

EFFECT OF RED GINSENG ON MICE EXPOSED TO VARIOUS STRESSES

Hiroshi Saito and Tiantong Bao*

*Experiment Station for Medicinal Plant Studies Faculty of Pharmaceutical Sciences
University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, 113 Tokyo, Japan*

** Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, China*

ABSTRACT

Effect of water extract of red ginseng (*Panax ginseng* C.A. Meyer) from Nagano prefecture on (1) forced exercise in mice using rope climbing test, (2) extinction of memory in hanging stressed mice and rectal temperature during the exposure to hanging stress, (3) sex and learning behavior of chronic hanging stressed mice, (4) sex cycle in the adult female mice using chronic hanging stress, and (5) motor coordination and one trial passive avoidance response in 40% alcohol administered mice using rotar-rod and step-through tests, were studied. Drugs tested were given orally.

(1) When it was given before the forced exercise, it potentiated the performance of the forced exercise. When it was given just after the forced exercise once a day for 2 weeks, it protected the mice against the reduction of the performance on the forced exercise. (2) When it was given just after the stress once a day for 4 days, it delayed the extinction of passive avoidance response in both step through and step-down tests, and protected the stressed mice against the decrease in rectal temperature slightly. (3) It protected the stressed mice against the decrease of sex behaviour and the increase in the failure of performance of passive avoidance response, and delayed the extinction of passive avoidanc

INTRODUCTION

In Chinese medicine, there are many crude drugs called Bu-Yaw, which are used to replenish vital energy and strengthen body resistance, and which we have no alternative technical terms in modern medicine. *Panax ginseng* which has been utilized for more than 5 thousands of years in China, is classified into a drug, Bu-chi-Yaw, and is now popular in the world as natural medicine. Red ginseng has been thought to be the most effective in ginseng products. Pharmacological properties of ginseng have been reported by a number of investigators, but those of red ginseng, a few. At the beginning of our pharmacological studies of red ginseng, clinical effect of ginseng reported in Chinese medical books gave us suggestions on new screening method to evaluate the effect of red ginseng. Especially, the effect of ginseng which was expected from the oldest Chinese traditional medical book, "Sheng-nung Pentsao Ching", introduced us into behavioural pharmacological studies of red ginseng using stressed animal. Now, we would like to show you our 5 experiments, titled i) Effect of red ginseng on forced exercise in mice, ii) Effect of red ginseng on extinction of passive avoidance response in mice, iii) Effect of red ginseng on sex and learning behaviours, adrenal gland weight and tyrosine hydroxylase activities of adrenal gland and hypothalamic regions in chronic stressed

mice, iv) Effect of red ginseng on sex cycle in chronic stressed female mice, and v) Effect of red ginseng on alcohol-induced abnormal behaviours in mice.

Effect of Red Ginseng on Forced Exercise in Mice

Rope climbing test was conducted by the use of a modification of the apparatus and method by Brekhman et al. In an experiment chamber of the apparatus, an endless rope is arranged so that it run downward at a constant rate of 4m/min, and a mouse was put on the rope. Electric shock was arranged to be delivered to mice when they failed to run up the endless rope at a corresponding speed and touched the floor. Only these mice, which managed to run up the endless rope more than 108 meters in 30 min, were used in the experiment conducted on the following day. Five weeks old ddy-strain male mice were used. The test was designed as follows.

First, mice were given drugs 30 min before the start of 1 hr rope climbing. The length of the rope which the mice had run up was recorded. Red ginseng at a dose of 125mg/kg apparently stimulated the rope climbing performance (Table 1).

Second, red ginseng was given immediately after 1 hr rope climbing exercise and the same procedure was repeated once daily at the same time of the day for the following 2 weeks. When rope climbing trial was repeated once daily for consecutive 2 weeks, rope climbing performance gradually lowered as shown in Fig. 1. When red ginseng was given at doses of 250 and 500mg/kg, it apparently suppressed the decrease in rope climbing performance.

