

Anxiolytic-Like Effects of Ginsenosides on the Elevated Plus-Maze Model in Mice

Hwa-Young CHA,^a Jeong-Hill PARK,^b Jin-Tae HONG,^a Hwan-Soo YOO,^a Sukgil SONG,^a
Bang-Yeon HWANG,^a Jae-Soon EUN,^c and Ki-Wan OH^{*,a}

^a College of Pharmacy, Chungbuk National University; Cheongju 361–763, Korea; ^b College of Pharmacy, Seoul National University; Seoul 151–742, Korea; and ^c College of Pharmacy, Woosuk University; Samrye 565–701, Korea.

Received January 26, 2005; accepted April 22, 2005

In a previous study, we reported that ginseng extract has anxiolytic-like effects in the elevated plus-maze model and that the ginseng saponin fraction plays an important role. This experiment was performed to investigate the anxiolytic-like effects of ginsenosides Rb₁, Rg₁, Rg₃-R, and Rg₃-S, and the Rg₅ and Rk mixture isolated from the ginseng saponin fraction in the elevated plus-maze. Furthermore, the anxiolytic-effects of Rb₁, Rg₁, Rg₃-R, Rg₃-S, and the Rg₅ and Rk mixture were compared with those of a well-known active anxiolytic drug (diazepam). The oral administration of ginsenoside Rb₁ significantly increased the number of open arm entries and the time spent on the open arm compared with those in the vehicle-treated group. Ginsenoside Rg₁ and the Rg₅ and Rk mixture also significantly increased the number of open arm entries and the time spent on the open arm. However, ginsenosides Rg₃-R and Rg₃-S did not increase the number of open arm entries or the time spent on the open arm. On the other hand, ginsenoside Rb₁ and the Rg₅ and Rk mixture decreased locomotor activity in a manner similar to diazepam. These data indicate that ginsenosides Rb₁, Rg₁, and the Rg₅ and Rk mixture have anxiolytic-like effects, but ginsenosides Rg₃-R and Rg₃-S do not in this model. We provide evidence that some ginsenosides may be useful for the treatment of anxiety.

Key words anxiolytic; elevated plus-maze; open arm; closed arm; ginsenoside; diazepam; locomotor activity

Anxiety affects one-eighth of the total population worldwide and has become a very important area of research interest in psychopharmacology during this decade.¹⁾ Benzodiazepines are still the most frequently used drugs for the treatment of generalized anxiety disorder despite their undesirable side effects such as muscle relaxation, sedation, physical dependence, memory disturbance, and interaction with other drugs.²⁾

In recent years, the development of new anxiolytics has been an area of interest. Various types of herbal medicines have been used as anxiolytic agents in different parts of the world.³⁾ The root of the kava plant from the tropical Pacific region, St. John's wort extract from Europe, and the saponin-containing fraction of the leaves of *Albizia lebbbeck* from India are known to have anxiolytic effects.^{4–6)} *Panax ginseng*, as a folk medicine, is one of the most commonly and widely used herbal medicines in Oriental countries such as Korea, China, and Japan. Ginseng has also long been used traditionally for the treatment of psychiatric disorders such as anxiety and depression. It was reported that *Panax ginseng* extract stabilized sleeping in food-deprived rats.⁷⁾ Ginseng saponins prolonged pentobarbital sleeping time and delayed the onset of convulsions when administered at high doses, effects that appear to be due to the GABA-benzodiazepine-chloride channel complex.⁸⁾ Ginseng saponins increased the affinity of specific binding of [³H]baclofen and [³H]flunitrazepam in crude synapse membrane from the rat frontal cortex.⁹⁾

On the other hand, we reported that ginseng produced anxiolytic-like effects and the saponin fraction played an important role in the plus-maze model in mice.¹⁰⁾ The present study was to know the anxiolytic potential of ginsenosides Rb₁, Rg₁, Rg₃-R, Rg₃-S, and the Rg₅ and Rk mixture in the elevated plus-maze, a behavioral test for anxiolytic drugs. Furthermore, the anxiolytic effects of ginsenosides and di-

azepam in the elevated plus-maze were compared to determine whether the behavioral profile of ginsenosides differed from that of the established anxiolytic diazepam.

