FECAL BOLI COUNT, A NEW CRITERIA FOR EVALUATING THE ANTI-STRESS EFFECT OF GINSENG

Y.S. Chang

School of Pharmacy, China Medical College, Taichung, Taiwan, R.O.C.

ABSTRACT

Panax ginseng has been reported to protect animals or to help them recover from physical, chemical, or biological stress. The antistress effects of ginseng were evaluated through the measurement of adrenal ascorbic acid, rectal temperature, and plasma level of glucose, lipids and corticosterone.

During the treadmill experiments of the antifatigue study, the groups of rats receiving *P. ginseng* or *P. quinquefolius* extracts were consistently found to leave fewer fecal boli on the wheel compared with controls. This phenomenon may be due to the reported antistress effects of ginseng. Another possibility could be that the *Panax* species examined produced anticholinergic effects which in turn inhibited the production of fecal boli. After an anticholinergic study, employing physostigmine and atropine as controls, anticholinergic effect was found not essential for the decrease of fecal boli number left on the wheels during antifatigue studies. The results were consistent with the antistress activity reported previously.

Even though the active constituents responsible for the antistress effects of ginseng remained to be determined, the fecal boli counts for stressed rats can be employed as a new protocol for evaluating the antistress effects of ginseng.

Key Words : Antistress ; Fecal boli count ; *Panax ginseng* ; *Panax quinquefolius*

INTRODUCTION

Stress is defined as any adverse stimulus, physical, mental, or emotional, internal or external that tends to disturb the organism's homeostasis[1]. *P. ginseng* has been reported to protect, or help animals recover, from physical, chemical, or biological stress[2].

Ginseng has been proved to exhibited antistress effects on various animal stress models such as high and low temperature[3], x-ray radiation[4], forced exercise[5,6], stress by hanging[7], Chemical stress[8] and biological stress[9].

During our initial antifatigue studies of ginseng using treadmill model[10], we found that the groups of rats receiving most doses of the *P. ginseng* and *P. quinquefolius* extracts were observed to excrete fewer fecal boli in the wheel chamber compared with the saline controls. This phenomenon might represent the reputed antistress effect of ginseng and lead us to further investigate the antistress effects of ginseng.

MATERIALS AND METHODS

1. Collection of *Panax* species

The roots of *P. quinquefolius* were provided by Kirkwood Associates, Chicago, IL., the roots of *P. ginseng* were provided by Prince of Peace Enterprises, San Francisco, California, U.S.A.

2. Preparation of ginseng extracts

Total ginsenoside extracts (saponin fraction) were obtained following a reported procedure[11]. One kg each of the dried, pulverized roots of *P. ginseng* amd *P. quinquefolius* were macerated with 2L methanol overnight, three times. The methanol extracts were combined and evaporated to dryness *in vacuo* on a rotavapor. The dried residue were redissolved in 800 ml water and partitioned with 800 ml water-saturated 1-butanol, three times. The 1-butanol fractions were combined and evaporated to dryness *in vacuo* on a rotavapor. The 1-butanol extracts of *P. ginseng* (KGB) and *P. quinquefolius* (AGB) were subjected to pharmacological studies.

The total water extracts of *P. ginseng* (KGW) and *P. quinquefolius* (AGW) were prepared by macerating the dried pulverized root powder with water overnight, three times ; the water extracts were combined and lyophilized.

In order to determine which constituents were responsible for the antistress activity, an additional 1 kg each of the dried pulverized roots of *P. ginseng* and *P. quinquefolius* was extracted and fractionated following the scheme shown in Figure 1. The lyophilized total water extracts were redissolved in 1 L water and poured into 4 L 95% ethanol to precipitate the polysaccharide fractions. The precipitates were redissolved in water, lyophilized, designated KGP and AGP, respectively, and stored in a desiccator in a cold room (5℃) for later pharmacological studies.

