EFFECT OF PANAX GINSENG ROOT ON EXHAUSTIVE EXERCISE IN MICE

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Abstract—Effects of extracts obtained from Panax Ginseng root on recovery from exhaustion, were studied using six methods: exploratory movement (EM), hole cross (HC), rotating rod (RR), sliding angle (SA), spring balance (SB) and rectal temp. (RT) tests. Four hr oscillation movements were used as enforced exercise. Drugs were injected i.p. immediately following the exercise. Water extract significantly accelerated the recovery of exploratory movements, increased motor activity index in EM test and elevated rectal temp. However, water extract decreased the index in HC test and grip tone in SB test. Anti-fatigue effects of Ginsenoside Rgl were obvious in every test. Lipophilic fraction significantly speeded up recovery from fatigue in EM, RR, RT and SB tests, but delayed recovery in HC and SA tests. Neutral saponins fraction had no effect on recovery in the 6 tests. In the EM tests, devised to measure two kinds of exploratory movements of fatigued mice and their spontaneous movement, methamphetamine (2 mg/kg), caffeine (25 and 50 mg/kg), 2-dimethylaminoethanol (25 mg/kg) and glucose (100 and 200 mg/kg) significantly speeded up the recovery of these two exploratory movements, and increased the spontaneous movement.

Panax Ginseng root, widely utilized in China, Korea and Japan, is believed to have anti-fatigue and anti-hypothernal activities. It has also been used to develop physical strength after illness. Petkov (1, 2, 3) reported that water-alcohol extracts of Ginseng root produced CNS-stimulant and analeptic activities. Brekhman and Dardymov (4) reported on the adaptogenic activity of Ginseng root and found that panaxosides and their genins produced unusually strong stimulant action. Few reports concerning anti-fatigue activity of Ginseng root have been published. The experiments previously done entailed mice swimming in water (5, 6, 7) and running up an apparently endless rope (8). In our laboratory, pharmacological properties of the extracts from Ginseng root have been studied (9). Slight CNS-stimulant action was found in a crude saponin fraction (GNo. 4) main component of which was Ginsenoside Rgl (GRgl), and lypophilic fraction (GNo. 5). These fractions were presumed to have anti-fatigue activity.

The present research was an attempt to determine a simple pharmacological screening method for anti-fatigue activity and an investigation of the effect of Ginseng root on exhausted animals.

MATERIALS AND METHODS

Preparation of Ginseng root extracts

Panax Ginseng C.A. Meyer from Nagano Prefecture, Japan, was extracted by Shibuta
et al. (10) as described in our previous report (9). Water extracts of Ginseng callus prepared by Furuya et al. (11) were also used.

**Animal treatment and exercise program**

Male mice (ddY-strain), each mouse weighing 20-23 g obtained from Shizuoka Farm Inc., were used for the experiments. They were divided into groups of 10, put in stainless steel cages (14×30×20 cm), and kept in a room with a constant temp. of 22±2°C. Oscillation movement was used as enforced exercise. Details of the oscillation apparatus has been reported by Takagi et al. (12).

**Methods employed**

- **Exploratory movement (EM) test**: The apparatus consisted of a rectangular, three-compartmented box (10×10×3.6 cm) made of transparent, black acryl plates. Both right and left transparent compartments were connected with the middle dark one by a round opening in a black plate 3 cm in diameter. The right hole with a shutter was at a height of 1.5 cm in the centre of the plate, the left one, without a shutter was at a height of 7.5 cm. In both transparent compartments, the animal was exposed to a fluorescent lamp (20 W) which was hung approx. 30 cm above the box. The animals were put into the right compartment individually. Five min later, the shutter was opened and the timers (A and B) were started. When the animal bridged the middle dark compartment, the shutter was closed. Timer A recorded the length of time the animal stayed on the floor in the middle room, and timer B, the length of time in the left compartment. The following three times were recorded: Time when the animal 1) passed through the right hole (L), 2) climbed on the left hole (M), and 3) moved into the left compartment (N). The number of times the mouse climbed on the left hole during a 30 min period was recorded at the same time as the index of motor activity (MA). The animals not given exercise were placed in the right compartment, and observed passing through the darkened compartment and moving into the left compartment for a period of 10 min. Tests on the same mice were conducted 5 min after oscillation exercise, at the same time and under the same conditions as the previous control trial. Motor activity (MA) index was counted as the percent ratio of the number of climbings of the exhausted mice onto the left hole to those of the control. Every 10 min, motor activity index was calculated as the percent ratio.