MATERIALS AND METHODS

Animals Male ICR mice (Samtako, Korea) weighing 20–28 g, in groups of 10–15, were used in all experiments. Groups of 10 mice were housed in acrylic cages (45×60×25 cm) with water and food available *ad libitum* under an artificial 12-h light/dark cycle (lights on at 07:00) and at a constant temperature (22±2 °C). To ensure adaptation to the new environment, the mice were housed in the departmental holding room for 1 week before experiments.

Experimental Compounds and Drug Ginsenosides Rb₁, Rg₁, Rg₃-S, and Rg₃-R, and the Rg₅ and Rk mixture were kindly provided by the Ginseng Science Inc. Rg₃-S, Rg₃-R, and the Rg₅ and Rk mixture, which are not usually found in white ginseng, were isolated in ginseng steamed at high temperature. Those compounds are unique constituents of red and sun ginseng, and more abundant in sun ginseng than in red ginseng.^{11–13)} Mice were given a single oral administration of each ginsenoside 60 min being placed on the elevated plus-maze. Diazepam (2 mg/kg, Dae-Won Pharmaceutical Co.) was administered orally 30 min before placement in the elevated plus maze. All compounds were dissolved in 0.9% physiologic saline and freshly prepared. Compounds were administered at a rate of 0.1 ml/10 g.

Measurement of Anxiolytic-Like Effects The elevated plus-maze test is described in detail elsewhere.^{14,15)} Briefly, the plus-maze apparatus is comprised of two open arms (30×5 cm) and two closed arms (30×5×15 cm) that extend from a common central platform (5×5 cm). The floor of each arm is wooden and the walls of the closed arms are clear Plexiglas. The entire maze is elevated to a height of 38 cm

* To whom correspondence should be addressed. e-mail: kiwan@chungbuk.ac.kr

above the floor level, as has been validated and described.¹⁶⁾ Experiments were conducted in a quiet room illuminated only by a dim light. Mice received a single administration of test compounds. Mice were placed on the plus-maze 30 min after diazepam and 60 min after ginsenosides. In a preliminary experiment, the effects of the agents were investigated at various time intervals. From the results of the preliminary experiment, we found that maximal effects were observed when diazepam was administered orally 30 min prior and ginsenosides 60 min prior to plus-maze placement. A standard 5-min test was employed for each mouse. The maze was thoroughly cleaned with damp and dry towels between mouse experimental periods. All experimental sessions were recorded with a video camera mounted vertically above the maze. The open arm activity was evaluated as: 1) time spent on the open arms relative to the total time spent in the plus-maze, expressed as a percentage ($100 \times \text{time spent on open arm} / \text{total time in the plus maze}$); and 2) the number of entries into both open and closed arms, expressed as a percentage ($100 \times \text{open} / \text{total entries}$). Four paws onto and two paws off of an arm constituted an arm entry and exit. The behavioral experiments took place under quiet conditions and low light (50 lux) and were carried out between 13:30–16:30 h.

Measurement of Locomotor Activity Since the plus-maze experiment was affected by changes in locomotor activity, an additional experiment was carried out with the specific aim of monitoring the activity. Separately from the experiment with the elevated plus-maze, spontaneous locomotor activity was measured automatically with a tilting-type ambulometer (AMB-10, O'Hara, Tokyo, Japan). Each mouse was placed in the activity cage (20 cm in diameter, 18 cm in height) and after an adaptation period of 10 min, the test compound administration protocol was implemented. Diazepam was administered orally 30 min prior to the experiment. Ginsenosides were administered orally 60 min prior to the experiment. Ambulatory activity was measured for 30 min after oral administration of the agents.

Statistics The data are expressed as mean \pm S.E.M. The significance of the effects of the compounds was assessed using analysis of variance (ANOVA). In case of significant variation, the individual values were compared with Dunnett's test.