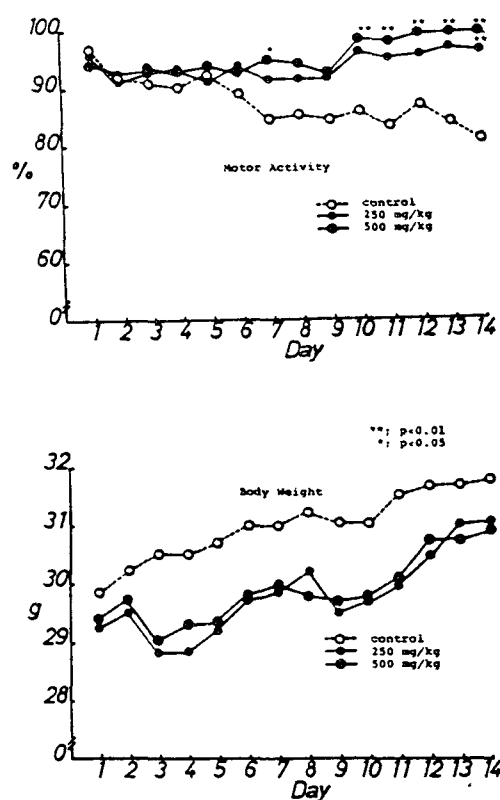


Fig. 1. Effect of red ginseng on rope climbing performance and body weight in the rope climbing test.

Effect of Red Ginseng on Exinction of Passive Avoidance Response in Stressed Mice

Hanging stress method reported by Avakian et al. was used as chronic stressor, and step through and step down tests were also used to observe the effect of red ginseng on extinction of passive avoidance response. The apparatus of step through test comprised an outer brightly illuminated compartment and inner darkened com-

Table 1. Effect of red ginseng on forced exercise in the rope climbing test

Dose (mg/kg)	0	125	250	500
Red Ginseng	70.3 ± 4.8 (10)	86.4 ± 2.6* (10)	81.4 ± 3.2 (10)	83.1 ± 3.8 (10)

Percent exercise of rope climbing for 1 hr is indicated as mean ± S.E.

Figures in parentheses indicate the number of animals tested.

*: Significantly different from control ($p < 0.05$).

Table 2. Effects of red ginseng on weights of organs and tyrosine hydroxylase activities in the stressed mice.

Groups	Body Weight (g)	Weights of Organs(mg)			TH Activity (pmol/hr/ug protein)	
		Adrenal	Testis	Prostate	Adrenal	Hypothalamus
Control	35.4 ± 0.7	7.3 ± 1.1	246 ± 10	10.5 ± 0.8	5.3 ± 0.8	0.19 ± 0.01
Stress Control	35.8 ± 0.7	10.6 ± 0.8*	267 ± 8	12.7 ± 1.0	10.4 ± 1.4**	0.23 ± 0.01**
Stress & Red Ginseng	34.8 ± 0.4	8.8 ± 0.5	263 ± 5	12.0 ± 0.9	13.9 ± 2.2**	0.28 ± 0.03**

** : Significantly different from control ($p < 0.01$) and *; ($p < 0.05$).

partment. The two compartments were partitioned by a wall with a round opening of 3cm in diameter in the middle. A timer connected with apparatus recorded the time elapsed before the mice entered dark compartment. First, a mouse was placed in the brighter compartment with its tail towards the opening. When the mouse stepped into the darkened compartment through the opening, a punishing shock of 36 volt was given to the mouse. The time required by the mouse before it entered the dark compartment (latency) was recorded for each mouse. Testing trial was conducted 24 hrs later, whereby latency and the percentage of mice which stepped through into the dark compartment, were recorded during the 5 min testing trial.

The apparatus of step down test comprised a box with an electric conductive grid floor designed to give 36 volt electric shock on touch. A rubber stand is provided in the centre. Each mouse was individually put on the rubber stand. As soon as mouse stepped down and touched the grid floor, punishing shock was given to the mouse. Then, the mouse was kept in this box for 10 min to complete "learning time. The number of mice which stepped down and the times of "stepping down" during the later half (5min) of the 10 min "learning time", were recorded to evaluate their capacity to learn. Three minutes testing trial was conducted 24 hrs later by the same procedure, and their response was recorded to evaluate their memory capacity. The results were rated by the percentage of mice which showed wrong response and the mean number of "stepping down".

