Further Evidence in Support of Psychotropic Action on Red Ginseng

Hiroyuki Yoshimura and Naoto Kimura*

Central Research Laboratory, Ehime University School of Medicine, Onsen-gun, Ehime 791-02, and
**Matsuyama Psychiatric Institute, Misawa-cho, Matsuyama-city, Ehime 790, Japan*

Abstract□Using an ethopharmacological technique, we demonstrated that saponin fraction from red ginseng root possessed a potent psychotropic actions on either intermale or maternal aggression models. A series of experiments clearly indicated that one of psychoactive ingredient is ginsenoside Rb1. Although a drug-induced debilitation of motor performance remains a possible cause of the antiaggressive effect of the drug, ginsenoside Rb1 did not alter the locomotor activity of the mice during agonistic confrontations. Thus, one can eliminate the possibility that the psychotropic effect of ginsenoside Rb1 might be concealed by a drug-induced impairment of motor performance. More recently, we developed a new model for copulatory disorder and introduced into the behavioral analysis of drug action. Male mice which has been housed individually from weaning for 5 weeks failed to manifest copulatory behavior when they encountered with the sexually-receptive females. Daily administration of crude ginseng saponin during isolation housing period prevented the development of copulatory disorder, whereas both ginsenoside Rb1 and Rg1 were ineffective. A further experiment may be needed to explore active ingredient of ginseng saponins.

Keywords□*Panax ginseng*, Korean red ginseng, psychotropic action, saponin, ginsenoside Rb1

Introduction

The root of *Panax ginseng*, especially Korean red ginseng, has played an important role in oriental medicine, and was first described in the oldest known Chinese medical book, Sheng-nong Ben-cao jing, in the first century. The ginseng has been used not only for the management of metabolic illnesses but also for the treatment of psychosomatic diseases such as anxiety neurosis, depressive state and insomnia. The psychotropic action of ginseng root has been demonstrated through its long history of use in oriental medicine, but experimental proof in support of clinical applications is limited.

In animals, as well as in humans, several behavioral effects of ginseng saponins have been reported: suppression of exploratory and spontaneous movement,[1-3] prolongation of hexobarbital sleeping time,[1,2] facilitation of recovery from exhausted state induced by forced running,[4] and inhibition of conditioned avoidance response.[5,6] Most of these early studies, however, were performed with a crude saponin, and the results obtained therefore appear inconsistent due to variations in both the quality and the quantity of active components.

Recently, more than 35 saponins have been isolated from ginseng root, and their chemical structures were identified. They can be classified into two major groups according to their sapogenins, that is, either the protopanaxadiol or protopanaxatriol groups. Ginsenoside Rb1 and ginsenoside Rg1 are representative of these two groups. At present, these pure ginseng saponins are supplied by Korea Ginseng and Tobacco Research Institute or Japan-Korea Red Ginseng Co., Ltd., on request of researcher.

We carried out a series of experiments on the effects of red ginseng on intermale aggression in mice administering the following drugs: crude ginseng saponins, pure ginsenoside Rb1 and ginsenoside Rg1.[7,8] Consequently, we demonstrated that both crude ginseng saponins and pure ginsenoside Rb_1 were significantly suppressed aggressive episodes in a dose-dependent manner without causing motor

dysfunction. Because ginsenoside Rg1 did not show any significant effect on male aggressive behavior, it is clear that ginsenoside Rb1 is one of the psychoactive ingredients of ginseng root. Comparing with the effect of other psychotropic drugs on intermale aggression, pharmacological profile of ginsenoside Rb1 is similar in nature to the effects of antidepressants.[9]

Using a female aggression model, maternal aggression in mice, we also found that crude ginseng saponins and ginsenoside Rb1, administered either acutely or chronically, had potent suppressive effects on maternal aggression.[7,8] In this model, ginsenoside Rg1 failed to manifest any significant effect on female behavior. Because solitary situation during pregnancy is critical to develop postpartum maternal aggression, it appears that ginsenoside Rb1 may alleviate such sociopsychological stress. Interestingly, ginseng-containing prescriptions such as Unkeitou (Wen-Jing-Tang) and Nyoshinsan (Nu-Shen-San) have been thought to be effective in the management of psychosomatic diseases in woman such as indefinite complaints, postpartum depressive states, and menopausal disorders. This paper will focus on the comparison of the effects of ginsenoside Rb1 and anxiolytic drugs on maternal aggression, and discuss concerning a possible mechanisms underlying the suppression of female aggression.

