PHARMACOLOGICAL STUDIES OF PANAX GINSENG ROOT:  
PHARMACOLOGICAL PROPERTIES OF A CRUDE SAPONIN FRACTION

Keijiro TAKAGI, Hiroshi SAITO and Moriyoshi TSUCHIYA  
Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences,  
University of Tokyo, Bunkyo-ku, Tokyo, Japan

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It is said in Chinese medicine that Ginseng root has an anti-fatigue, anti-hypothermia,  
antidiabetic and sedative activities. It has been also utilized to develop physiological  
strength and to increase spontaneous movement of digestive system.  

Several papers concerning Ginseng root have been reported in the pharmacological  
fields, stating that some fractions of Ginseng root have central nervous system stimulant  
and analeptic activities (1), or stimulate a motility of digestive system (2). Petkov (3, 4)  
reported that water-alcohol extracts of Ginseng root produced smooth muscle contraction  
and had stimulant effects on central nervous system with the pole climbing test. Shibata  
et al. (5) have studied the chemical structure of Ginseng saponins and their sapogenins.  

In this paper, pharmacological properties of a crude saponin fraction (GNo. 3) of  
Ginseng root are discussed and effects of GNo. 3 on central nervous system and digestive  
system were investigated.

MATERIALS AND METHODS

Ginseng root was extracted with methanol and to the methanolic extracts was added  
ether to give precipitate (GNo. 3). GNo. 3 contains mainly neutral saponins (GNS) and  
also other components (10). A detail of the separation was described by Shibata et al.  
(5). The solution of GNo. 3 was prepared with physiological saline.

The following methods were employed.

1) Neuropharmacological observations in mice

Neuropharmacological observations in mice were performed by the method of Smith  
(6). GNo. 3 was intraperitoneally administered to pairs of male mice (ddY-strain) weighing  
18–20 g, in ascending logarithmic doses (from the dose which induce symptoms similar to  
that of control animal to approximate LD50). Mice receiving the test substance were ob-  
served continuously for 2 hr, and overnight mortality was recorded.

2) Acute toxicity in mice

Groups of 10 male mice, weighing 15–18 g, were used in order to determine the intra-  
peritoneal LD50. Statistics of mortality were recorded 72 hr after administration. LD50  
was calculated by the method of Litchfield-Wilcoxon (7).
3) Intracerebral test in mice

Method for observation of the effect of test substance introduced directly into the brain in the unanaesthetized mouse, was employed. Groups of 6 male mice, weighing 24–26 g, were used. GNo. 3 of 0.02 ml was injected. For technical details see the report of Haley et al. (8).

4) Potentiation of hexobarbital sleeping time in mice

Male mice, weighing 20–24 g, were used in a group of 10. GNo. 3 was administered intraperitoneally, and 30, 60 and 120 min later 70 mg/kg of hexobarbital sodium was injected into the same route. Duration of loss of the righting reflex was measured.

5) Climbing test in mice

The effect of GNo. 3 on exploratory movement was observed in mice with climbing test. Details have been described by Sandburg (9). Male mice, weighing 18–20 g, were employed in a group of 10. Mice were placed in a test cage for 10 min and the number of animals climbing the net was counted. The test was performed 4 times: 30, 60, 90 and 120 min after the intraperitoneal administration of the preparation.

6) Writhing induced by 0.7% acetic acid

Male mice in groups of 10, weighing 20–23 g, were given GNo. 3 orally, and 30 min later an intraperitoneal injection of 0.7% acetic acid. The number of writhes per mouse was recorded for a period of 20 min after administration of acetic acid.

7) Shuttle box test in rats

Experimental details are described in another paper (13). Conditioned male rats (Wistar strain), weighing 180–200 g, were employed for the study on conditioned response (CR). Conditioned rats were exposed to 5 consecutive trials after i. p. injection of saline as control, and trained 20 min a day for 3 days. GNo. 3 in doses of 100 and 200 mg/kg were then given into the same route, and the rats exposed to 5 consecutive trials.

8) Shay method in rats

Groups of 8 male rats, weighing 180–200 g, were fasted for 24 hr, the pylorus then being ligated under light ether anaesthesia. The rats were then returned to their individual cages and deprived of food and water until sacrificed 16 hr later by a blow on the head. A small incision was made in the greater curvature of the stomach. Gastric contents from each animal were centrifuged and volumes noted. Free and total acidity were determined by titrating the samples with 0.1 N NaOH using Töpfer's reagent and phenolphthalein as indicators. GNo. 3 in a dose of 500 mg/kg was administered subcutaneously before pylorus ligation.

9) Lesions induced by the stress in rats

Male rats, weighing 200–240 g, were subjected to the restraint and water immersion stress for 20 hr. Details have been described by Takagi et al. (11). GNo. 3 in a dose of 50 mg/kg was administered orally for three days and 500 mg/kg subcutaneously 30 min before immersion. Determination of the lesion index was made as described by Takagi et al. (12).