Red ginseng was tested for its effect on

normal learning and memory process and on stress-induced abnormal learning and memory process. Red ginseng was given to mice i) 10 min or 30 min before learning trial, ii) immediately after learning trial, or iii) 10 min or 30 min before testing trial, for the evaluation of its influence on acquisition, consolidation and retrieval of memory process.

Hanging stress was also given to mice once for 1 hr i) 90 min or 60 min before learning trial, ii) immediately after learning trial, or iii) 90 min or 23 hr before testing trial, for the evaluation of its influence on acquisition, consolidation and retrieval of memory, respectively. Red ginseng was given 10 min before the start of stress or just after the end of stress. Stress had no influence on acquisition and consolidation of memory. But stress induced impairment in retrieval of memory significantly, and red ginseng showed the

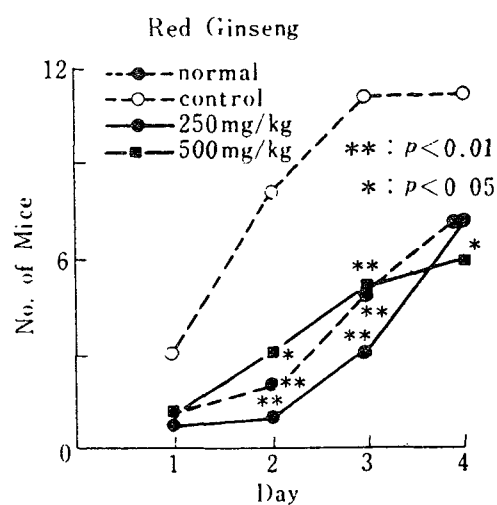


Fig. 2. Effect of red ginseng on extinction of memory in the step through test in the stressed mice.

effect on stress-induced deficit of retrieval of memory slightly. Then, hanging stress was given 1 hr once daily at the same time of day for the following 4 days. Red ginseng was given just after the exposure to stress every day. "Learning trial"s in both step through and step down tests were performed between 2 hr and 1 hr before the start of the first hanging stress. Five minutes "testing trial" in the step through test and 3 minutes "testing trial" in the step down test were performed between 21 hr and 22 hr after the end of stress for 4 days, for the evaluation of its influence on extinction of memory. Fig. 2 shows the effect of red ginseng on extinction of memory in the step through test. Red ginseng at doses of 250 and 500mg/kg, apparently delayed the extinction of learning behaviour. Red ginseng also showed the same effect shown in the step through test in the step down test.

Effect of Red Ginseng on Sex and Learning Behaviours, Adrenal Gland Weight, and Tyrosine Hydroxylase Activities of Adrenal Gland and Hypothalamic Regions in Chronic Stressed Male Mice.

We used the hanging stress method as chronic stressor, and then, we succeeded in introducing a decrease of sex behaviour (licking, mounting and intromission), and increase in failure of learning behaviour (extinction and retrieval of passive avoidance response) in mice. An enlargement of adrenal gland and an increase of tyrosine hydroxylase activities in the adrenal gland and hypothalamic regions in chronic stressed mice, were also recognized 24 hr after the last exposure to hanging stress. On the other hand, no change in various parameter (rectal temperature,

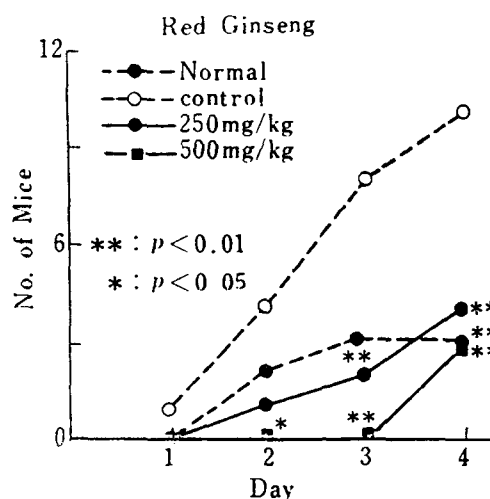


Fig. 3. Effect of red ginseng on extinction of memory in the step down test in the stressed mice.