On the other hand, ever since the introduction of ginseng root into oriental medicine, it has been considered that ginseng root replenishes a vital energy leading to the strengthening of sexual behavior in males. To our knowledge, there has been little experimental approach in which the effect of ginseng root on copulatory disorder in males were clearly demonstrated. We noticed the fact that prolonged isolation housing altered the social communication between individuals in rodents, and developed a new animal model for research on copulatory behavior. In this symposium, I will describe the detail of the method and show whether or not ginseng saponins can prevent the development of copulatory disorder induced by isolation housing in male mice.

General Methods

Subjects: The animals used were ICR albino mice obtained originally from Clea Inc. (Osaka, Japan) and inbred in this laboratory. All subjects had free access to food and water, and were handled once per week for cage cleaning. Their cage floors were covered with wood shavings. The temperature in the vivarium was maintained at 23 + 1 °C, and a 12 hr light-dark cycle was automatically controlled (lights on at 7:00 a/m., off at 7:00 p.m.).

Apparatus: Behavioral testing was carried out in a clear polycarbonate cage (21 × 32 × 14 cm) with wood shavings. Each test was recorded using a video monitor system; at a later time, several behavioral elements were scored, with the aid of a computerized event recorder. The observer, who did not know the drug condition, depressed one key on a console for as long as each behavioral element occurred.

Drugs: All ginseng saponins were given intraperitoneally in an isotonic saline vehicle in a volume of 0.1 m*l* per 10 g body weight.

Results and Discussion

Maternal aggression in female mice

Each female mouse was housed together with an agematched male for 4 days, and then housed alone during pregnancy and lactation.[10] Maternal aggression was studied in the home cages of the female mice. Agonistic confrontations were performed on the 5th and 7th postpartum days, and the pups were not removed during the testing period. The test was started immediately after the introduction of a male intruder into the female's home cage, and lasted for 5 min from the time the first attack bite occurred. The test was terminated if the female did not attack an intruder within 5 min. Since a drug-induced debilitation of motor performance remains a possible cause of the anti-aggressive effect of the drug, the locomotor activity of the female mice during the 5 min testing period was also scored by measuring the number of ambulations from one position to