10) Test on the isolated guinea-pig ileum
A strip of male guinea-pig ileum about 3 cm long was suspended in a 10 ml organ bath filled with Tyrode's solution bubbled with air. After constant response to acetylcholine had been obtained, the experiment was performed.

RESULTS

1) Neuropharmacological observations in mice

GNo. 3 in doses of 100, 200, 500 and 1000 mg/kg were given intraperitoneally. GNo. 3 in a dose of 100 mg/kg had no effects on behavioral changes in mice. The following behavioral changes were observed by both 200 and 500 mg/kg. Decrease of spontaneous movement was found within 10 min after injection, and produced ataxia, loss of startle response and piloelection 30 min after administration, and continued for 2 hr. Muscle relaxation, loss of righting reflex and decrease of pain response could not be ascertained for 2 hr. GNo. 3 in a dose of 1000 mg/kg produced death in two animals. Decrease of spontaneous movement, stretch of legs and ataxia were produced and the belly of mouse touched the floor. Depth of breathing increased and clonic convulsions resulted in death.

2) Acute toxicity in mice

The LD₅₀ and the 95% confidence limit were 910 mg/kg (861.7-960.9 mg/kg).

3) Intracerebral test in mice

Animals administered 0.02 ml of saline, remained quiet for approx. 2 min, and then showed constant exploratory walking. GNo. 3 in a dose of 10 µg/animal caused mice to remain quiet for 5 min with only respiratory excitation. Twenty µg/animal induced increased spontaneous movement 1 to 2 min after injection. Some animals had clonic seizures or running convulsions immediately after injection. Respiratory excitation also resulted, and 6 to 7 min later became depressed. Fifty µg/animal induced convulsions with agitated spurs and death with extensor convulsions, or assumed a hunched position with clonic convulsions.

4) Potentiation of hexobarbital sleeping time in mice

GNo. 3 in a dose of 100 mg/kg produced a significant prolongation of sleeping time when hexobarbital was administered 30, 60 and 120 min after the injection of GNo. 3.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Time</th>
<th>30</th>
<th>60</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Time</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GNo.3, 12.5 mg/kg</td>
<td>25</td>
<td>17.1±3.1</td>
<td>25.9±5.4</td>
<td>43.5±6.9*</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>23.4±4.5</td>
<td>45.6±5.3*</td>
<td>41.8±4.3*</td>
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<td></td>
<td>100</td>
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<td>54.9±6.8*</td>
<td>51.5±7.2*</td>
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<tr>
<td></td>
<td>CPZ</td>
<td>2</td>
<td>52.3±7.6*</td>
<td>64.5±7.5*</td>
</tr>
</tbody>
</table>

Groups of 10 mice were employed.
a) average min. S.E.
b) time of hexobarbital administration after injection of GNo. 3
* P<0.05 when compared with control.
Fifty mg/kg produced a significant potentiation of sleeping time 60 and 120 min after injection, and 25 mg/kg, 120 min after injection. In a dose of 12.5 mg/kg, no effect was seen on sleeping time (Table 1).

5) Climbing test in mice

GNo. 3 produced significant inhibition of exploratory movement (Fig. 1). Twenty five mg/kg decreased exploratory movements by about 50%. Depression of spontaneous movement was observed for 2 hr after administration of GNo. 3.

6) Writhing induced by 0.7% acetic acid

GNo. 3 had no inhibitory effects on writhing induced by 0.7% acetic acid. Aspirin, in doses of 100 and 200 mg/kg given orally, had a significant inhibiting effect on writhing to approx. 60 and 15% respectively.

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**FIG. 1.** Effect of GNo. 3 on exploratory movement in mice.
Abscissa: doses of GNo. 3
Ordinate: Percent of mice climbing the net within 2 hr.

**FIG. 2.** Effect of GNO. 3 on conditioned response in rats.
Abscissa: time after GNO. 3 administration
Ordinate: percent of a specific and non-specific block

- : percent of a group given saline which failed to respond to conditioned stimulus (CS) but still responsive to unconditioned stimulus (US)
- : percent of a group given GNo. 3 which failed to respond CS but still responsive to US.
- : percent of a group of which was blocked both CR and UR.

Groups of 8 rats were employed.
7) **Shuttle box test**

Results are shown in Fig. 2. GNo. 3 in a dose of 100 mg/kg produced a slight specific blocking action of CR. Maximal effect on CR occurred immediately after administration, and blocking effects were abolished approx. 1 hr later. Two hundred mg/kg produced a significant specific block of CR for 100 min (P=0.05). It was found that 200 mg/kg produced a significant decrease of motor activity in all animals and a block of the unconditioned response (UR) in two animals. It had no effect on motor incoordination and myorelaxation in rats for 2 hr after the administration in a dose of 200 mg/kg.

8) **Shay method in rats**

Following administration of GNo. 3, no significant changes were observed in the volume of gastric juice, free and total acid outputs or lesion index, in comparison with the control. Atropine sulfate in a dose of 10 mg/kg subcutaneously, was found to have a suppressant effect on gastric secretion.

9) **Lesions produced by the stress in rats**

GNo. 3 had no inhibiting effect on the appearance of stress ulcer. Chlorpromazine hydrochloride in a dose of 10 mg/kg prevented stress ulcer production.