motor coordination, body tone, grip tone, spontaneous movement and exploratory movement) were seen in mice every 4 hr after the exposure to the stress. Weight of organs (testis and prostate gland), and serum and urine components (blood sugar, total protein, albumin, corticosterone, testosterone and 17-ketosteroids) also did not change in chronic stressed mice 24 hr after the last exposure to hanging stress. The rectal temperature of mice during the exposure to stress were measured at the same time to find preventive effect for fatigue of red ginseng against the decrease of rectal temperature induced by the exposure to stress.

IV-CS strain adult male mice, 9 weeks old, breeding in a private cage, were used throughout these experiments. After a week breeding, 3 estrogenic adult female mice induced by the administration of estradiol benzoate, were put into the

Table 3. Effects of red ginseng on blood glucose, total protein, albumin and inorganic phosphorus in the stressed mice.

Groups	Blood Glucose (mg/dl)	Total Protein (g/dl)	Albumin (g/dl)	Inorganic P. (mg/dl)	A/G (g/dl)
Control	158 ± 11	7.2 ± 0.3	3.5 ± 0.3	8.1 ± 0.6	3.6 ± 0.5
Stress Control	151 ± 10	6.1 ± 0.6	3.6 ± 0.3	6.7 ± 0.6	2.5 ± 0.5
Stress & Red Ginseng	157 ± 12	7.5 ± 0.5	3.5 ± 0.2	8.0 ± 0.8	3.9 ± 0.8

private cage of male mouse for 30 min every 5 days. A group of 10 male mice which succeeded to intromit every day, were used in the experiment. In the opening day, male mouse was put into the cage for 10 min where 10 estrogenic female mice induced by the administration of estradiol benzoate were breeding. The number of mice which showed sex behaviours was observed. From the next day on, we observed sex behaviours with the same conditions at the same time of the day for 2 weeks. The test was performed from 7:30 a.m. to 11:00 a.m.

The acquisition of passive avoidance response was performed in the opening day, using the step down test. From the next day on, the mouse was put on the rubber stand for 3 min every day with the same conditions at the same time of the day for 2 weeks to observe the retrieval of memory. The test was performed from 11:00 a.m. to 12:00 a.m. Mice which stepped down onto the floor were recorded. The number of mice which failed in retrieval of memory once and twice, respectively, were also recorded. After the end of the experiment, the number of days when the mouse stepped down on the floor were recorded as error.

Hanging stress was given at 1:00 p.m. every day for 30 min from the opening day to 3rd day, 60 min-hanging stress, from 4th day to 6th day, 90 min-hanging stress, 7th day to 9th day, 120 min-hanging stress, 10th day. During the exposure

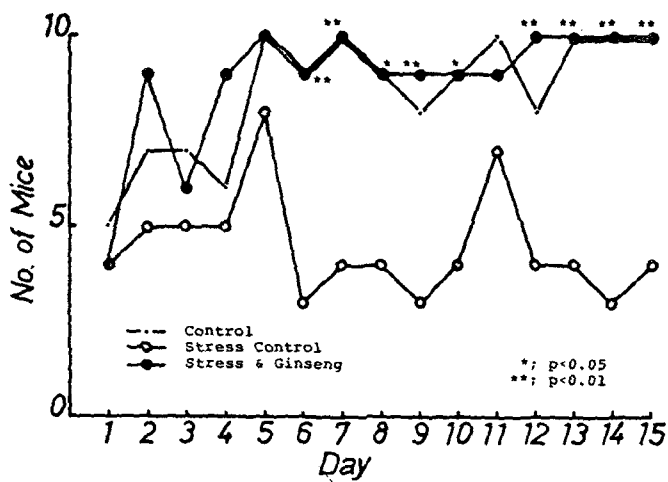


Fig. 4. Effect of Red ginseng on mounting behaviour in the stressed mice.

to stress, rectal temperature of mice were recorded every 30 min. Red ginseng was given every day just after the exposure to stress. Twenty four hr after the last exposure to stress, mice were killed and blood, a pair of adrenal glands and hypothalamic regions were removed for the determination of tyrosine hydroxylase activities and contents of serum components.