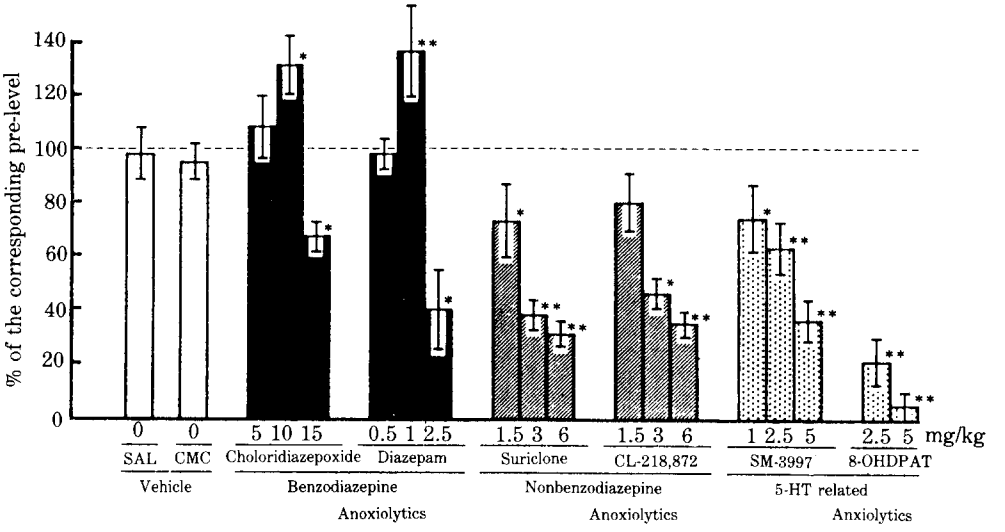

Fig. 1. Acute effect of various anxiolytic drugs on maternal aggression in female mice. Each column shows the post-drug score expressed as a percentage of the corresponding pre-drug scores. Significant differences from the corresponding pre-drug levels are indicated by $^{*}p<0.05$ and $^{**}p<0.01$

another. Behavioral testing was conducted between 13:00 and 16:00 h.

Fig. 1 shows acute effect of various anxiolytic drugs on maternal aggression in female mice. To clarify a pharmacological profile of ginseng saponins, we used the following three different kinds of anxiolytic drugs: benzodiazepine derivatives such as chlordiazepoxide and diazepam, nonbenzodiazepine cyclopyrrolone derivatives such as suriclone and zopiclone, and nonbenzodiazepine pyrimidinylpiperazine derivatives such as SM-3997 and 8-OH-DPAT. Either cyclopyrrolone or pyrimidinylpiperazine derivatives has not benzodiazepine structure. Cyclopyrrolone shows high binding affinity to benzodiazepine receptor, while pyrimidinylpiperazine binds to 5-HT-1A receptor without binding affinity to benzodiazepine receptor. As shown in Fig. 1, benzodiazepine anxiolytics increased the frequency of attack bite at small doses, but higher dosage of these drugs decreased the attack frequecy with suppression of locomotor activity. Cyclopyrrolone and pyrimidinylpiperazine derivatives manifested only suppressive effect on maternal aggression. They did not increase the frequency of attack bite. There was slight difference in the effect on locomotor activity between cyclopyrrolone and pyrimidinylpiperazine; the former showed a significant suppression of locomotion, while the latter did not show any significant changes. Since ginsenoside Rb1 manifests suppression of attack bite without causing any motor dysfunction, acute effect of the drug is similar to the effect of pyrimidinylpiperazine anxiolytics.

As shown in Fig. 2, acute administration of crude ginseng saponins and ginsenoside Rb1 significantly suppressed maternal aggression in a dose-dependent manner, whereas ginsenoside Rg1 was ineffective. None of the treatments employed significantly affected locomotor activity during agonistic confrontations, except 100 mg/kg crude ginseng saponins which reliably decreased locomotion. Compared with the characteristics of anxiolytic drugs mentioned above, it appears that ginsenoside Rb1 shares several pharmacological characteristics of nonbenzodiazepine 5-HT related anxiolytics.

Chronic administration of benzodiazepine derivatives failed to prevent the development of maternal aggression. A significant suppression of maternal aggression was found in mice which were treated with either cyclopyrrolone or pyrimidinylpipera-

