Cold stress.

condensed and freeze-dried.

Study animal

The study animal used was 88 5-week-old female ICR mice obtained from the central lab (Seoul Korea). The mice were reared at 22 ± 2°C with relative humidity of 50 ± 5% by keeping the light cycle of 12 h. One-week at the lab was allowed to all animals to adopt to the laboratory conditions. They were freely fed on solid feed (Samyang Fee Co.) and water. Four animals were placed in each polycarbonate cage.

Stress application

As shown in Fig. 1, the mice in cold stress group (CS group) were kept at 4°C for consecutive 4 days. On the first day, they were placed in the cold room for 4 h, the second day for 7 h, the third day for 15 h, and the fourth day for 24 h. The mice in heat stress group (HS group) were kept at 37°C for consecutive 4 days for 45 min each day (Kimura *et al.*, 1996). The mice in cold water swimming stress group (CWSS group) were placed in a 24×20 ㎠ polycarbonate cage containing water for 15 min each day to apply stress (Chae *et al.*, 1996). These mice were given the herbal medicine extract during the 4-day period at a constant interval and allowed to feed freely. By 24 h after giving the last feed, they were sacrificed. Then, blood samples were taken and organs were taken out for the study as shown in Fig. 2.

Blood analysis and weighing of organs

By 24 h after giving the last feed and applying stress, 1 ㎖ of blood sample was collected from the venous plexus behind the eye. It was placed in a micro-test tube treated with EDTA, which was placed in a blood cell counter (Oxford Science Co.) to analyze blood composition. After leaving 1.5 ㎖ of blood sample for 30 min, it was centrifuged at 3,000 rpm for 15 min to separate the supernatant containing serum. The collected serum was placed on DT slides (Johnson & Johnson, Co.) to auto-analyze blood components using a blood chemistry analyzer (DIMEMSION). We examined the levels of alkaline phosphatase (ALP), glucose (GLU) and cholesterol (CHOL) which were reported to be related with stress (Bowers *et al.*, 1966; Flegg *et al.*, 1973).

Then, the mouse was dissected in the abdomen, the spleen was taken out, and fatty tissue surrounding the organs was removed and weighed. The liver and both adrenal glands were taken out and weighed after removing their outer-membranes completely (Brekhman, 1969; Kim *et al.*, 1979).

RESULTS AND DISCUSSION

WBC analysis

The study was done using the freeze-dried extracts of *A. senticosus*, *R. coreanus*, *F. japonica* root, and *A. fruticosa* fruit. As shown in Table 1, the analysis of blood component showed that the best result was shown with *R. coreanus*. As shown in Fig. 3, the number of WBC is decreased in mice exposed to stress. In standard control mice not exposed to stress, the number of WBC was 10.34 per ㎕ of serum. In the control group of CWSS group, the WBC count was 7.94 (K/㎕). According

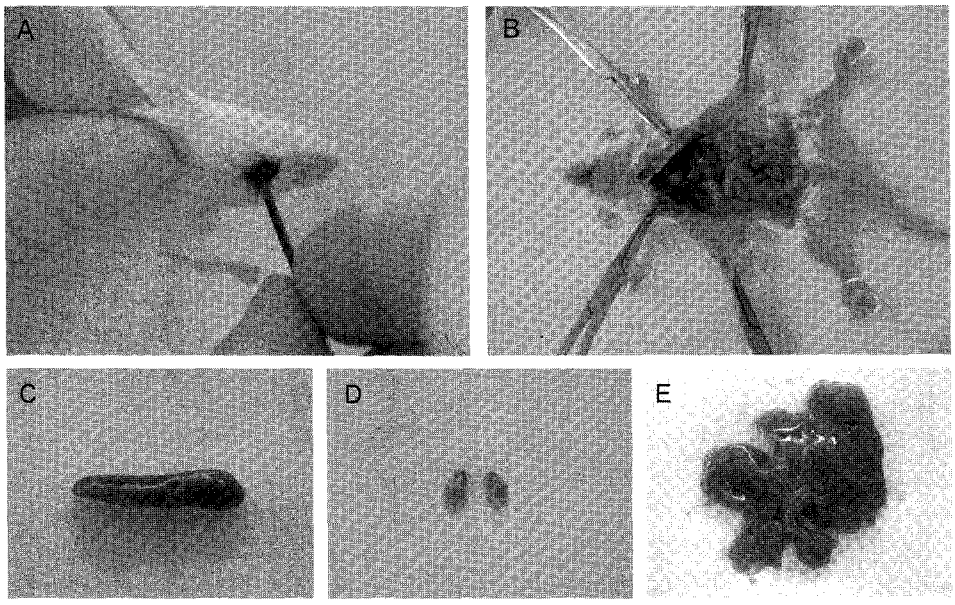

Fig. 2. Pictures of drawing the bloods (A) and macroscopic findings (B,C,D,E) in ICR mice. A: eye-punch, B: mice anatomize, C: spleen D: adrenal, E: liver.