Fig. 4 and 5 show the effect of red ginseng on mounting.

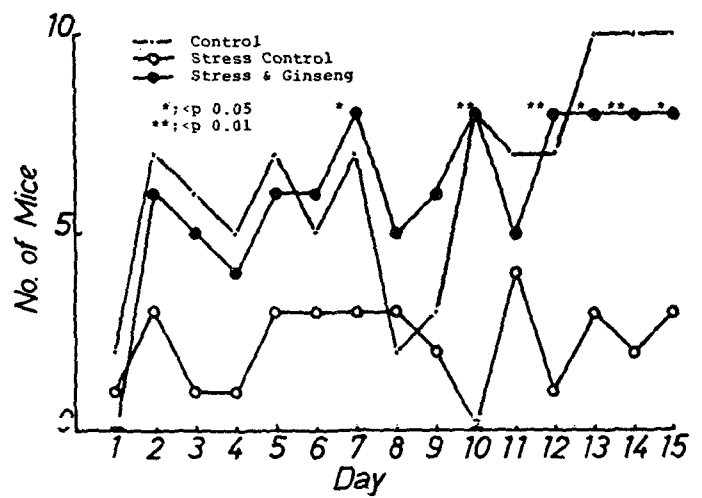


Fig. 5. Effect of red ginseng on intromission behaviour in the stressed mice.

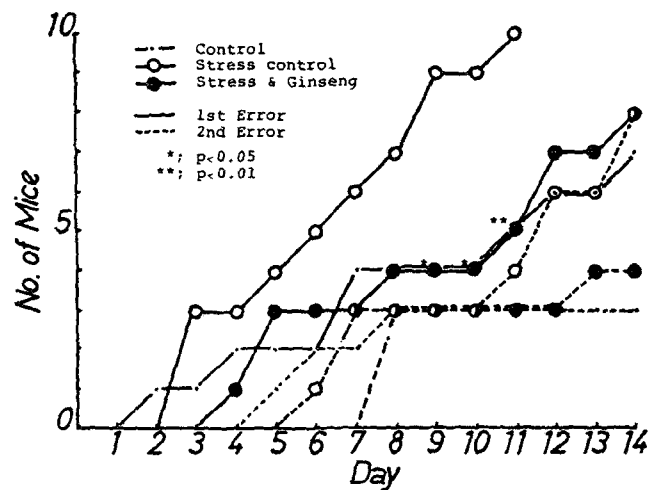


Fig. 6. Effect of red ginseng on extinction of memory in the step down test in the stressed mice.

Effect of Red Ginseng on Sex Cycle in Chronic Stressed Female Mice.

The schedule of hanging stress used was similar to those described in the above experi-

ment. After a week breeding, sear test was performed at 8:00 a.m. every day for 22 days, and after 8th smear test, hanging stress was given to mice at 9:00 a.m. every day for 2 weeks. Red ginseng was given just after the exposure to stress every day; IV-CS strain adult female mice, 9 weeks old, were used in the experiment. Table 4 shows the effect of red ginseng on disturbance of sex cycle by chronic hanging stress in female mice. The number of sex cycles and percent ratios of diestrus, proesterus, esterus, and metesterus days for 8 days before the exposure to stress and the administration of red ginseng, and those 2 weeks during the exposure to stress and the administration of red ginseng, were observed. Chronic hanging stress introduced a decrease of 4 days sex cycles and a decrease of diesterus and esterus days. Significant difference was seen in the number of 4 days sex cycles between stress control group and red ginseng treated group, but there were no differences in percent ratios of diesterus and esterus days between them. That is, red ginseng showed the protective effect against the decrease of normal sex cycle induced by chronic hanging stress.