The aqueous ethanol fractions were evaporated on a rotavapor *in vacuo* to remove the ethanol. The ethanol-free water solutions (c.a. 800ml) were each partitioned 4 times against 500 ml water-saturated 1-butanol. The 1-butanol fractions were combined and evaporated close to dryness *in vacuo* ; the residues were redissolved in water and lyophilized. These fractions represent the total ginsenosides of the roots and were found by HPLC to have a composition similar to those of the extracts obtained previously.

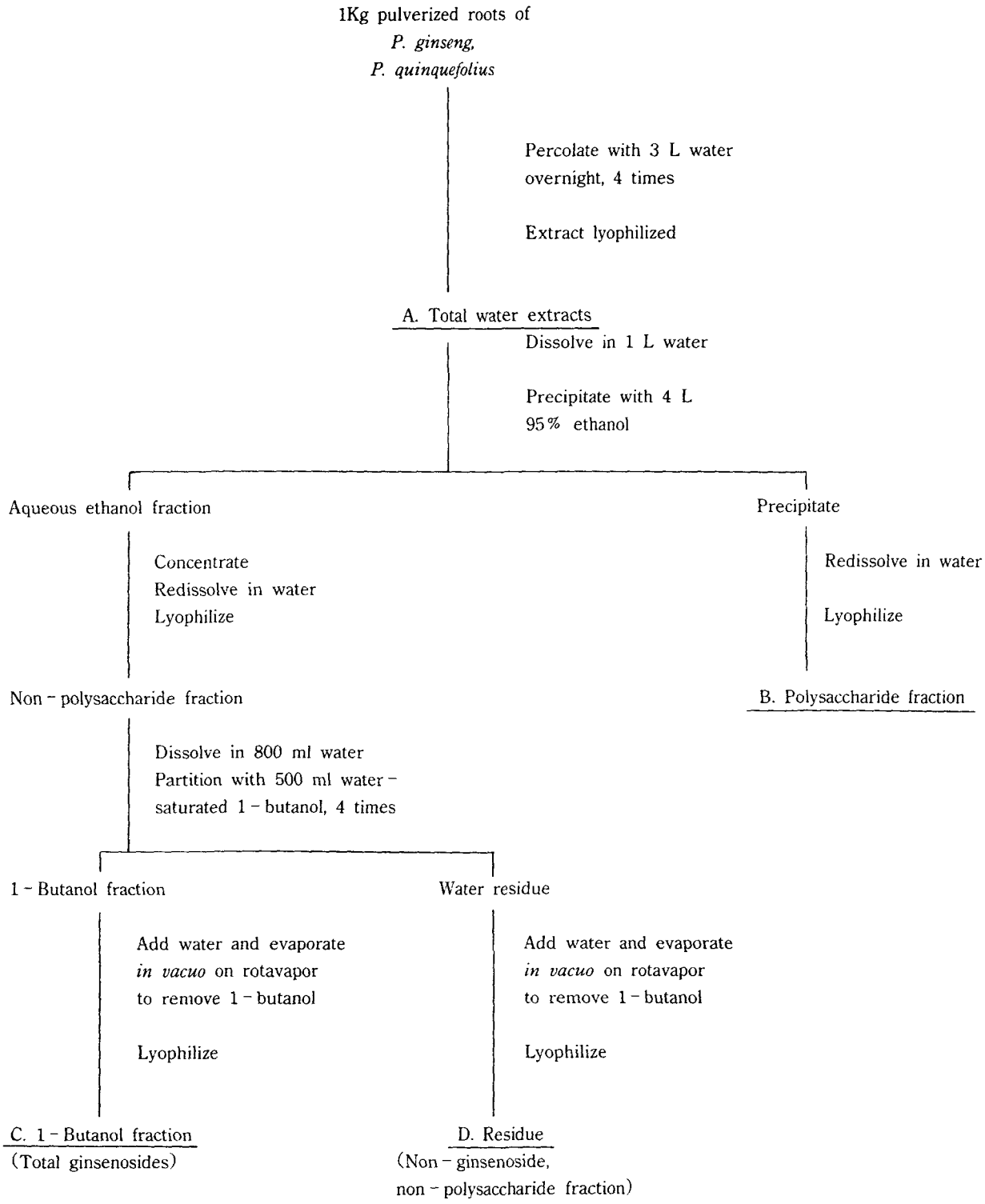

Figure 1. Fractionation scheme of *Panax* species.