Table 1. Hematological values after exposure to stress groups in ICR mice after feeding the extracts from *Acanthopanax senticosus, Rubus coreanus, Fatsia japonica* of roots and *Amorpha fruticosa* of fruit

Parameters		WBC† (K/μℓ)	NE (K/μℓ)	LY (K/μℓ)	MO (K/μℓ)	EO (K/μℓ)	BA (K/μℓ)
Standard control (no stress)		10.34	1.54	6.77	1.00	0.45	0.40
CWSS‡	No addition	7.94	2.08	4.41	0.93	0.41	0.10
	A. senticosus	8.48	1.98	5.11	0.86	0.43	0.23
	R. coreanus	8.07	1.84	5.05	0.53	0.48	0.31
	F. japonica of roots	8.02	1.74	5.01	0.40	0.42	0.28
	A. fruticosa of fruits	8.24	1.86	5.22	0.48	0.41	0.31
Cold stress	No addition	7.94	2.16	4.41	0.73	0.41	0.10
	A. senticosus	8.18	1.71	4.47	0.94	0.59	0.16
	R. coreanus	7.96	1.86	4.54	0.81	0.58	0.17
	F. japonica of roots	8.06	1.60	4.51	0.79	0.52	0.12
	A. fruticosa of fruits	8.12	1.72	4.62	0.57	0.41	0.13
Heat stress	No addition	8.64	2.98	6.01	0.73	0.28	0.23
	A. senticosus	9.34	2.05	6.37	0.72	0.35	0.33
	R. coreanus	8.82	2.14	7.11	0.92	0.45	0.32
	F. japonica of roots	9.32	2.01	4.51	0.79	0.32	0.22
	A. fruticosa of fruits	9.13	1.84	4.62	0.57	0.41	0.39

†WBC; White Blood Cell, NE; Neutrophile, LY; Lymphocyte, MO; Monocyte, EO; Eosinophile, BA; Basophile.
‡CWSS: cold water swimming stress.

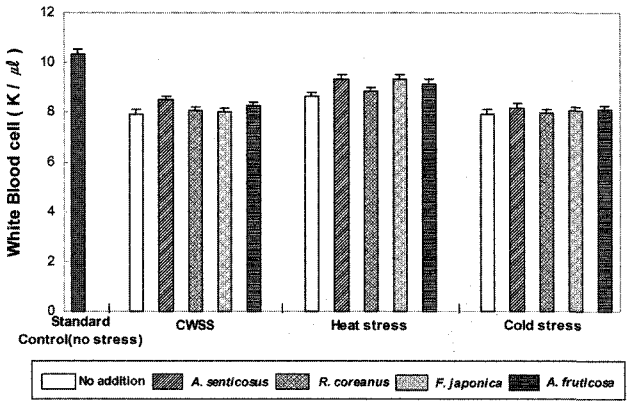

Fig. 3. White Blood Cell values after exposure to each stress groups in ICR mice after feeding the extracts from *Acanthopanax senticosus, Rubus coreanus, Fatsia japonica* of roots and *Amorpha fruticosa* of fruit.

to the extracts used, it was 8.02 (K/μℓ) with *F. japonica* root extract and 8.07 (K/μℓ) with *R. coreanus* extract. It was not significantly different between *F. japonica* and *R. coreanus* extracts. Compared with other extracts, the WBC count returned best to the normal level. In the control group of CS group, the WBC count was 7.94 (K/μℓ). It was 7.96 (K/μℓ) with *R. coreanus* extract, showing the most anti-stress effect in cold stress group. In the control group of HS group, the WBC count was 8.64 (K/μℓ) with the lowest count seen with *R. coreanus* at 8.82 (K/μℓ). The lymphocyte count was also lowest with this extract at 5.01 (K/μℓ). Along with the total WBC count, the neutrophile and lymphocyte counts were decreased in mice exposed to stress as the reversal effect of stress. This result would be sufficient to presume the correlation between stress and immune actions.

Biochemical analysis of Blood

The serum levels of cholesterol and alkaline phosphate (ALP) would increase, whereas the level of glucose would decrease with stress. Table 2 shows the results of the changes in serum cholesterol, glucose and ALP levels in ICR mice with different stress applied. Among the stress control groups, the level of serum cholesterol was 85 mg/dℓ in CWSS group, 104 mg/dℓ in CS group, and 103 mg/dℓ in CS group. However, it was 55 mg/dℓ in the standard control group not applied with stress. Thus, we could tell that the serum cholesterol level would increase with stress. It was also 55 mg/dℓ in mice treated with *F. japonica* root extract extracted with ethanol after cold stress, similar to that in the standard control group. This result suggests that the root extract showed anti-stress activities. Furthermore, it was also significantly changed with all other extracts. As for another stress factor, the level of serum glucose was increased compared with 108 mg/dℓ seen in the standard control group. As in the case of serum choles-

Table 2. The effects of extracts on serum biochemical level in restraint stress induced in ICR mice