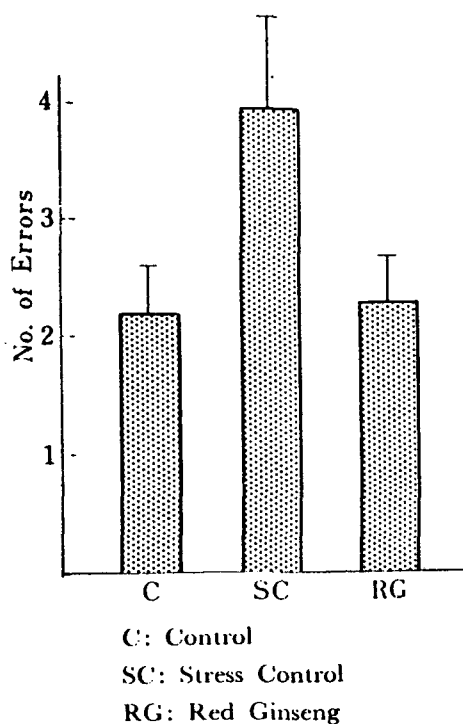


Fig. 7. Effect of red ginseng on performance of memory in the step down test in the stressed mice.

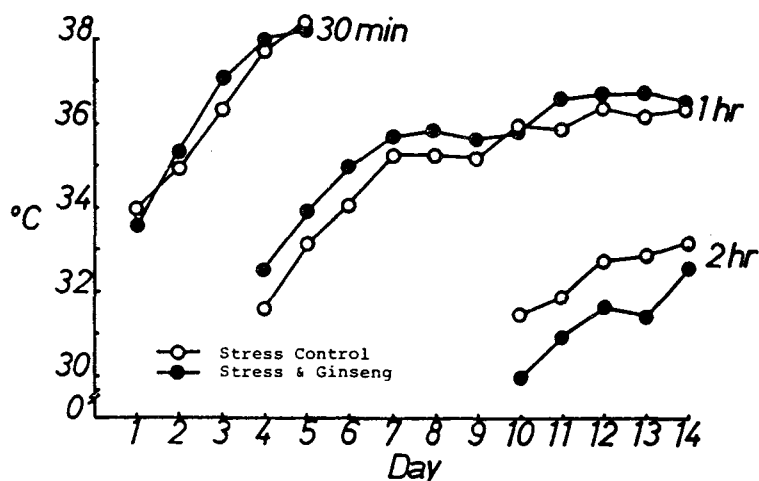


Fig. 8. Effect of red ginseng on rectal temperature in the stressed mice

Fig. 9 shows the effect of red ginseng on rectal temperature during the exposure to stress. Significant differences were seen in rectal temperature between them. That is, red ginseng showed the protective effect against the decrease of body temperature induced by stress.

Effect of Red Ginseng on Alcohol-induced Abnormal Behaviour in Mice.

Fourty % alcohol was given to mice orally at a dose of 0.1ml/10g of body weight in this experiment. Day-strain male mice, 5 weeks old, were used in the experiment. The test was designed as follows.

In the rotating rod test, only those mice which were capable of staying on a rotating rod

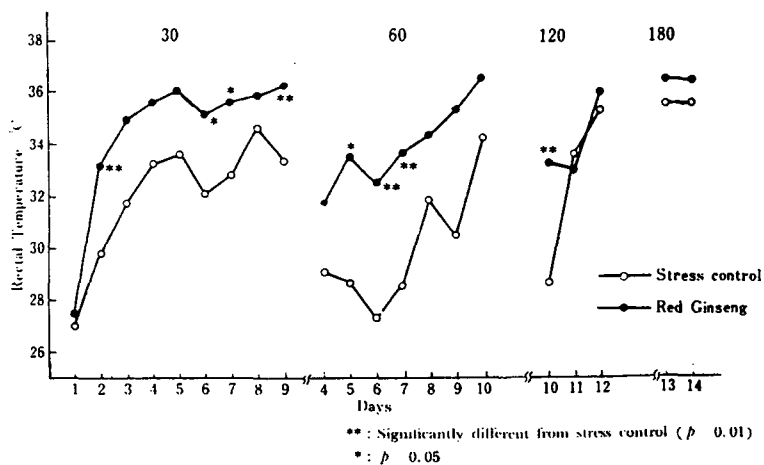


Fig. 9. Effect of red ginseng on rectal temperature in the stressed female mice.

