PHARMACOLOGICAL STUDIES OF PANAX GINSENG LEAVES

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Accepted 9 August 1972

Abstract—Pharmacological properties of a crude saponin fraction (GF-DS-I) and saponins (GF-DS-II) which were obtained from Panax Ginseng leaves, were estimated by blind screening consisting of three tests: 1) neuropharmacological observations in mice, 2) tests on the respiratory and cardiovascular system in rats, and 3) tests on the guinea-pig isolated ileum. GF-DS-I appeared to have CNS-depressive, neuroleptic, analgesic, hypertensive, cholinergic, and histamine-like activities, while GF-DS-II, had CNS-depressive, neuroleptic, analgesic, hypotensive, atropine-like and papaverine-like activities.

These neuroleptic activities, were examined and confirmed by tests on motor activity, exploratory movements, muscle tone, motor coordination, hypothermia and potentiation of CNS-depressant, and pole climbing, anticonvulsant and analgesic tests. No effects were seen on hemolysis.

Pharmacological studies on extracts of Ginseng root, have been reported by a number of investigators (1-9), but a report on Ginseng leaves has not heretofore been seen. Chemical research on Ginseng leaves has been reported by Shibata et al. (10, 11). We have investigated Ginseng root systematically, and, in particular, pharmacological properties of saponins in Ginseng root. A crude saponin fraction (G. No. 3) and GNS being a mixture of neutral saponins consisting mainly of Ginsenoside-Rb1, -Rb2 and -Rc have been reported, in particular the pharmacological properties (12-15). In this paper, pharmacological properties of Ginsenoside-F (GF), the main component of Ginseng leaves has been studied, and compared with those of saponin fractions from Ginseng root.

MATERIALS AND METHODS

Preparation of extracts from Ginseng leaves

Ginseng leaves were treated as shown in Fig. 1, and GF-DS-I (crude saponin fraction) and GF-DS-II (saponins prepared from GF-DS-I) were used. On acid hydrolysis, GF-DS-II afforded panaxadiol and panaxatriol. Details of the fractionation of these components from Ginseng leaves, is described by Shibata et al. (10, 11). The solution of these two components was dissolved in physiological saline.

The following methods were employed.

1) Neuropharmacological observations in mice

This method modified by Takagi et al. (16), is based on the work of Irwin (17). Test extracts were administered i.p. to male mice (ddy-strain), weighing 18-20 g. A useful
logarithmic series for dosing is: ... 1, 2, 5, 10, ... mg/kg (from the dose which induce symptoms similar to those of a control animal to approx. LD₅₀). Observations were made for 2 hr and then after 24 and 48 hr. Results were recorded in tabular forms (12).

2) *Acute toxicity in mice*

Male mice (ddy-strain), weighing 18–20 g, were used in order to determine i.v., i.p. and oral LD₅₀. Mortality was recorded 72 hr after treatment. The LD₅₀ was calculated by the up and down method.

3) *Tests on the respiratory and the cardiovascular systems in the rat*

After the rat had been anaesthetized with urethane-chloralose by the i.p. route, both arterial blood pressure, heart rate, and responses of respiratory system were recorded (12). Effects of extracts on respiration and cardiovascular system to a series of stimuli: acetylcholine chloride (ACh: 2 μg/kg), epinephrine hydrochloride (Epi: 5 μg/kg), histamine dihydrochloride (His: 5 μg/kg), electrical stimulation (5 V, 2 sec, 30 c.p.m.) of vagal stump, and occlusion of both carotid arteries for 20 sec, were studied. Effects of atropine sulfate (Atr: 2 mg/kg), diphenhydramine hydrochloride (Diph: 3 mg/kg), propranolol hydrochloride (Prop: 250 μg/kg), phentolamine mesylate (Phent: 5 mg/kg) and hexamethonium bromide (C₆: 5 mg/kg) on the responses of respiration and cardiovascular system to extracts, were also studied.

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**FIG. 1. Separation of Panax Ginseng leaves.**

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4) **Tests on the guinea-pig isolated ileum**

2.5–3.0 cm of intestine, excised at approx. 20 cm from pylorus was suspended in Tyrode's solution and bubbled with air in a 10 ml organ bath kept at 37°C. The antagonism to the contraction induced by ACh, His (cumulatively added), nicotine tartarate (Nic: $2 \times 10^{-8}$ g/ml) and serotonin creatine sulfate (5-HT: $3 \times 10^{-8}$ g/ml), or the antagonism of Atr ($10^{-8}$ g/ml) and Diph ($10^{-8}$ g/ml), to the contraction induced by test extract were studied.