The remaining water residues representing the non - ginsenoside, non - polysaccharide fractions of the roots were lyophilized, designated KGX and AGX, respectively, and stored in a desiccator in a cold room (5℃) for pharmacological studies.

Ginsenosides Rg_1, Rb_1 Rd and Re were isolated and purified from 1 - butanol fraction[11] and identified by comparing their physical and spectral data with that of the authentic samples.

3. Animals

Experiments were carried out with male Sprague - Dawley rats (body weight 200 - 224 g) that were housed in temperature - controlled rooms with a 12 - hr light/dark cycle (lights on 7 a.m. to 7 p.m.), and allowed free access to water and food.

4. Fecal Boli Count on Treadmill

An Omni - Stress Modified Activity Wheel, Model XP (Omnitech Electronics, Inc. ; Columbus, Ohio) was used for evaluating the antifatigue activity of the ginseng extracts and pure compounds. Thirty minutes after i.p. administration of the drugs, the rats were placed individually into the enclosed wheels (18" diameter, 44" circumference, and 3.5" width) and the door closed and latched. The wheels were then placed on the base unit. The motor was set to run at the maximum speed of 15 mpm(meters per minute) with a 5 - minute accelerating time to reach that speed. The rats ran against the movement of the wheel until they failed to keep pace. The time up to the point at which the rats failed to pass the center line of the wheels was recorded as the fatigue time. All rats were kept on the running machine until the 20 - minute preset interval ended. Rats were then unloaded from the wheels.

At the end of each experiment, the number of fecal boli left by the rats were counted and recorded. The wheels were cleaned before starting the next experiment.

5. Anticholinergic effects of 1 - butanol and water extracts of *P. ginseng* and *P. quinquefolius*

During the antifatigue study, groups of rats receiving most doses of the *P. ginseng* and *P. quinquefolius* extracts were observed to excrete fewer fecal boli in the wheel chamber compared with the saline controls (Table I). This phenomenon might represent the reputed antistress effect of ginseng. Another possibility might be that the *Panax* species tested produce anticholinergic activity which in turn inhibited the production of fecal boli. In an effort to rule out the latter possibility, the following experiments were designed.

The three doses of each ginseng extract used in the antifatigue study (KGB, AGB, KGW, and AGW) were administered to rats to test their effects on fecal excretion that had been stimulated by the administration of a cholinergic agonist, physostigmine. The anticholinergic agent, atropine, was used as a positive control, and caffeine was tested as well. Each substance, including saline, was tested both with and without the administration of physostigmine.

Forty - eight rats were used for this study. Six groups, consisting of eight rats each, were initially injected i.p. with saline, one of three doses (100, 200, and 400 mg/kg) of a ginseng extract, atropine (0.5 mg/kg), or caffeine (10 mg/kg) ; 30 minutes later, four rats from each group received an i.p. injection of saline (subgroup a), while the other four in each group received the cholinergic challenge with physostigmine (0.3 mg/kg i.p.) (subgroup b). The rats were then placed in empty cages without bedding, one rat per cage, and observed for five hours, for the

Table I. Summary of antifatigue and antistress effects of 1 - butanol and water extracts of *P. ginseng* and *P. quinquefolius*