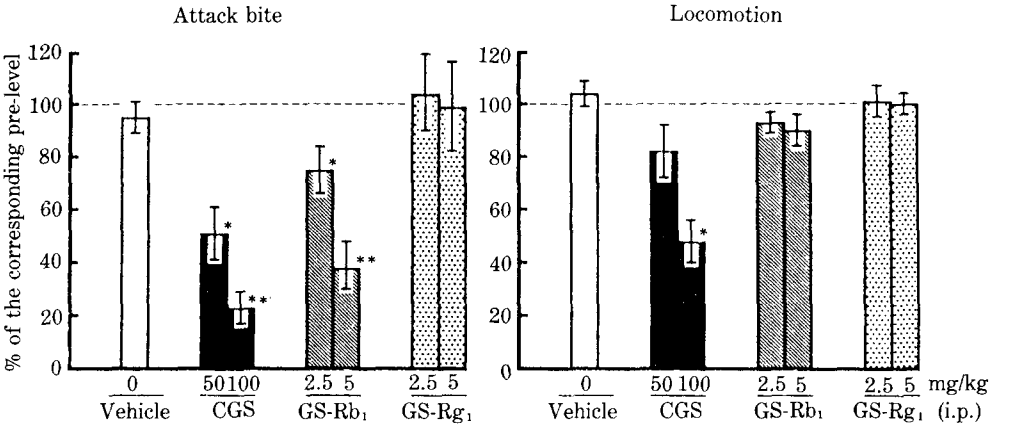

Fig. 2. Acute effect of various ginseng saponins on maternal aggression and locomotor activity in female mice. Each column shows the post-drug scores expressed as a percentage of the corresponding pre-drug scores. Significant differences from the corresponding pre-drug levels are indicated by $^{*}p<0.05$ and $^{**}p<0.01$. Note that ginsenoside Rb1 significantly suppressed the frequency of attack bites without causing any motor dysfunction.

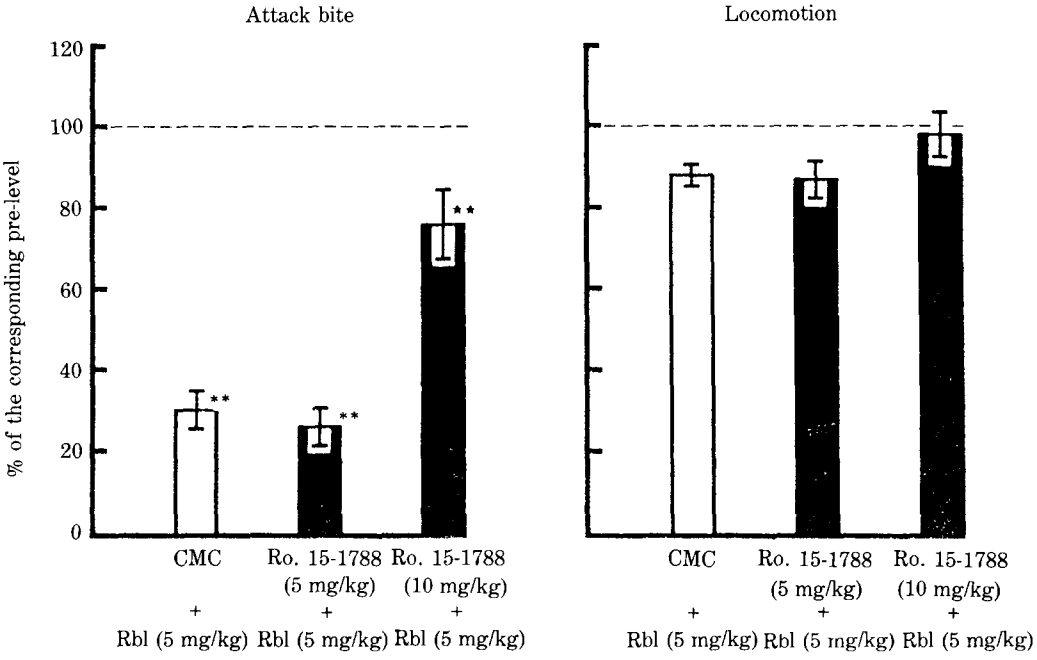

Fig. 3. Effect of Ro. 15-1788 pretreatment on ginsenoside Rb1-induced suppression of maternal aggression in female mice. Note that ginsenoside Rb1 suppression of maternal aggression was significantly antagonized by pretreatment of 10 mg/kg Ro. 15-1788.

zine derivative. All drugs did not affect the locomotor activity during agonistic confrontation. Because daily administration of ginsenoside Rb1 significantly suppressed the development of maternal aggression, chronic effect of ginsenoside Rb1 is similar to those of nonbenzodiazepine anxiolytics.