5) **Hemolytic test**

Effects of test extracts on hemolytic activity were observed by the method of Fujita et al. (18). Pure saponins (Merck) was used as the standard.

6) **Intracerebral injection in mice**

The method for observing the effect of test substance introduced directly into the brain in the unanaesthetized mouse in groups of 6, weighing 24–26 g, were employed. Technical details have been described by Haley et al. (19). Test extracts were dissolved in saline and 0.02 ml was injected.

7) **Motor incoordination and muscle relaxation in mice**

1) Rotating rod, 2) sliding angle, and 3) spring balance tests described by Takagi et al. (20) were employed for measuring the motor incoordination and myorelaxation in groups of 8 male mice weighing 20–22 g.

8) **Motor activity in mice**

1) An activity wheel and 2) a hole cross tests described by Takagi et al. (21) were employed for motor activity. Groups of 10 male mice in a hole cross test, or a mouse in an activity wheel test, weighing 20–22 g, was placed in the test cage 10 min after the administration. Countings were continued for 1 hr, and recorded as a percent for the value which was counted in the same animal or the same group at the same time one day before the test.

9) **Climbing test in mice**

The effect of test extracts on exploratory movement was observed in male mice in groups of 10, weighing 18–20 g, using the climbing test described by Sandberg (22). Mice were placed in a test cage for 10 min every 30 min after the administration and the number of animals climbing the net was counted. This was repeated 4 times.

10) **Potentiation of hypnotic action of hexobarbital in mice**

Groups of 6 male mice, weighing 22–25 g, were administered test extracts by the i.p. route, and 30 min later 70 mg/kg of hexobarbital sodium was injected by the same route. Duration of loss of the righting reflex was measured.

11) **Analgesic tests in mice**

i) **Writhing induced by 0.7% acetic acid**

Groups of 8 male mice, weighing 20–23 g, were given test extracts orally, and 30 min later an i.p. injection of 0.7% acetic acid was given. The number of writhings per mouse was recorded for a period of 10 min, beginning 10 min after administration of acetic acid.

ii) **Tail pressure test**

The method has been described by Takagi et al. (23). Groups of 10 male mice, weighing
12) Anticonvulsant tests in mice
   i) Convulsions induced by electroshock
      Groups of 6 male mice, weighing 20–23 g, were given test extracts i.p., and 30 min
      later subjected to the maximum electroshock seizure (corneal electrodes charged with 25
      mA, 0.17 sec), in order to observe tonic extension of hind legs and lethal action.
   ii) Convulsions induced by chemicals
      Groups of 6 male mice, weighing 22–26 g, were given test extracts by the i.p. route
      30 min before an i.v. injection of pentylenetetrazol (60 mg/kg) or an i.p. injection of strych-
      nine nitrate (2 mg/kg). Three endpoints of time were taken: the first appearance of clonic
      convulsions, tonic extension of hind limbs and lethal action.
13) Hypothermia in mice
    Groups of 6 male mice, weighing 22–26 g with a rectal temp. of 37–38°C, were given
    test extracts i.p. and rectal temp. was recorded every 30 min for 2 hr after the treatment.
14) Ratio of reflexes in the mouse
    This method was described by Witkin et al. (24). Male mice in groups of 10, weigh-
    ing 18–20 g, were given test extracts by the i.p. route. Corneal and pinna reflexes were
    observed by using a pig hair, 15, 30, 60, 90 and 120 min after administration.
15) Pole climbing test in rats
    Details of apparatus and training have been described in a previous paper (13). Con-
    ditioned male rats in groups of 10 (Wistar-strain), weighing 150–180 g, were tested for
    a period of 120 min after an i.p. injection of physiological saline. Three days later, rats
    given test extracts by the same route, were tested at the same intervals.
16) Motor incoordination and muscle relaxation in rats
    Rotating rod and suspension tests described Takagi et al. (13), were employed for
    measuring the motor deficit and myorelaxation in rats.

RESULTS

1) Neuropharmacological observations in mice
   From the number of survivors in each group, approx. LD_{50} of GF-DS-I and II (i.p.)
   was estimated to be between 200 and 500 mg/kg. Lethal doses produced the following
   common symptoms for 2 hr after administration: a decrease of alertness, grooming,
   traction, spontaneous movement, touch response, grip tone, body tone and body temp.
   an appearance of passivity, piloerection, ptosis and abnormal gait. Disappearance of
   pain reflex, corneal reflex and pinna reflex were observed approx. 30 min after administra-
   tion. A slightly extended posture with abdomen touching floor was also observed. These
   behavioral changes continued for 2 hr and mice died approx. 24 hr after the treatment.
   Effects on mice were produced with extracts in doses of less than LD_{50}. These changes
   of behavior continued for 2 hr with mice showing renewed vitality 24 hr after. These ef-
   fects were dose-dependent and results are shown in Fig. 2.
2) Acute toxicity in mice