- **Hole cross (HC), rotating rod (RR), sliding angle (SA), spring balance (SB) and rectal temp. (RT) tests**: Details of the five methods have been reported by Takagi et al. (12).

**RESULTS**

**Exploratory movement test**

Determination of exercising time: Periods of exercising time required to produce pronounced exhaustion were determined. Groups of 10 mice were exercised each for 1/4, 1/2, 1, 2, 3 and 4 hr, and the test was done as described above. No drugs were administered. Results are shown in Fig. 1. Significant delay of N was observed in the groups which were oscillated for more than 30 min, and significant delays of M and L were
seen in the groups after exercise for more than 2 hr and 3 hr, respectively. Significant
decrease in motor activity was also observed in the groups oscillated for more than 30
min. A 4 hr exercise was adopted to produce exhausted states (see previous report Takagi
et al. (12)). Drugs were injected i.p. immediately after the exercise.

![Graph](image)

**Fig. 1.** Effects of oscillation movements measured by exploratory movement (EM)
test method after various exercise periods. Each value is the mean of values
obtained from 30 mice. Values significantly different from control (p<0.05,
Student's t-test) are indicated by *.

A : The lowest portion of each bar shows the time it took the mouse to pass
through the middle darkened compartment after the shutter was opened (L).
The middle bar shows the time it took the animal to climb into the next hole (M). The top bar shows the time it took the mouse to move into the second
lightened compartment (N).

B : The lowest, middle and top portions of each bar show the percent ratio
of the number of times the mouse climbed up and into the second hole during
the 10, 20 and 30 min test periods respectively to those of control trial for 30
min.

**Fig. 2.** Effects of drugs on exploratory and spontaneous movements after the mice
were oscillated for 4 hr. For expression, see Fig. 1. Each value is the mean
of values obtained from 12 mice.
Effects of drugs on exhaustion recovery: Methamphetamine hydrochloride (MA): 1, 2, 4 and 8 mg/kg; caffeine (CAF), used as complex with sodium benzoate: 25, 50, 100 and 200 mg/kg; 2-dimethylaminoethanol (DMAE): 25, 50, 100 and 200 mg/kg, pH was adjusted to neutral with HCl; morphine hydrochloride (MOR): 0.2, 1, 5 and 25 mg/kg were dissolved in physiological saline. Glucose (GL): 100, 200, 400 and 800 mg/kg in distilled water were used. Results are shown in Fig. 2. MA significantly shortened times L and M at a dose of 2 mg/kg. The 1 mg/kg treated group showed a significant speed-up of time N. MA 2 and 4 mg/kg increased the motor activity index. CAF, at all doses used significantly speeded up times L and M, and increased motor activity index. It reduced time N significantly in the lower two doses. DMAE significantly shortened times L and M, and increased the index at 25 mg/kg. GL in the lower two doses shortened times L and M and increased the index, but the higher two doses treated groups showed only a significant speed-up of time L. MOR had no effect.

Effects of Panax Ginseng root on exhaustion recovery: Water extracts of Ginseng root and callus (NW and CW) significantly increased exploratory movements. NW and CW in the lower doses used, significantly introduced the speed-up of times L and M. A significant speed-up of time L was seen in crude saponin fraction (NS) 100 mg/kg, and crude neutral saponin fraction (GNo. 3) 25 and 50 mg/kg treated groups. Lyophilic fraction (GNo. 5) significantly shortened time M at doses of 100 and 400 mg/kg. GRgI significantly increased the speed of times L and M at the higher three doses. No fractions were seen which increased the speed of time N within 30 min. In contrast, MeOH extract (NM) at the highest dose introduced a significant delay of times L and M. Neutral saponin fraction (GNS) delayed time L at the higher three doses. Results are shown in Fig. 3.