Group No.	Drugs	Dose (mg/kg)	No.of Rats	Fatigue time(min) Mean± S.E.M.	Value[a]	No.of Fecal Boli Mean± S.E.M.	Value[a]
1	Saline	-	125[b]	7.56 ± 0.26		4.23 ± 0.17	
2	AGB Extract	100	10	8.55 ± 1.03		2.20 ± 0.59	<0.01
3	AGB Extract	200	10	10.85 ± 1.59	<0.02	0.70 ± 0.33	<0.001
4	AGB Extract	400	10	10.25 ± 0.94	<0.04	0.40 ± 0.22	<0.001
5	AGW Extract	100	10	9.90 ± 1.30		3.00 ± 0.54	<0.04
6	AGW Extract	200	9	12.22 ± 1.42	<0.001	2.22 ± 0.81	<0.002
7	AGW Extract	400	10	12.05 ± 1.56	<0.001	2.20 ± 0.49	<0.001
8	KGB Extract	100	9	8.61 ± 1.26		1.33 ± 0.41	<0.001
9	KGB Extract	200	10	9.30 ± 1.55		0.40 ± 0.15	<0.001
10	KGB Extract	400	9	9.78 ± 1.70		0.22 ± 0.54	<0.001
11	KGW Extract	100	10	6.00 ± 0.91		4.00 ± 0.93	
12	KGW Extract	200	9	9.43 ± 1.57		2.56 ± 0.41	<0.006
13	KGW Extract	400	10	13.08 ± 1.82	<0.001	2.40 ± 0.54	<0.002
14	Caffeine	10	133[b]	11.23 ± 0.40	<0.001	4.54 ± 0.15	

[a] Level of significance compared with saline vehicle control. Only p values <0.05 are listed.
[b] The results for saline and caffeine controls are totaled for the entire antifatigue/antistress study.

Table II. Summary of the anticholinergic effects of 1-butanol and water extracts of *P. ginseng* and *P. quinquefolius*

Group No.	Drug	Challenge Drug	No of Observations	No of Fecal Boli at 1 hr Mean± S.E.M.	*p* Value	No of Fecal Boli at 5 hr Mean± S.E.M.	*p* Value
1a	Saline	Saline	32	0.23± 0.09		1.03± 0.29	
1b	Saline	Physostigmine	32	2.31± 0.41	<0.001[a]	3.75± 0.52	<0.001[a]
2a	Atropine	Saline	32	0.00± 0.00		1.38± 0.38	
2b	Atropine	Physostigmine	32	0.35± 0.19	<0.001[b]	1.57± 0.47	<0.005[b]
3a	Caffeine	Saline	32	0.84± 0.26		3.94± 0.67	<0.001[a]
3b	Caffeine	Physostigmine	32	2.73± 0.51		6.09± 0.47	<0.001[b]
4a	KGB(100 mg/kg)	Saline	8	0.00± 0.00		0.88± 0.58	
4b	KGB(100 mg/kg)	Physostigmine	8	0.88± 0.40	<0.03[b]	0.88± 0.40	<0.01[b]
5a	AGB(100 mg/kg)	Saline	8	0.00± 0.00		1.38± 0.63	
5b	AGB(100 mg/kg)	Physostigmine	8	1.38± 0.65		1.38± 0.65	<0.04[b]
6a	KGB(200 mg/kg)	Saline	8	0.13± 0.13		2.25± 1.22	
6b	KGB(200 mg/kg)	Physostigmine	8	1.13± 0.48		2.25± 0.62	
7a	AGB(200 mg/kg)	Saline	8	0.13± 0.13		1.63± 0.91	
7b	AGB(200 mg/kg)	Physostigmine	8	2.00± 0.68		2.63± 0.91	
8a	KGB(400 mg/kg)	Saline	8	0.00± 0.00		2.50± 0.96	
8b	KGB(400 mg/kg)	Physostigmine	8	1.00± 0.38	<0.05[b]	2.88± 1.11	
9a	AGB(400 mg/kg)	Saline	8	0.13± 0.13		0.38± 0.26	
9b	AGB(400 mg/kg)	Physostigmine	8	2.00± 0.60		2.00± 0.60	
10a	KGW(100 mg/kg)	Saline	8	0.13± 0.13		3.38± 1.08	<0.04[a]
10b	KGW(100 mg/kg)	Physostigmine	8	2.38± 0.53		3.38± 0.92	
11a	AGW(100 mg/kg)	Saline	8	0.63± 0.32		2.63± 0.89	
11b	AGW(100 mg/kg)	Physostigmine	8	4.00± 0.91	<0.02[b]	5.00± 0.98	
12a	KGW(200 mg/kg)	Saline	8	0.25± 0.25		4.13± 1.27	<0.01[a]
12b	KGW(200 mg/kg)	Physostigmine	8	3.50± 0.80		6.25± 1.46	<0.03[b]
13a	AGW(200 mg/kg)	Saline	8	0.50± 0.33		2.00± 1.25	
13b	AGW(200 mg/kg)	Physostigmine	8	1.38± 0.75		3.88± 1.33	
14a	KGW(400 mg/kg)	Saline	8	0.00± 0.00		3.25± 0.80	<0.05[a]
14b	KGW(400 mg/kg)	Physostigmine	8	3.25± 1.18		8.75± 1.42	<0.001[b]
15a	AGW(400 mg/kg)	Saline	8	0.25± 0.25		2.50± 0.85	
15b	AGW(400 mg/kg)	Physostigmine	8	1.00± 0.42	<0.05[b]	2.75± 0.88	