Table 4. Effect of red ginseng on disturbance of sex cycle by chronic hanging stress in female mice before stress for 1 week

Groups	Total Cycles	No. of Cycles			% of Days			
		≤ 3 Days	4 Days	≥ 5 Days	D	P	E	M
Control	17	2(11.8%)	11(64.7%)	4(23.6%)	41	5	48	8
Stress Control	15	2(13.3%)	11(73.3%)	2(13.3%)	40	1	51	8
Stress & Red Ginseng	17	1(5.9%)	12(70.6%)	4(23.6%)	39	4	49	9

During 30 min - 3 hr Stress for 2 Weeks

Groups	Total Cycles	No. of Cycles			% of Days			
		≤ 3 Days	4 Days	≥ 5 Days	D	P	E	M
Control	32	3(9.4%)	25(78.1%)**	5(15.6%)*	43	3	49	6
Stress Control	31	7(22.6%)	11(35.5%)	13(41.9%)	37	11	37	15
Stress & Red Ginseng	27	3(11.15%)	18(66.7%)**	6(22.7%)	32	9	42	17

Red ginseng (500mg/kg p.o.) were given just after stress.
 **: Significantly different from stress control ($p < 0.01$).
 D: Diestrus, P: Proestrus, E: Estrus & M: Metestrus

for 3 min in 2 successive trials, were used. This selection was performed 24 hr before the actual test. Red ginseng and alcohol were simultaneously given to mice 30 min before the test, and effect of red ginseng on alcohol-induced motor incoordination was evaluated for 3 min every 30 min for 3 hr. As shown in Fig. 10, alcohol-induced motor incoordination was observed for almost 3 hrs in mice. Red ginseng at a dose of 500 mg/kg accelerated recovery of motor coordination in alcohol-treated mice significantly.

Table 5 shows the effect of red ginseng on alcohol-induced inhibition of acquisition and retrieval of passive avoidance response in the step through test. The apparatus and method of evaluation used were similar to those described in the above experiment. The test was designed as follows. First, red ginseng was given to mice, and 30 min later, alcohol was given. After another 30 min, learning trial was given to those mice. Second, red ginseng was given to mice 1 hr before testing trial. Thirty minutes later, alcohol was given to mice. After another 30 min, testing trial was given to those mice. When alcohol was given 30 min before the learning trial, "step

through latency" shown by those alcohol-treated mice was shorter than that of normal mice in learning trial, and more mice stepped through the opening into the darkened compartment in the subsequent testing trial. Corresponding results were obtained in testing trial, when alcohol

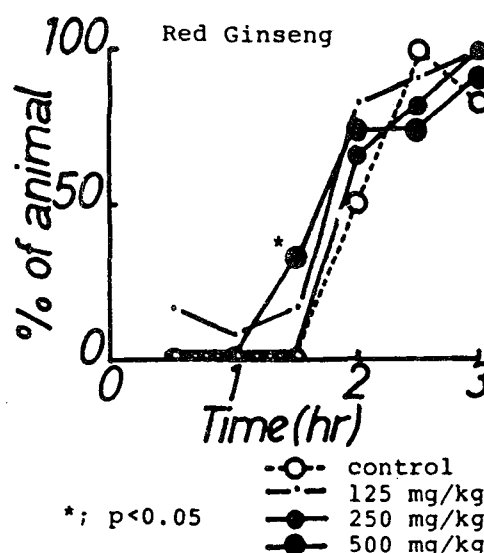


Fig. 10. Effect of red ginseng on motor coordination in the 40% alcohol treated mice in the rotating rod test.

Table 5. Effect of red ginseng on exploratory movement, registration of memory, and retrieval of memory in 40% alcohol-administered mice in step through test.