RESULTS

Anxiolytic Like-Effects of Ginsenosides Behavior observed in the elevated plus-maze confirmed the anxiolytic activity of diazepam reported previously.^{17–20)} As the positive control, diazepam 2 mg/kg increased open arm entries and time spent on open arms.

Ginsenosides Rb₁ (10, 25, 50 mg/kg) and Rg₁ (10, 25, 50 mg/kg) and the Rg₅ and Rk mixture (25, 50 mg/kg) increased the percentage of open arm entries compared with those in the vehicle control animals, respectively (Figs. 1, 2, 5). On the other hand, ginsenosides Rb₁ (25, 50 mg/kg) and Rg₁ (50 mg/kg) and the Rg₅ and Rk mixture (50 mg/kg) increased the percentage of time spent on open arms (Figs. 1, 2, 5). However, ginsenosides Rg₃-S and Rg₃-R did not increase the percentage of open arm entries and percentage of time spent on open arms (Figs. 3, 4).

Effects of Ginsenosides on Spontaneous Locomotor Activity Locomotor activity was significantly decreased by diazepam (2 mg/kg). Locomotor activity was also decreased in animals pretreated with ginsenoside Rb₁ (50 mg/kg) and the Rg₅ and Rk mixture (50 mg/kg) compared with that in the vehicle group. However, ginsenoside Rg₁ (50 mg/kg) did not decrease locomotor activity (Fig. 6). Ginsenosides inhibited locomotor activity to a lesser extent than diazepam and thus had a better profile for anxiolytic agents.

DISCUSSION

The elevated plus-maze is a well-established animal model for testing anxiolytic drugs.^{21,22)} Diazepam, a standard anxiolytic used clinically, is also employed in behavioral pharmacology as a reference compound for inducing anxiolytic-like effects, even when the compound being screened does not act *via* benzodiazepine receptors.²³⁾

In a previous study, we reported that ginseng produced anxiolytic-like effects and that the saponin fraction played an important role in the plus-maze model in mice because the water extract of ginseng, which contains less ginsenosides, did not produce anxiolytic effects.¹⁰⁾ On the other hand, we were interested in the anxiolytic-like effects of ginsenosides such as Rb₁, Rg₁, Rg₃-R, Rg₃-S, and the Rg₅ and Rk mixture

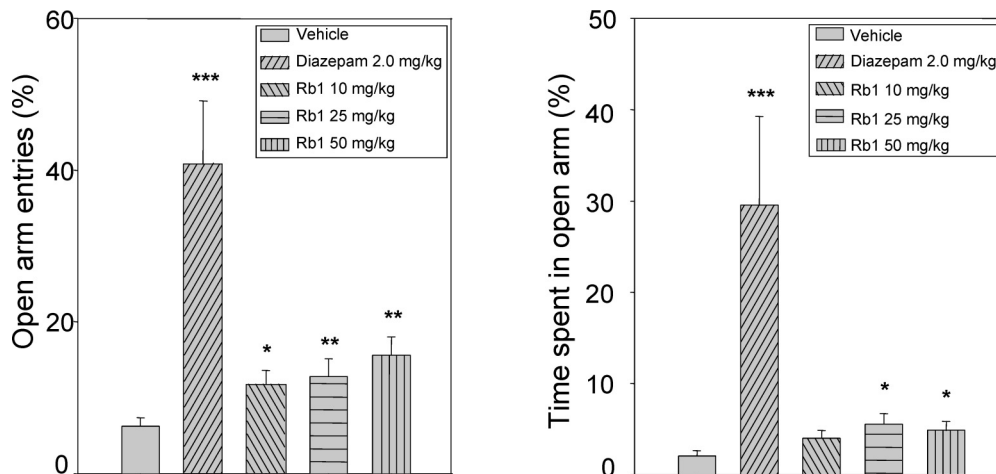


Fig. 1. Effects of Diazepam and Ginsenoside Rb₁ on the Percentage of Open Arm Entries and Time Spent in Open Arms on the Elevated Plus-Maze. Data are expressed as mean values (\pm S.E.M.) from each group of at least 10 mice. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, compared with the vehicle-treated control.