	Parameters	CHOL (mg/dℓ)†	GLU (mg/dℓ)	ALP (units/dℓ)
Standard Control (no stress)		55	108	103
CWSS‡	No addition	85	173	154
	A. senticosus	67	170	126
	R. coreanus	97	91	114
	F. japonica of roots	57	138	129
	A. fruticosa of fruits	49	125	107
Heat stress	No addition	104	168	169
	A. senticosus	78	110	132
	R. coreanus	76	123	155
	F. japonica of roots	67	160	129
	A. fruticosa of fruits	61	132	140
Cold stress	No addition	103	165	175
	A. senticosus	81	129	119
	R. coreanus	85	134	161
	F. japonica of roots	55	121	133
	A. fruticosa of fruits	68	138	125

†CHOL; cholesterol, GLU; glucose, ALP; alkaline phophatase, ‡CWSS: cold water swimming stress.

terol level, the level of serum glucose was also increased with stress. With the treatment of different extracts, the level of serum glucose was decreased. We could confirm that the levels of cholesterol and glucose would increase when stress would increase the level of serum corticosteroid, affecting the metabolism of lipid and carbohydrate (Cho *et al.*, 1998). With *R. coreanus* extract that affected glucose level most significantly, the glucose level was most close to that of normal level in HS group at 110 mg/dℓ. The level of ALP was 107 units/dℓ in CWSS group treated with *A. fruticosa* fruit. Different levels were shown in different stress groups according to different factors measured. In HS group, the anti-stress level was relatively high in HS group with A. senticosus treatment. The similar result was shown with A. senticosus in studies that examined anti-fatigue (Davydov *et al.*, 2000) and stress effects (Gaffney *et al.*, 2001).

Results of weighing internal organs

We measured the weight of spleen, liver and adrenal gland related with immunity. The results are shown in Fig. 4~5 and Table 3. The liver and adrenal gland were hypertrophied as the animal was exposed to stress. However, the weight of spleen was decreased (Cho *et al.*, 1995).

Among different organs that we examined, the weight of spleen was decreased due to stress but reached the normal

Table 3. The effects of extracts from *Acanthopanax senticosus, Rubus coreanus, Fatsia japonica* and *Amorpha fruticosa* derivatives on adrenal weight in restraint stress-induced ICR mice.

	CWSS (mg)	Heat stress (mg)	Cold stress (mg)
Standard control (no stress)	7.8	7.8	7.8
A. senticosus	12.3	13.7	14.7
R. coreanus	14.0	14.1	14.5
F. japonica	11.0	10.2	14.2
A. fruticosa	8.2	11.3	14.4
No addition	13.0	15.2	15.1

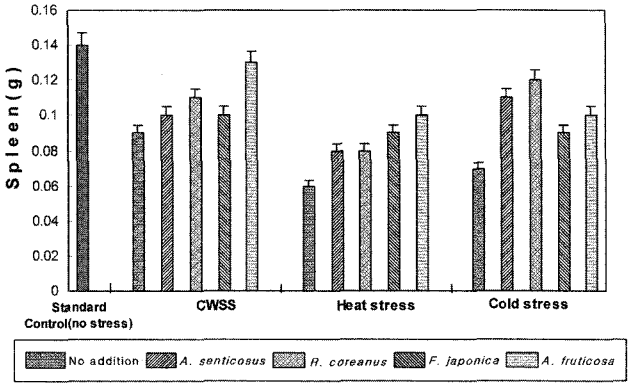

Fig. 4. Change of spleen weight agter exposure to each stress groups in ICR mice after feeding the extracts from *Acanthopanax senticosus, Rubus coreanus, Fatsia japonica* and *Amorpha fruticosa.*

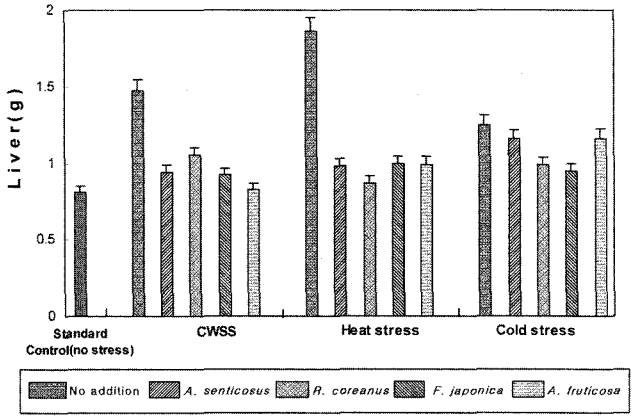

Fig. 5. Change of liver weight after exposure to each stress groups in restraint stress induced ICR mice after feeding the extracts from *Acanthopanax senticosus, Rubus coreanus, Fatsia japonica* and *Amorpha fruticosa.*

weight in mice treated with each extract. Especially, it was similar to the normal weight with the treatment of *R. coreanus* extract in CS group. It was decreased most significantly with hot stress and recovered less with *R. coreanus* extract (0.08 g)

compared with other stress extracts. Unlike the spleen, the adrenal gland was hypertrophied. Wrona *at al.* (2004) reported that the adrenal gland is related with changes in serum hormone levels. We also confirm the hypertrophy of adrenal gland in this study. The adrenal gland was hypertrophied the most in HS group and recovered most effectively with *R. coreanus* extract. Furthermore, among anti-stress results from the liver, the effects of *R. coreanus* extract were the best.

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