10) **Test on the isolated guinea-pig ileum**

Though GNo. 3 in a dose of 10^-6 g/ml produced no contraction of guinea-pig ileum, 10^-5 g/ml produced an occasional slight contraction. A significant contraction took place in 10^-4 and 3 x 10^-4 g/ml, however, these same concentrations produced no contraction in a few experiments (Fig. 3). The contraction, produced at 10^-4

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**FIG. 3. Effect of GNo. 3 on the isolated guinea-pig ileum.**

**FIG. 4. Effect of GNo. 3 on the contraction produced by acetylcholine.**
g/ml, was completely inhibited by $10^{-3}$ g/ml of atropine sulfate, but not by diphenhydramine hydrochloride. GNo. 3 in lower concentrations ($3 \times 10^{-5}$ to $10^{-1}$ g/ml) induced significant potentiation of the contraction caused by acetylcholine chloride, whereas it showed inhibition of the acetylcholine contraction in higher concentration ($10^{-2}$ g/ml) (Fig. 4). The same situations were also recognized in the influence of GNo. 3 on histamine contraction. Results are shown in Fig. 5.

![Fig. 5. Effect of GNo. 3 on the contraction produced by histamine.](image)

### DISCUSSION

In a previous report (10), it was confirmed that GNS which was isolated from GNo. 3 and consisted mainly of neutral saponins, Ginsenoside-Rb1, -Rb2 and -Rc, had possible tranquilizing and papaverine-like activities. Cholinergic, histamine-like and hypertensive activities were ascertained in the fractions of GNo. 4 and GNo. 5. Possibility of CNS-stimulant action was also confirmed in GNo. 4 fraction.

In neuropharmacological observations in mice, it was estimated that GNo. 3 had CNS-depressant activity, and the possibility of undesirable actions which were estimated from symptoms of abnormal gait, staggering gait, muscle relaxation and loss of righting reflex, was thought to be slight. Results were compared when the agent was introduced directly into the brain, and similarities were observed.

CNS-depressant activity was confirmed from the test on hexobarbital sleeping time and exploratory movement. The elongation of hexobarbital sleeping time by GNo. 3 was confirmed in a small dose of 25 mg/kg. The action was thought to appear slowly and continue for a few hr. GNo. 3 decreased the exploratory activity in mice in a small dose of 25 mg/kg, which dose induced behaviour similar to that of control animal injected saline. GNo. 3 had no analgesic activity.

GNo. 3 in a dose of 100 mg/kg produced a specific blocking action of CR in rats. It was also confirmed that 100 mg/kg of GNo. 3 decreased spontaneous movement but did not produce motor incoordination and decrease of muscle tone. From these results, it was presumed that GNo. 3 had sedative or tranquilizing activity.
Actions of GNo. 3 on digestive system were studied, but no effects were seen on gastric secretory volume or free and total acid outputs, and it did not prevent production of stress ulceration in a dose of 500 mg/kg though a central depressant agent such as chlorpromazine inhibited the appearance of the lesions.

GNo. 3 produced the contraction of the isolated guinea-pig ileum in a moderate dose of $10^{-4}$ g/ml, but $10^{-3}$ g/ml had no stimulation on smooth muscle preparations. This contraction by $10^{-4}$ g/ml of GNo. 3 was antagonized by atropine sulfate but not by diphenhydramine hydrochloride. GNo. 3 in doses of less than $10^{-4}$ g/ml increased the contraction produced by smaller doses of acetylcholine or histamine but inhibited in higher concentrations the contraction by the two agonists. It was thought that GNo. 3 has at least two different pharmacological active components from the test on the isolated guinea-pig ileum, that is, cholinergic and papaverine-like components. The possibility of histamine-like activity also remained.

It was reported that GNS, a main component of GNo. 3, had no contractive effect on the isolated guinea-pig ileum but did have papaverine-like activity (10). Papaverine-like activity of GNo. 3 can be attributed to GNS. Choline has been found in Ginseng root by Takatori et al. (14). We also found cholinergic and histamine-like activities in other fractions of Ginseng root (10). It was thought choline-like components contained in Ginseng root had been distributed into these fractions.

SUMMARY

Pharmacological studies of a crude saponin fraction which contained Ginsenoside-Rb and -Rc mainly, were performed. This fraction (GNo. 3) showed CNS-depressed states in neuropharmacological observations in mice. Prolongation of hexobarbital sleeping time, decrease of exploratory activity by GNo. 3 in mice, and a specific blocking action of the conditioned response by GNo. 3 in rats, were confirmed significantly in small doses. Loss of righting reflex, ataxia, abnormal gait, staggering gait and decrease of muscle tone were not produced. GNo. 3 had no effects on secretory volume or free and total acid outputs of gastric juice, and no preventive effect on lesions produced by stress. From the test on the isolated guinea-pig ileum, GNo. 3 was estimated to contain at least two pharmacological active components, i.e. cholinergic substance and GNS indicated by papaverine-like activity.

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