![Fig. 3. Effects of Panax Ginseng root on exploratory movements after a 4 hr oscillation by EM test. For expression, see Fig. 1. Each value is the mean of values obtained from 12 mice.](attachment:image-url)
GRgl at the higher three doses, GNo. 5 at all doses used, 50 mg/kg of GNo. 3 and NS, and 100 mg/kg of NW and CW, significantly increased the motor activity index. A significant decrease was seen in the groups treated F, fraction, which is obtained from the CHCl₃-eluant of the column chromatography of GNo. 4 on silica gel and does not contain saponins. Results are shown in Fig. 4.

**FIG. 4.** Effects of Panax Ginseng root on spontaneous movement after a 4 hr oscillation by EM test. For expression, see Fig. 1.

Hole cross test

GNo. 3 at the lower two doses and GRgl at the lowest dose produced a significant increase of the motor activity index, while NS, GNS and GNo. 4 at all doses used, F₄ at the higher two doses, NW 200 mg/kg and GNo. 5 100 mg/kg, significantly decreased the index. Results are shown in Fig. 5.
Rotating rod test

NS at a dose of 100 mg/kg for the second measurement and 400 mg/kg of GNo. 5 for the first measurement, considerably prevented the mice from falling. 50 mg/kg of GNo. 4 accelerated the falling for the second measurement. Results are shown in Fig. 6.

![Graph showing effects of Panax Ginseng root on measurements of RR test](image)

**Fig. 6.** Effects of Panax Ginseng root on measurements of RR test after a 4 hr oscillation. Ordinates represent percentage of mice passing the 3 min test. Each value is the mean of values obtained from measurement of 20 mice. Solid lines represent results obtained from tests performed 30 min after exercise ended. Broken lines, 2 hr after exercise.

Sliding angle test

NS significantly promoted the recovery of body tone at doses of 25 and 100 mg/kg 30 min after the exercise ended and again at 25 mg/kg for the second measurement. Significant increase of sliding angle was seen 2 hr after the administration of 50 mg/kg of GRg1.
and 100 mg/kg of GNo. 4, while GNo. 3 at all doses used showed a significant decrease in sliding angle for both measurements. In the GNo. 5 400 mg/kg and the GNS 12.5 mg/kg injected groups, a significant decrease of sliding angle was seen for the first measurement. Results are shown in Fig. 7.

**Spring balance test**

Significant increases of grip tone were seen 30 min and 2 hr after the administration of GRgl at a dose of 50 mg/kg, and 30 min after 50 mg/kg of NS and 200 mg/kg of GNo. 5. Significant delays of recovery were observed for both measurements after the administration of NW at a dose of 200 mg/kg and GNo. 4 at all doses. GNo. 3 at a dose of 25 mg/kg and GNS 12.5 and 50 mg/kg significantly decreased the recovery of grip tone for the first measurement. Results are shown in Fig. 8.

![Figure 8](image.png)

**Fig. 8.** Effects of Panax Ginseng root on grip tone, measured by SB test, after a 4 hr oscillation. Ordinates represent grip tone in grams. For expression, see Fig. 6.

![Figure 9](image.png)

**Fig. 9.** Effects of Panax Ginseng root on hypothermia after a 4 hr oscillation. Ordinates represent rectal temp. in °C. For expression, see Fig. 6.
Rectal temperature test

As shown in Fig. 9, hypothermia due to the 4 hr oscillation was significantly elevated by administration of NW at a dose of 200 mg/kg, GNo. 3 100 mg/kg and GRgl 200 mg/kg for the second measurement, and GNo. 5 at the lower two doses for the first one. On the other hand, significant delays of recovery of rectal temp. were seen for both measurements after NS at the lower two doses and F4 at the highest dose.