In order to know the mechanisms of psychtropic action of ginsenoside Rb1, we invesigated how this action is modified by pretreatment with Ro. 15-1778, benzodiazepine receptor blocker. As

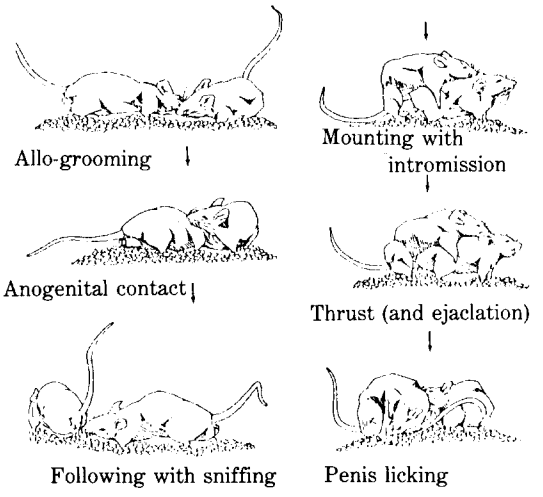

Fig. 4. Behavioral pattern of social interaction between male and receptive female mice.

shown in Fig. 3, 10 min before administration of ginsenoside Rb1 (5 mg/kg) Ro.15-1778 (5 and 10 mg/kg) was injected intraperitoneally. Females which were pretreated with Ro.15-1778 (10 mg/kg) prior to administration of ginsenoside Rb1 showed a significantly higher frequency compared to the control females pretreated with CMC ($p<0.01$), and these animals showed a similar frequency of attack bite to those of pre-level. This finding suggests that suppressive effect of ginsenoside Rb1 may mediate through benzodiazepine receptor. To our knowledge, there has been no other study in which the interaction between ginsenoside and benzodiazepin receptor has neurochemically demonstrated. More recently, we investigated the effect of methysergide pretreatment on ginsenoside Rb1 suppression of maternal aggression, and found that methysergide did not antagonize the action of ginsenoside Rb1. Since methysergide is a specific antagonist at serotonin receptors, our result indicates that endogenous serotonin mechanisms may not be responsible for the suppression of maternal aggression induced by ginsenoside Rb1.

Copulatory disorder in male mice

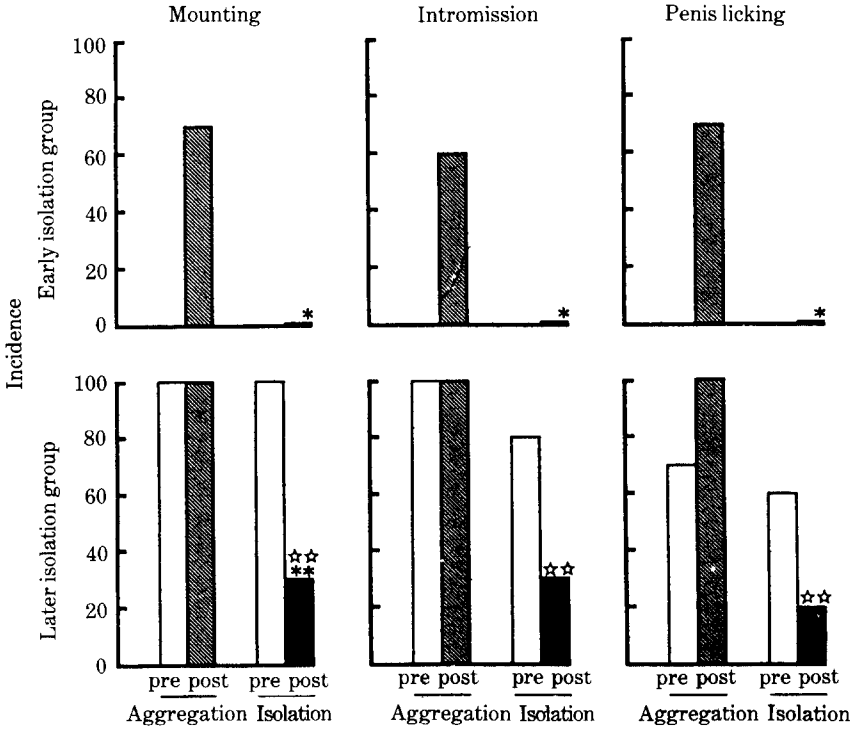

Fig. 5. Effect of isolation housing on copulatory behaviors in male mice. Upper panel shows copulatoty behaviors in early isolation group, and lower panel indicates copulatory behaviors in later isolation housing.