[a] Level of significance compared with control group 1a.
[b] Level of significance compared with control group 1b.

production of fecal boli. The number of fecal boli were counted at 30 min, 1 h, 2 h, 3 h, 4 h, and 5 h after the second injection. The rats were then allowed four days to recover before starting the next experiment.

The results are summarized in Table II. Subgroup b of each dose which received physostigmine (0.3 mg/kg) as a challenge drug showed a significant increase in the number of fecal boli compared to subgroup a which received no cholinergic challenge. Atropine, 0.5 mg/kg, blocked the increase in fecal boli caused by physostigmine. Of the ginseng extracts tested, only KGB induced a significant decrease in excretion of fecal boli at 1 and 5 hours. KGB and AGW decreased fecal boli number at 1 hour, but not at 5 hours. AGB, 100 mg/kg, decreased fecal boli excretion at 5 hours, but not during the first hour of observation. Additional, higher doses of AGB did not show any decrease in fecal boli. Furthermore, KGW at doses of 100, 200 and 400 mg/kg, and AGW at 100 mg/kg showed a significant increase in fecal boli. Caffeine significantly increased fecal boli at 5 hours.

RESULTS

1. Fecal boli count on treadmill :

As shown in Table I, groups of rats receiving all doses of the various ginseng extracts (except for the lowest dose of KGW) left fewer fecal boli in the wheels compared with the saline controls. This phenomenon may be due to the antistress

effect of ginseng. The group of rats receiving caffeine did not leave fewer fecal boli than did those receiving saline.

After fractionation, polysaccharides and non - ginsenoside, non - polysaccharides fractions and pure ginsenosides Rg_1, Rb_1, Rd and Re were also evaluated for their effects on fecal boli counts on treadmill treated rats. The results were summarized in Table III. and 4. Except for lower doses of AGX, KGP, and KGX, rats receiving all other doses of ginseng extracts showed a significant decrease in fecal boliexcretion compared with saline controls. Higher doses of ginsenosides Rg_1, Rd, and Re all decreased fecal boli excretion. No decrease in fecal boli number was observed in rats receiving ginsenoside Rb_1.