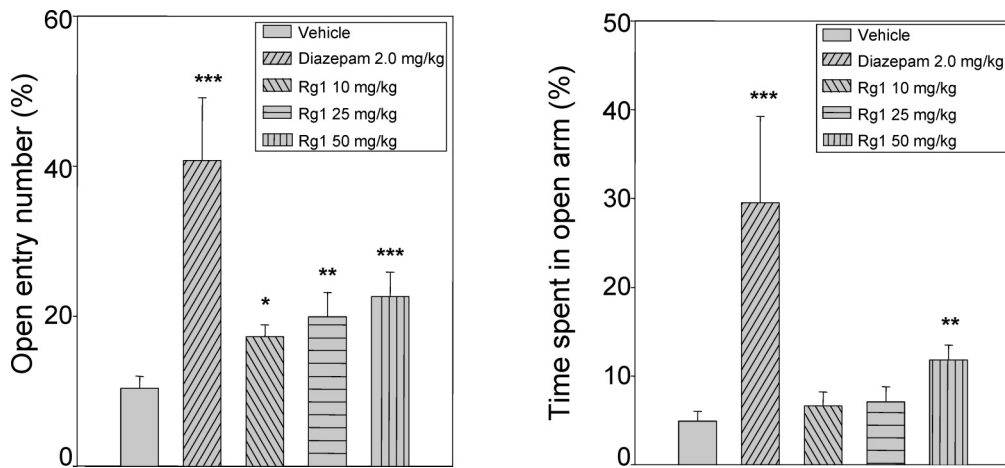


Fig. 2. Effects of Diazepam and Ginsenoside Rg₁ on the Percentage of Open Arm Entries and Time Spent Time in Open Arms on the Elevated Plus-Maze
 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ compared with the vehicle-treated control.

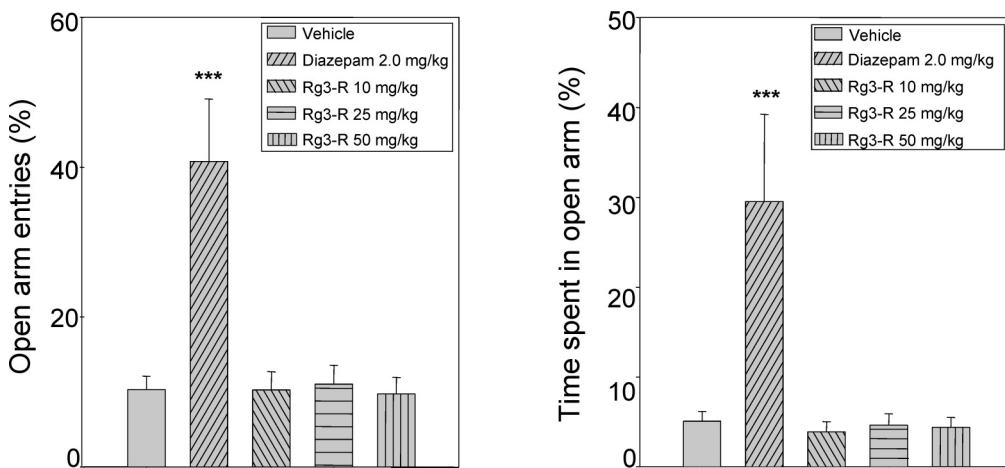


Fig. 3. Effects of Diazepam and Ginsenoside Rg₃-R on the Percentage of Open Arm Entries and Time Spent Time in Open Arms on the Elevated Plus-Maze
 *** $p < 0.005$ compared with the vehicle-treated control.