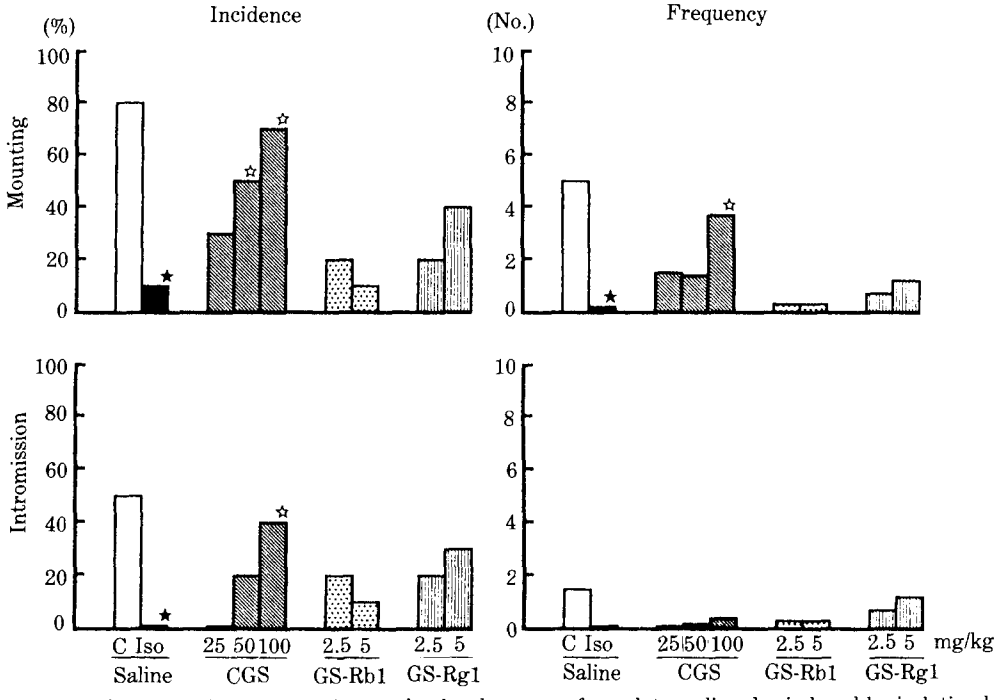

Fig. 6. Effect of various ginseng saponins on the development of copulatory disorder induced by isolation housing in male mice. Each drug was administered daily during the isolation housing period. Note that daily administration of crude ginseng saponins significantly prevented the development of couplatory disorder.

Social interactions between male and female mice was observed in a clear polycarbonate cage with wood shavings. To produce a receptive state, all female mice were pretreated with estrogen and progesterone. Because a hyperactivity of exploratory behavior (rearing and jumping) of the female mouse to the novel cage frequently disturbed male copulatory behavior, each female has placed in the cage for 24 hr before testing. The test was started immediately after the introduction of a male mouse into the female's cage and lasted for 10 min. The following behavioral elements were scored: mounting, intromission, penis licking, allo-grooming, anogenital contact, following, locomotion, rearing, self-grooming and digging. A typical behavioral pattern of social interaction between male and receptive female mice were shown in Fig. 4. Social confrontations were arranged between 19:00 and 22:00 h.