2. Anticholinergic effects of 1 - butanol and water extracts of *P. ginseng* and *P. quinquefolius*

The three doses of each ginseng extract used in the antifatigue study (KGB, AGB, KGW, and AGW) were administrered to rats to test their effects on fecal excretion that had been stimulated by the administration of a cholinergic agonist, physostigmine. The anticholinergic agent, atropine, was used as a posi-

Table III. Summary of antifatigue and antistress effects of polysaccharide and non - ginsenoside, non - polysaccharide farctions of *P. ginseng* and *P. quinquefolius*

Group No.	Drugs	Dose (mg/kg)	No.of Rats	Fatigue time(min) Mean± S.E.M.	P Value[a]	No.of Fecal Boli Mean± S.E.M.	P Value[a]
1	Saline	-	125[b]	7.56 ± 0.26		4.23 ± 0.17	
2	AGP Extract	100	9	6.28 ± 0.47		2.78 ± 0.36	<0.02
3	AGP Extract	200	9	7.39 ± 0.79		2.89 ± 0.51	<0.03
4	AGP Extract	400	9	8.39 ± 1.26		2.00 ± 0.55	<0.001
5	AGX Extract	100	9	11.10 ± 1.61	<0.007	3.80 ± 0.74	
6	AGX Extract	200	9	12.45 ± 1.60	<0.001	2.60 ± 0.76	<0.006
7	AGX Extract	400	9	13.55 ± 1.56	<0.001	1.10 ± 0.41	<0.001
8	KGP Extract	100	9	6.28 ± 0.69		4.00 ± 0.50	
9	KGP Extract	200	9	6.00 ± 0.53		2.89 ± 0.51	<0.03
10	KGP Extract	400	9	6.17 ± 0.51		2.67 ± 0.55	<0.01
11	KGX Extract	100	9	6.22 ± 0.39		4.00 ± 0.37	
12	KGX Extract	200	9	6.44 ± 0.59		3.00 ± 0.41	<0.05
13	KGX Extract	400	9	12.17 ± 1.74	<0.001	2.78 ± 0.49	<0.02
14	Caffeine	10	133[b]	11.23 ± 0.40	<0.001	4.54 ± 0.15	

[a] Level of significance compared with saline vehicle control. Only p values <0.05 are listed.
[b] The results for saline and caffeine controls are totaled for the entire antifatigue/antistress study.

Table IV. Summary of antifatigue and antistress effects of ginsenosides Rg_1, Rb_1, Re, and Rd

Group No.	Drugs	Dose (mg/kg)	No.of Rats	Fatigue time(min) Mean± S.E.M.	P Value[a]	No.of Fecal Boli Mean± S.E.M.	P Value
1	Saline	-	125[c]	7.56 ± 0.26		4.23 ± 0.17	
2	Ginsenoside Rg_1	10	9	7.61 ± 1.26		2.89 ± 0.63	<0.04[a]
3	Ginsenoside Rg_1	30	9	7.33 ± 0.82		3.00 ± 0.55	
4	Ginsenoside Rg_1	100	9	6.78 ± 0.70		2.67 ± 0.55	<0.02[a]
5	Ginsenoside Rb_1	10	9	6.50 ± 0.55		3.22 ± 0.85	
6	Ginsenoside Rb_1	30	9	6.94 ± 0.57		4.67 ± 0.47	
7	Ginsenoside Rb_1	100	9	6.94 ± 1.03		3.33 ± 0.53	
8	Ginsenoside Rd	10	9	7.17 ± 0.57		4.44 ± 0.96	
9	Ginsenoside Rd	30	10	8.11 ± 1.16		2.44 ± 1.00	
10	Ginsenoside Rd	100	9	8.94 ± 1.44		1.00 ± 0.29	<0.002[b]
11	Ginsenoside Re	10	10	8.40 ± 1.48		1.70 ± 0.56	<0.02[b]
12	Ginsenoside Re	30	10	6.95 ± 0.53		2.90 ± 0.59	
13	Ginsenoside Re	100	10	6.15 ± 0.65		1.80 ± 0.39	<0.02[b]
14	PVP	200	19	8.03 ± 0.72		3.63 ± 0.45	
15	Caffeine	10	133[c]	11.23 ± 0.40	<0.001[a]	4.54 ± 0.15	

[a] Level of significance compared with saline vehicle control. Only p values <0.05 are listed.
[b] Level of significance compared with PVP control.
[c] The results for saline and caffeine controls are totaled for the entire antifatigue/antistress study.

tive control, and caffeine was tested as well. Each substance, including saline, was tested both with and without the administration of physostigmine.