DISCUSSION

It is generally recognized that mice are inclined to move from a bright place into a dark place. It was noted in our report (13), that oscillated animals delayed movement into the dark compartment. Moreover different behavioral properties of animals are thought to exist between exploratory movements when entering into a dark place, and movements when going out into a light one. EM test was originally devised to study the recovery of two different exploratory movements and motor activity of exhausted animals due to drugs. MA at a dose of 2 mg/kg, DMAE 25 mg/kg and GL 100 and 200 mg/kg significantly speeded up the recovery of two exploratory movements of exhausted animals, and increased motor activity. Effects of CAF at doses of 25 and 50 mg/kg were obvious in every measurement undertaken. MOR had no effect on recovery from exhausted states in this test. Extracts of Panax Ginseng root are thought to contain many bioactive constituents, and therefore, do not lend themselves well to exact pharmacological evaluation. Moreover, evaluation methods of anti-fatigue effect or pharmacological methods to investigate effects of drugs on exhausted states of animals have not been generally established. In our report (12), five methods were utilized to measure states of fatigue: hole cross (HC), rotating rod (RR), sliding angle (SA), spring balance (SB) and rectal temp. (RT) tests. Of the three exercising methods, oscillation, treadmill running and high frequency sound wave, oscillation produced hypothermia, motor incoordination, and decrease of spontaneous movement, body tone and grip tone.

The effects of Panax Ginseng root on recovery from fatigued states were studied using the 6 tests described above. NW and CW significantly shortened the recovery of two exploratory movements and increased motor activity of exhausted animals in the EM test. Recovery from exhausted states in the RT test, was also recognized in NW treatment, but decrease of motor activity and grip tone was seen in HC and SB tests. In crude sapo- nin fractions, NS significantly accelerated the recovery of the exploratory movement of mice entering into the dark compartment and increased motor activity index in EM test, but decreased the index in HC test. Recovery of motor coordination, grip tone and body tone in RR, SA and SB tests was also reported in NS treatment, but there was a delay of recovery of rectal temp. in the RT test. GNo. 3, containing mainly neutral saponins (GNS) as well as other components, significantly shortened time L and increased the indices in EM and HC tests. Moreover, acceleration of recovery of rectal temp. was also observed, but GNo. 3 delayed the recovery from exhaustion in SA and SB tests. GNo. 4, containing mainly Ginsenoside Rg1, Rg2 and Rg3 as well as other components, accelerated
the recovery from exhausted states in SA test, but delayed in HC, SB and RR tests.

GNS had no effect on promotion of recovery from exhaustion. On the contrary, GNS delayed the recovery of spontaneous movement in EM and HC tests, motor coordination and rectal temp. in RR and RT tests. GRg1 significantly accelerated the recovery from exhausted states in EM, HC, SA, SB and RT tests. GNo. 5 also accelerated recovery in EM, RR, RT and SB tests, but delayed it in HC and SA tests.

It is clear from the present study and previous research that Ginseng root has many pharmacologically opposite properties, for example, CNS-stimulant to CNS-depressant, cholinergic and histamine-like to papaverine-like, and hypertensive to hypotensive properties. Effects of Ginseng root on recovery from fatigue are too complex to explain from the results of studies to date. However, the present investigation showed that Ginseng root contains two opposite properties, acceleration to delay, on recovery from exhaustion. Effects of GRg1 and GNo. 5 were obvious in almost every test undertaken. Moreover, the possibility also remains that substances contained in GNo. 3 and not saponins (GNS), accelerated the recovery from exhausted states. On the contrary, GNS and F4 delayed the recovery from exhaustion in almost every test. In other data (14), however, these were observed to have neuroleptic properties, so it cannot be easily concluded that these produce delay of recovery from fatigued states. Anti-fatigue effect of Ginseng root is thought to be complicated as it contains a number of pharmacologically active substances. GRg1 and GNo. 5 probably play an important role in the anti-fatigue effects of Ginseng root. Further experiments are required in order to establish precise pharmacological methods for screening anti-fatigue activity.

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