Equal number of male mice (n = 10) were randomly assigned to aggregation and isolation groups at weaning (21-22 days of age). Mice in aggregation group were housed 10 animals per cage, while mice in isolation group were housed individually in a polycarbonate cage (12 × 20 × 11 cm). Behavioral testing began 5 weeks after the start of each housing. Fig. 5 shows effect of early isolation housing on copulatory behaviors in male mice. Compared to aggregation group, incidence of copulatory behaviors (mounting, intromission and penis licking) was significantly low in isolation group ($p<0.05$). There was significant difference in the frequency of copulatory behavior between isolation and aggregation groups: mounting ($p<0.01$), intromission ($p<0.05$) and penis licking ($p<0.01$). The duration of each copulatory elements was also significantly different between two groups. When these isolated mice were re-socialized by housing together with 3 group-housed males in the same cage, copulatory disorder was recovered as a function of the length of group housing.

Fig. 6 shows effect of ginseng saponins on the

development of copulatory disoder induced by isolation housing in male mice. Chronic drug treatment (once per day) was started immediately after the start of isolation housing, and was terminated 24 hrs before behavioral testing. Daily administration of crude ginseng saponins prevented the development of copulatory disorder significantly in a dose-dependent manner. However, ginsenoside Rb1 and Rg1 failed to prevent the development of copulatory disorder. Therefore, it is most likely that ginseng saponins other than ginsenoside Rb1 and Rg1 may participate in the prevention of copulatory disorder.

In conclusion, crude ginseng saponins and ginsenoside Rb1 administered intraperitoneally have potent psychotropic effects. These pieces of evidence provide support for a possible therapeutic use of ginseng saponins in psychiatric medicine.

Acknowledgements

This research was supported in part by a grant from the Medical Society for Red Ginseng Research. The assistance of Kayo Nishimoto, Shinzo Kimura, and Tomomasa Kajikawa is gratefully acknowledged. Japan-Korea Red Ginseng Co., Ltd. (Koube) and Korea Ginseng and Tobacco Research Institute geneously supplied the ginseng saponins.

Literature Cited

1. Takagi, K., Saito, H. and Nabata, H.: Pharmacological studies of *Panax ginseng* root: Estimation of pharmacological actions of *Panax ginseng* root. *JPN J. Pharmacology*, **22**, 245 (1972).
2. Takagi, K., Saito, H. and Tsuchiya, M.: Pharmacological studies of *Panax ginseng* root: Pharmacological properties of a crude saponin fraction. *JPN J. Pharmacology*, **22**, 339 (1972).
3. Takagi, K., Saito, H. and Tsuchiya, M.: Effect of *Panax ginseng* root on spontaneous movement and exercise in mice. *JPN J. Pharmacology*, **24**, 41 (1974).
4. Saito, H., Yoshida, Y. and Takagi, K.: Effects of *Panax ginseng* root on exhaustive exercise in mice. *JPN J. Pharmacology*, **24**, 119 (1974).
5. Nabata, H., Saito, H. and Takagi, K.: Pharmacological studies of neutral saponins (GNS) of *Panax ginseng* root. *JPN J. Pharmacology*, **23**, 29 (1973).
6. Saito, H., Tsuchiya, M., Naka, S. and Takagi, K.: Effects of *Panax ginseng* root on conditioned avoidance response in rats. *JPN J. Pharmacology*, **27**, 509 (1977).
7. Yoshimura, H., Watanabe, K. and Ogawa, N.: Psychotropic effects of ginseng saponins on agonistic behavior between resident and intruder mice. *European J. Pharmacology*, **146**, 291 (1988).
8. Yoshimura, H., Watanabe, K. and Ogawa, N.: Acute and chronic effects of ginseng saponins on maternal aggression in mice. *European J. Pharmacology*, **150**, 319 (1988).
9. Yoshimura, H.: Studies contrasting drug effects on reproduction induced agonistic behavior in male and female mice, in: Ethopharmacology of Agonistic Behavior in Animals and Humans, eds. B. Olivier, J. Mos and P.F. Brain (Martinus Nijhoff Publishers, Holland) pp. 94-104 (1987).
10. Yoshimura, H. and Ogawa, N.: Acute and chronic effects of psychotropic drugs on maternal aggression in mice. *Psychopharmacology*, **97**, 339 (1989).