LD$_{50}$ of GF-DS-I and II (i.v.) was 381 and 299 mg/kg respectively. Behavioral changes observed in lethal doses: GF-DS-I produced extended posture with abdomen touching floor and abnormal gait a few min after treatment. A sedative state was seen for several min. Approx. 10 min later, swimming convulsions appeared and mice died after 15 to 25 min. GF-DS-II produced these behavioral changes faster than GF-DS-I and mice died within several min. LD$_{50}$ (i.p.) was 402 and 306 mg/kg respectively, and oral LD$_{50}$ was estimated to be more than 5 g/kg. Surviving mice showed no change in behavior the following day.

3) Tests on the respiratory and the cardiovascular systems in the rat

GF-DS-I: The arterial blood pressure of the rat lowered transiently with i.v. injection of GF-DS-I in doses between 0.5 and 2.0 mg/kg, and rose transiently with more than 5 mg/kg as shown in Fig. 3. One mg/kg lowered the mean arterial blood pressure by 12% that of normal however 5 mg/kg raised it by 9%, in 5 rats respectively. A slight decrease in heart rate was also detected in doses of more than 1 mg/kg, but effect was not seen on respiration after these doses. Tachyphylaxis was not detected with an injec-
tion of GF-DS-I.

Hypotensive or hypertensive doses of GF-DS-I did not alter the characteristic blood pressure responses to ACh, His, Epi, bilateral vagotomy and carotid occlusion. Pre-administration of Atr and Diph reversed the hypotensive response to GF-DS-I to that of a transient hypertensive response, and eliminated the slight increase of heart rate. Prop did not eliminate the hypotensive response to GF-DS-I. Hypertensive responses to GF-DS-I in a dose of more than 5 mg/kg and in a dose of 1 mg/kg after the simultaneous administration of Atr and Diph, were not eliminated by Phent and C6.

GF-DS-II: The arterial blood pressure showed a prolonged hypotension with injection of GF-DS-II at a dose of more than 2 mg/kg (Fig. 3). In 5 rats, 10 mg/kg lowered the mean arterial blood pressure by 24% that of normal. Effects on respiration and heart rate were not seen after these doses. Characteristic blood pressure responses produced by the above five stimuli were not altered. Pre-administration of Atr, Diph and Prop did not eliminate the hypotensive response to GF-DS-II in 5 rats.

4) Tests on the guinea-pig isolated ileum

GF-DS-I caused contractions of the ileum in high concentrations (10^{-5}–10^{-4} g/ml). The contraction produced at 10^{-4} g/ml was completely inhibited by Atr and partially by Diph. High concentration (10^{-4} g/ml) of GF-DS-I induced significant inhibition of the contraction caused by ACh and His in higher concentrations. Effects on 5-HT and Nic contractions were not seen (Fig. 4–1). GF-DS-II produced no contraction, and inhibited ACh, His, 5-HT and Nic contractions in higher concentrations (10^{-5}–10^{-4} g/ml) (Fig. 4–2).
Fig. 4-1. Effect of GF-DS-I on guinea pig isolated ileum.

Fig. 4-2. Effect of GF-DS-II on guinea pig isolated ileum.
5) **Hemolytic test**

Hemolytic activities of both GF-DS-II were be observed. Hemolytic index of pure saponins (Merck) was approx. 50,000.

6) **Intracerebral injection in mice**

Control animals given saline remained quiet for 2 to 3 min and then showed constant exploratory walking. GF-DS-II, in a dose of 10 μg/animal had no effect on mice. In a group given 20 μg/animal, half became depressed for about 7 to 10 min after the administration, then resumed normal activity. The other half walked and ran for 3 to 5 min then became depressed for another 10 to 20 min. Fifty μg/animal induced clonic convulsions plus running immediately after the injection, after which the animals died.

7) **Motor incoordination and muscle relaxation in mice**

No significant change in sliding angle was seen after i.p. administration of GF-DS-II. In the spring balance test, significant decrease of grip tone was seen 90 min after the i.p. injection of 400 mg/kg of GF-DS-II. In the rotating rod test, dose-dependent increase of inhibition was observed 1 hr after i.p. injection, and mice could hardly stand on the rotating rod with doses of 200 and 400 mg/kg 30 min after the treatment (Fig. 5).