The results are summarized in Table II. Subgroup b of each dose which received physostigmine (0.3 ㎎/㎏) as a challenge drug showed a significant increase in the number of fecal boli compared to subgroup a which received no cholinergic challenge. Atropine, 0.5 ㎎/㎏, blocked the increase in fecal boli caused by physostigmine. Of the ginseng extracts tested, only KGB induced a significant decrease in excretion of fecal boli at 1 and 5 hours. KGB and AGW decreased fecal boli number at 1 hour, but not at 5 hours. AGB, 100 ㎎/㎏, decreased fecal boli excretion at 5 hours, but not during the first hour of observation. Additional, higher doses of AGB did not show any decrease in fecal boli. Furthermore, KGW at doses of 100, 200 and 400 ㎎/㎏, and AGW at 100 ㎎/㎏ showed a significant increase in fecal boli. Caffeine significantly increased fecal boli at 5 hours.

DISCUSSION

Stress is defined as any adverse stimulus, physical, mental, or emotional, internal or external that tends to disturb the organism's homeostasis[1]. Many models of stress (foot shock stress, heat plate stress, hanging stress, etc.) have been reported for studying the effect of drugs on animals. Various biochemical parameters, such as ACTH, prolactin, glucose, and free fatty acids, as well as body temperature, have often been measured to indicate the degree of stress[12]. Defecation score, however, has been the most commonly used parameter[12-17], and has been found to have a direct relationship with corticosterone excretion [12].

Panax ginseng has been reported to protect animals or to help them recover from physical, chemical, or biological stress[2-7]. As seen in Table II, groups of rats receiving all doses of the various ginseng extracts, except for the lowest dose of KGW, left fewer fecal boli in the wheels compared with the saline controls, this phenomenon perhaps demonstrating the reputed antistress effect of ginseng ; the group of rats receiving caffeine did not leave fewer fecal boli than did those receiving saline. Another possibility is that these *Panax* species might possess anticholinergic activity which in turn would inhibit the production of fecal boli[18]. To rule out this latter possibility, the anticholinergic effect of ginseng on fecal excretion was evaluated.

As was seen in Table II, pretreatment with atropine, as expected, significantly decreased the production of fecal boli stimulated by physostigmine challenge (2b versus 1b). Although the results following pretreatment with a few doses of the various ginseng extracts also showed a statistically significant difference when compared with the results obtained for subgroup 1b, no dose response effect was seen (see subgroups 4b and 8b versus subgroup 6b), and in some cases (see groups 12 and 14 as well as the caffeine treated group 3), the differences represented significant increase in the production of fecal boli. It thus seems unlikely that an anticholinergic effect was responsible for the decreased number of fecal boli found in the activity wheels after their use by rats treated with the water or 1-butanol extracts of *P. quinquefolius* or *P. ginseng*. This phenomenon, which likewise was seen following treatment of rats with most doses of the polysaccharide and non-ginsenoside, non-polysaccharide fractions obtained from these species (Table III), seems more likely to be a manifestation of the reputed antistress effect of ginseng.

Even though the active constituents responsible for the antistress effects of ginseng remained to be determined, the fecal boli counts for stressed rats can be employed as a new protocol for evaluating the antistress effects of ginseng.