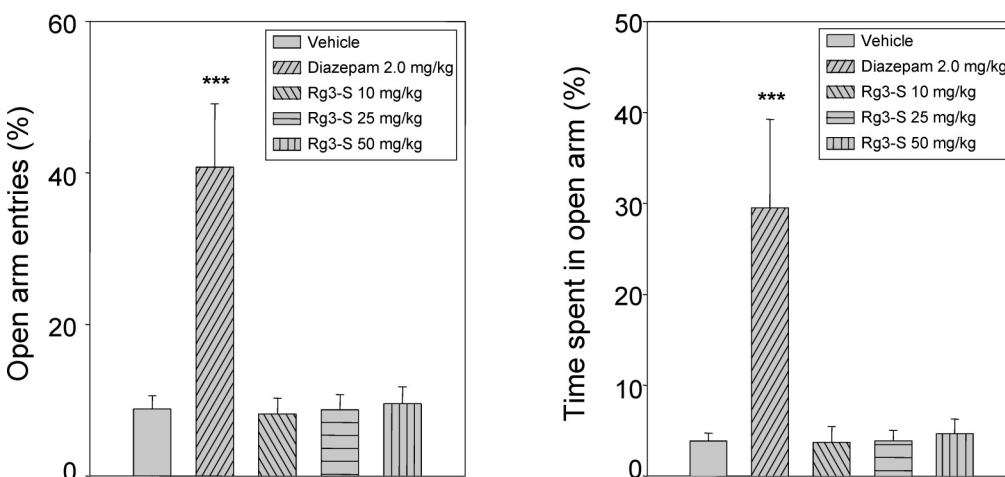


Fig. 4. Effects of Diazepam and the Ginsenoside Rg₃-S on the Percentage of Open Arm Entries and Time Spent in Open Arms on the Elevated Plus-Maze
 *** $p < 0.005$ compared with the vehicle-treated control.

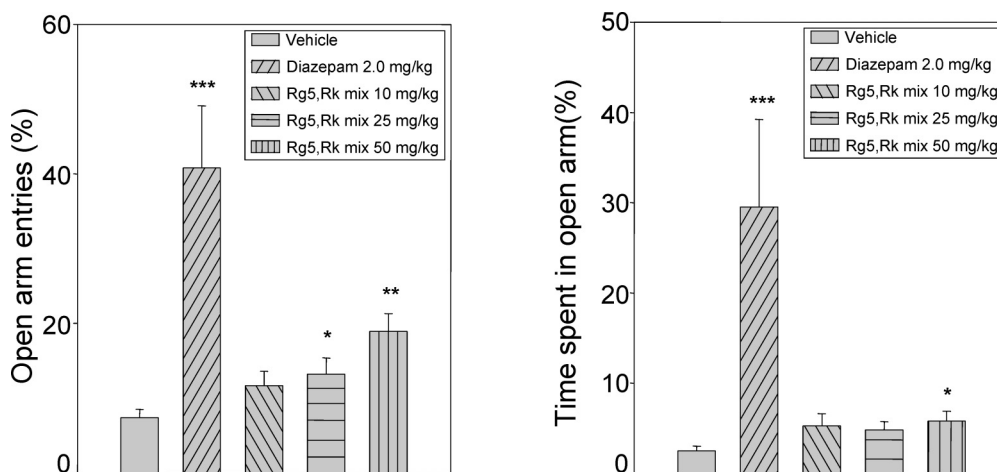


Fig. 5. Effects of Diazepam and Ginsenoside Rg₅ and Rk mixture on the Percentage of Open Arm Entries and Time Spent in Open Arms on the Elevated Plus-Maze

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ compared with the vehicle-treated control.

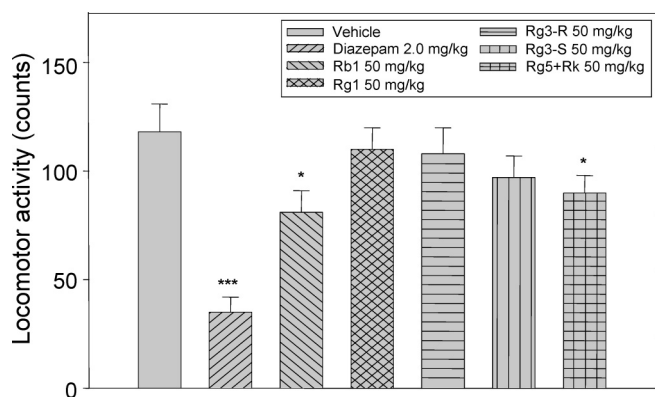


Fig. 6. Effects of Diazepam and Ginsenosides on Locomotor Activity in Mice

Data are expressed as mean values (\pm S.E.M.) from groups of at least 10 mice. * $p < 0.05$, *** $p < 0.005$ compared with the vehicle-treated control.