**Fig. 5. Effect of GF-DS-II on motor coordination and muscle tone in mice.**

1) Activity wheel test  
2) Hole cross test

**Fig. 6. Effect of GF-DS-II on motor activity of mice.**
8) Motor activity in mice
   i) Activity wheel test: Motor activity of control animals given saline increased on the test day by approx. 100% as compared to that of the previous day. More than 100 mg/kg produced a significant decrease of motor activity (P<0.05) (Fig. 6-1).
   ii) Hole cross test: Twenty mg/kg of GF-DS-II given i.p. produced a significant decrease of exploratory movement by approx. 39% that of control (Fig. 6-2). The inhibition of exploratory movement was recognized at a dose of 50 mg/kg immediately after administration and continued for a few hr.

9) Climbing test in mice

GF-DS-I in a dose of more than 12.5 mg/kg, and GF-DS-II in a dose of more than 25 mg/kg, produced a significant inhibition of this exploratory behavior (Fig. 7), which was quite evident 30 min after administration.

![Graph showing effects of GF-DS-I and II on climbing test of mice.]

**Fig. 7.** Effects of GF-DS-I and II on climbing test of mice.

**Table 1.** Effects of GF-DS-I and II on hexobarbital induced sleeping time of mice.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Dose (mg/kg)</th>
<th>No. of animals</th>
<th>Time±S.E. (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>6</td>
<td>16.7±2.0</td>
</tr>
<tr>
<td>CPZ</td>
<td>4</td>
<td>6</td>
<td>44.4±3.0*</td>
</tr>
<tr>
<td>GF-DS-I</td>
<td>100</td>
<td>6</td>
<td>15.2±2.3</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>6</td>
<td>23.7±2.9</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>6</td>
<td>34.7±2.5*</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>6</td>
<td>17.0±2.2</td>
</tr>
<tr>
<td>CPZ</td>
<td>4</td>
<td>6</td>
<td>36.3±5.3*</td>
</tr>
<tr>
<td>GF-DS-II</td>
<td>50</td>
<td>6</td>
<td>16.6±1.0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>6</td>
<td>19.6±1.2</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>6</td>
<td>31.5±2.3*</td>
</tr>
</tbody>
</table>

* significant at P=0.01
CPZ: chlorpromazine hydrochloride
10) Potentiation of hypnotic action of hexobarbital in mice

GF-DS-I in a dose of 400 mg/kg, and GF-DS-II in a dose of more than 200 mg/kg produced a significant elongation of sleeping time of hexobarbital. CPZ in a dose of 4 mg/kg showed potentiation to that of 200 mg/kg of GF-DS-II and 400 mg/kg of GF-DS-I (P<0.05) (Table 1).

11) Analgesic tests in mice

i) Writhing induced by 0.7% acetic acid: Significant inhibition was seen in doses of 200 and 400 mg/kg of GF-DS-I and II (P<0.05) (Table 2).

ii) Tail pressure test: Significant increase of maximum pain threshold was seen in a dose of 400 mg/kg of GF-DS-I and over 100 mg/kg of GF-DS-II (Fig. 8). Maximal effect was seen 30 min after administration.

12) Anticonvulsant tests in mice

i) Convulsions induced by electroshock: GF-DS-I and II had no inhibitory effect on tonic flexor, tonic extensor, clonic convulsions and mortality.

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**TABLE 2. Effects of GF-DS-I and II on writhing induced by 0.7% acetic acid.**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Dose (mg/kg)</th>
<th>No. of animals</th>
<th>No. of writhings ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>7</td>
<td>30.4±2.5</td>
</tr>
<tr>
<td>Aminopyrine</td>
<td>200</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>GF-DS-I</td>
<td>100</td>
<td>7</td>
<td>22.7±4.8</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>7</td>
<td>16.3±4.5***</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>7</td>
<td>7.1±4.5***</td>
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<tr>
<td>Control</td>
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<td>8</td>
<td>0</td>
</tr>
<tr>
<td>GF-DS-II</td>
<td>50</td>
<td>8</td>
<td>16.4±6.8</td>
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<tr>
<td></td>
<td>100</td>
<td>8</td>
<td>14.6±4.4</td>
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<tr>
<td></td>
<td>200</td>
<td>8</td>
<td>6.5±1.4*</td>
</tr>
</tbody>
</table>

* significant at P=0.05
** significant at P=0.02
*** significant at P=0.01

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**Fig. 8. Effects of GF-DS-I and II on tail pressure in mice.**
ii) Convulsions induced by chemicals: GF-DS-I in a dose of 200 mg/kg elongated the time leading to clonic and tonic extensor convulsions and to death induced by strychnine. Two hundred mg/kg of GF-DS-II also elongated the time leading to these effects but not significantly (Table 3). The time leading to clonic and tonic extensor convulsions induced by pentylenetetrazol was elongated by GF-DS-II in a dose of 100 mg/kg. Decrease of mortality was also seen after this dose (Table 3).