Compounds	Doses(mg/kg)	No. of Mice	Time ^{a)} (sec.)	(1) Time ^{b)} (sec)	No. of ^{c)} Mice	Time ^{b)} (sec)	(2) No. of ^{c)} Mice
Control		40	14.5 ± 1.3	180	40	176.2 ± 1.0	38
EtOH		40	8.7 ± 1.2**	11.7 ± 0.9**	0**	69.5 ± 12.1**	3
EtOH+Red Ginseng 250		40	9.2 ± 1.5*	29.1 ± 6.3**	1**	132.2 ± 10.3** ###	6
EtOH+Red Ginseng 500		40	8.9 ± 1.1**	21.0 ± 5.7**	1**	138.6 ± 15.1** ###	10

(1): Drugs were given 30 min before the learning trial (Registration of Memory).

(2): Drugs were given 30 min before the testing trial (Retrieval of Memory).

a): Step through latency (sec) in the learning trial (Exploratory Movement).

b): Step through latency (sec) in the testing trial.

c): No of mice which did not step through the opening into the dark compartment in the testing trial.

** : Significantly different from control (P < 0.01) and * : (p < 0.05).

: Significantly different from EtOH (p < 0.01).

was given 30 min before the testing trial. As shown in Table 5, administration of red ginseng 1 hr before testing trials alleviated the effect of alcohol. That is, red ginseng counteracted significantly the alcohol-induced motor incoordination and impairment of the retrieval of passive avoidance response in mice.

Above these results showed that red ginseng had definite effect on stress-affected mice; It prevented decreases in physical and neural strengths induced by physical and chemical stressors, and promoted recovery from affected conditions.

I.P. Lee: I'm interested in your stress application to the female mice with respect to any changes in the estro-cycle. Have you synchronized estro-cycle for those female mice to applied to stress conditions?

Saito: Yes. Behaviors of female and male mice are different. For example, female mice had more adaptability than the male for stress. But as for the learning behavior, male mice learn the passive avoidance behavior more quickly.

홍삼이 스트레스에 노출된 생쥐의 행동에 미치는 영향

Hiroshi Saito and Tiangtong Bao*

Faculty of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan *Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, China

홍삼 추출물이 스트레스에 노출된 생쥐의 행동에 미치는 영향을 다음과 같은 실험을 통하여 연구하였다.

1. 줄타기 테스트와 같은 강제적 운동
2. 매달리기와 같은 스트레스에 노출된 생쥐의 기억력의 감퇴 및 직장 체온의 변화
3. 매달리기와 같은 만성적 스트레스에 노출된 생쥐의 성행동능과 학습행동능
4. 매달리기와 같은 만성적 스트레스에 노출된 성숙한 암컷쥐의 성주기 변화
5. 40%의 알콜을 투여한 후 rotar-rod 및 step-through테스트를 통한 운동자율능과 수동성 시도 기피반응

홍삼의 투여는 경구투여 방법을 택하였다.

1. 강제적 운동을 시키기 전에 인삼을 투여하면, 강제적 운동수행능이 증가되었다. 2주동안 하

루에 1회 강제적 운동을 시킨 후 바로 인삼을 투여하면, 생쥐의 강제적 운동수행능의 감소가 억제되었다.

2. 4일동안 하루에 1회 스트레스에 노출시킨 후 인삼을 경구 투여하면 step-through와 step-down 테스트에 대한 수동성 기피반응의 쇠퇴를 지연시켰으며, 직장온도의 감소를 억제하였다.
3. 인삼투여는 성능력의 감퇴와 수동성 기피반응

수행능의 감퇴를 개선하였고, 수동성 기피반응의 쇠퇴를 지연시켰다. 인삼의 투여는 스트레스에 노출된 후 하루에 한번씩 2주동안 투여하였다.

4. 인삼투여로 스트레스에 의한 성주기의 파괴와 직장온도가 감소되는 것이 방지되었다.
5. 인삼투여로 알콜유발성 운동실조와 기억의 습득 및 회복의 저해가 보호되었다.