REFERENCES

1. Dorland, W.A.(1981) *Dorland's Illustrated Medical Dictionary.* Saunders Company, 26th edn., Philadelphia, p. 1259.
2. Bae, H.W.(1978) *Korea Ginseng,* Korea Ginseng Research Institute, 2nd edn., Seoul.
3. Bombardelli, E., Cristoni, A., and Lietti, A. : The effect of acute and chronic ginseng saponins treatment on adrenal function : biochemical and pharmacological aspects. In : *Proceedings of the Third International Ginseng Symposium, 1980, Seoul, Korea.* pp. 9-16, Seoul, Korean Ginseng Research Institute, Republic of Korea, 1980.
4. Park, D.L. : Effect of P. ginseng on X-ray irradiation and synergistic study on nitromin. Insam Munhun Teukjip 2 : 55-65, 1964.
5. Brekhman, I.I. and Dardymov, I.V.(1969) Pharmacological investigation of glycosides from ginseng and *Eleutherococcus. Lloydia* 32, 46-51.
6. Takagi, K., Saito, H., and Tsuchiya, M. (1974) Effect of *Panax ginseng* root on spontaneous movement and exercise in mice. *Japanses Journal of Pharmacology* 24, 41-48.
7. Saito, H. (1985) Neuropharmacological studies of *Panax ginseng.* In : H.M. Chang, H.W. Yeung, W.W. Tso and A. Koo (eds), *Advances in Chinese Medicinal Materials Research,* World Scientific, Singapore, pp. 509-518.
8. Choi, Y.C. : Effect of *Panax ginseng* on carbon tetrachloride induced liver injury and X-irradiation damage in rats. Soul Uidae Chapchi 13 : 1-14, 1972.
9. Wang, B., Shu, T., Tuan, W., and Men, S. (1985) *Jen-Shen Research.* Tianjin Scientific Press, Tianjin.
10. Chang, Y.S., Schlemmer, R.F. Jr., Pezzuto, J.M., Fong, H.H.S., Farnsworth, N.R. and I.M. Chang(1990) : Comparative Antifatigue Studies of *Panax ginseng* and *Panax quinquefolius.* The 6th International Congress of Oriental Medicine, Tokyo, Japan. October 19-21.
11. Sanada, S., Kondo, N., Shoji, J., Tanaka, O., and Shibata, S. (1974) Saponins of ginseng. I. Structures of ginsenosides Ro, Rb_1, Rb_2, Rc and Rd. *Chemical and Pharmaceutical Bulletin* 22, 421-428.
12. Gentsch, C., Lichtsteiner, M., Driscoll, P., and Feer, H.(1982) Differential hormonal and physiological reponses to stress

in Roman high - and low - avoidance rats. *Physiology Behavior* 28, 259 - 263.

13. Broadhurst, P.L.(1957) Determination of emotionality in the rat. I. Situational factor. *British Journal of Psychology* 48, 1 - 12.
14. File, S.E. and Vellucci, S.V.(1979) Behavioural and biochemical measures of stress in hooded rats from different sources. *Physiology Behavior* 22, 31 - 35.
15. Hall, C.S.(1934) Emotional behavior in the rats. I. Defecation and urination as measures of individual differences in emotionality. *Journal of Comparative Physiology* 18, 385 - 403.
16. McCarty, R. and Kopin, I.(1979) Stress - induced alterations in plasma catecholamines and behavior of rats : effects of chlorisondamine and bretylium. *Behavior Neural Biology* 27, 249 - 265.
17. McCarty, R. Chiueh, C., and Kopin, I.(1978) Behavioral and cardiovascular responses of spontaneously hypertensive and normotensive rats to inescapable footshock. *Behavior Biology* 22, 405 - 410.
18. Weiner, N.(1980) Atropine, scopolamine, and related antimuscarinic drugs. In : A.G. Gilman L.S. Goodman, and A. Gilman (eds), *The Pharmacological Basis of Therapeutics.*