13) Hypothermia in mice

Significant lowering of rectal temperature was seen in a dose 400 mg/kg of GF-DS-I and II 30 min after administration and continued for 2 hr. 200 mg/kg also lowered the rectal temp. 30 min after administration, however not significantly.

14) Ratio of reflexes in the mouse

No change was seen on the corneal reflex for 2 hr after administration of GF-DS-I and II. Disappearance of pinna reflex could be observed in 2 animals on 200 mg/kg of GF-DS-II, 9 animals on 400 mg/kg, and 7 animals on 400 mg/kg of GF-DS-I, 1 hr after administration and continued for 2 hr.

15) Pole climbing test in rats

GF-DS-II in doses of 10 and 30 mg/kg produced a significant specific block of the CR and elongation of response latency (P<0.01), within 40 min after administration. Maximal effects were produced within 10 min after treatment, and effects on the CR and response latency were not seen until 100 min after. 90 mg/kg showed a significant block
of the unconditioned response 20 min after the treatment and continued for 2 hr. Two animals died 3 days after the treatment (Fig. 9).

16) Motor incoordination in rats

Groups of 8 male rats, weighing 150–180 g, were used and GF-DS-II was injected i.p.

i) Rotating rod test: 10 and 30 mg/kg produced no effect on motor deficit and 90 mg/kg produced a slight inhibition for 1 hr after administration. Maximal effect was seen in the first trial 30 min after injection. 180 mg/kg produced a significant inhibition for 2 hr after administration.

ii) Suspension test: 30 mg/kg produced a slight muscle relaxation within 30 min after administration but no effect was seen 90 min later. 90 and 180 mg/kg produced a significant decrease of muscle tone for 2 hr after treatment. Loss of pinna, corneal and righting reflexes was not seen for 2 hr.
DISCUSSION

Pharmacological properties of GF-DS-I and II preliminarily estimated from blind screening consisted of 3 tests: 1) neuropharmacological observations in mice, 2) tests on the cardiovascular and respiratory system in the rat, and 3) tests on the isolated guinea-pig ileum.

From these results, GF-DS-I appeared to have effects such as CNS-depression, tranquillization, analgesic, cholinergic, histamine-like, and hypertensive activities. GF-DS-II also appeared to have CNS-depression, tranquillization, analgesic, hypotensive, atropine-like and papaverine-like activities.

Neuroleptic properties were examined using the tests as shown in this paper. GF-DS-I and II produced CNS-depression as revealed by their effects on reduction of spontaneous and exploratory movements and potentiation of hypnotic action of hexobarbital. These effects occurred with doses which did not produce motor incoordination and muscle relaxation in mice. Analgesic and anticonvulsant activities were also confirmed. Moreover, GF-DS-II inhibited conditioned avoidance response in the pole climbing test. The specific blocking effect on CR was transient and appeared 1 hr after administration. The inhibition of CR induced by GF-DS-II occurred within doses which did not produce depression of motor coordination and muscle tone. 90 mg/kg of GF-DS-II inhibited unconditioned response and produced a significant decrease of grip tone in rats. In addition to these actions, results from rotating rod test, hypothermia and ratio of two reflexes strongly indicate neuroleptic activity of GF-DS-II.

In previous reports (12–14), it was proposed that G.No.3 extracted from Ginseng root and containing mainly neutral saponins (GNS) and other components, had possible tranquillizing, cholinergic, histamine-like, papaverine-like and hypertensive activities, and that tranquillizing and papaverine-like activities were ascertained in GNS isolated from G.No.3 and consisted mainly of neutral saponins, Ginsenoside-Rb1, -Rb2 and -Rc. Neuroleptic activity of G.No.3 and GNS was also confirmed (13, 14). Similarities in pharmacological properties were observed between GNS and GF-DS-II, and between G.No.3 and GF-DS-I. It was confirmed that Ginsenoside-Rg, the saponins from Ginseng root, which afforded panaxatriol on acid hydrolysis, does not have CNS-depressive activity. Neuroleptic activity of GF-DS-II is attributed to neutral saponins which are similar to main components of GNS and afforded panaxadiol on acid hydrolysis.

Acknowledgements: We are grateful to Prof. S. Shibata, Faculty of Pharmaceutical Sciences, University of Tokyo, and Prof. O. Tanaka, School of Medicine, Hiroshima University for kindly providing GF-DS-I and II.

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