from ginseng saponins. In this study, we found that Rb₁, Rg₁, and the Rg₅ and Rk mixture increased the percentage of open arm entries and time spent in open arms and thus showed anxiolytic effects in this model. The anxiolytic effects of drugs such as benzodiazepines are accompanied by decreased locomotor activity and sedation.²⁴⁾ We previously reported that ginsenosides Rb₁ and Rg₁ inhibited psychostimulant-induced hyperactivity.^{25–28)} In agreement with previous reports, ginsenoside Rb₁ inhibited locomotor activity in this experiment. However, ginsenoside Rg₁ inhibited locomotor activity to a lesser extent than diazepam, and thus has a better profile for an anxiolytic agent. There is considerable interest in the development of new anxiolytics that do not induce sedative effects and do not inhibit locomotion.

Drugs derived from traditional herbs may have possible therapeutic relevance in the treatment of anxiety.²⁹⁾ Research has been conducted to investigate natural anxiolytic agents in the search for an alternative, more specific, and perhaps cost-free therapy. Various types of herbal medicines have been used as anxiolytics in different parts of the world. The root of the kava plant from the tropical Pacific region, St. John's wort extract from Europe, and the saponin-containing fraction of the leaves of *A. lebeck* from India are known to have anxiolytic effects.^{4–6)} In addition, ginsenosides from *P. gin-*

se are the active pharmacognostics responsible for enhancing cognitive behavior.³⁰⁾

Although the active mechanism of ginseng is still unclear, its anxiolytic effects appear to be related to the GABA-benzodiazepine-chloride channel receptor complex. We reported that ginsenosides interact with the ligand-binding to the GABA_A and GABA_B receptors. In particular, ginsenosides enhance specific [³H]flunitrazepam binding and increased the affinity of [³H]flunitrazepam binding.⁹⁾ In addition, the level of [³H]muscimol binding was strongly elevated in almost all regions of the frontal cortex after administration of ginsenoside Rc, but was decreased after ginsenoside Rg₁.³¹⁾ Ginseng saponins administered at high doses prolonged the pentobarbital sleeping time and delayed the onset of convulsions in behavioral studies.⁸⁾ Ginseng induces sedative effects at higher doses and anxiolytic-like effects at lower doses. Therefore, the anxiolytic-like effects of ginseng may involve GABAergic mechanisms. The exact underlying mechanism of action remains to be elucidated.

Thus it is concluded that ginsenosides from *P. ginseng* that contains saponins has anxiolytic activity. The GABAergic transmission mechanism may be responsible for the anxiolytic activity of the ginsenosides.

Acknowledgement The authors are grateful for the support of the Center for Healthcare Technology Development from Korean Ministry of Science and Technology.

REFERENCES

- 1) Eisenberg R. B., Davis S. L., Ettner S., Appel S., Wilkey M., Rompay V., Kessler R. C., *J. Am. Med. Assoc.*, **280**, 1569–1575 (1998).
- 2) Griffiths R. R., Sannerud C. A., "Abuse of and Dependence on Benzodiazepines and Other Naxiolytic/sedative Drugs," *Psychopharmacology*, 3rd ed., Raven Press, New York, 1987, pp. 1535–1540.
- 3) Heinrich M., Gibbons S., *J. Pharm. Pharmacol.*, **53**, 425–432 (2001).
- 4) Rex A., Morgenster E., Fink H., *Progress in Neuro-Psychopharmacol. & Biol. Psychiatry*, **26**, 855–860 (2002).
- 5) Friede M., Freudenstein J., *Eur. Psychiatry*, **17**, 96 (2002).
- 6) Une H. D., Sarveiya V. P., Pal S. C., Kasture V. S., Kasture S. B., *Pharmacol. Biochem. Behav.*, **69**, 439–444 (2001).
- 7) Lee S. P., Honda K., Rhee Y. H., Inoue S., *Neurosci. Lett.*, **111**, 217–221 (1990).
- 8) Jung N. P., Jin S. H., *J. Ginseng Sci.*, **20**, 431–471 (1996).

- 9) Kimura T., Saunders P. A., Kim H. S., Rhee H. M., Oh K. W., Ho I. K., *Gen. Pharmacol.*, **25**, 193—199 (1994).
- 10) Cha H. Y., Seo J. J., Park J. H., Choi K. J., Hong J. T., Oh K. W., *J. Ginseng Res.*, **28**, 132—135 (2004).
- 11) Kwon S. W., Han S. B., Park I. H., Kim J. M., Park M. K., Park J. H., *J. Chromatogr. A*, **921**, 335—339 (2001).
- 12) Park I. H., Kim J. M., Piao L. Z., Kwon S. W., Kim N. Y., Han S. B., Park M. K., Park J. H., *Arch. Pharm. Res.*, **25**, 837—841 (2002a).
- 13) Park I. H., Kim N. Y., Han S. B., Kim J. M., Kwon S. W., Kim H. J., Park M. K., Park J. H., *Arch. Pharm. Res.*, **25**, 428—432 (2002b).
- 14) Hogg S., *Pharmacol. Biochem. Behav.*, **54**, 21—30 (1996).
- 15) Rodgers R. J., Johnson N. J. T., *Pharmacol. Biochem. Behav.*, **59**, 221—232 (1997).
- 16) Pellow S., File S. E., *Pharmacol. Biochem. Behav.*, **24**, 525—529 (1986).
- 17) Rex A., Stephens D. N., Fink H., *Pharmacol. Biochem. Behav.*, **53**, 1005—1011 (1996).
- 18) Pellow S., Chopin P., File S. E., Briley M., *J. Neurosci. Methods*, **14**, 149—167 (1985).
- 19) Dalvi A., Rodgers R. J., *Pharmacol. Biochem. Behav.*, **62**, 727—734 (1999).
- 20) Fernandes C., Arnot M. I., Irvine E. E., Bateson A. N., Martin I. L., File S. E., *Psychopharmacology*, **145**, 251—259 (1999).
- 21) Dawson G. R., Tricklebank M. D., *Trends Pharmacol. Sci.*, **16**, 33—36 (1995).
- 22) Kulkarni S. K., Reddy D. S., *Methods Find. Exp. Clin. Pharmacol.*, **18**, 219—230 (1996).
- 23) Soderpalm R., Hjorth S., Engel J. A., *Pharmacol. Biochem. Behav.*, **32**, 259—265 (1989).
- 24) Treit D., *Neurosci. Biobehav. Rev.*, **9**, 203—222 (1985).
- 25) Tokuyama S., Oh K. W., Kim H. S., Takahashi M., Kaneto H., *Jpn. J. Pharmacol.*, **59**, 423—425 (1992).
- 26) Kim H. S., Hong Y. T., Oh K. W., Seong Y. H., Rhee H. M., Oh S., Park W. K., Jang C. G., *Gen. Pharmacol.*, **30**, 783—789 (1998a).
- 27) Kim H. S., Hong Y. T., Jang C. G., *J. Pharm. Pharmacol.*, **50**, 555—560 (1998b).
- 28) Kim H. S., Kim K. S., Oh K. W., *Pharmacol. Biochem. Behav.*, **63**, 407—412 (1999).
- 29) Beaubrun G., Gray G. E., *Psychiatr. Serv.*, **51**, 1130—1134 (2000).
- 30) Kim W. Y., Kim J. M., Han S. B., Lee S. K., Kim N. D., Park M. K., Kim C. K., Park J. H., *J. Nat. Prod.*, **63**, 1702—1704 (2000).
- 31) Kim H. S., Hwang S. L., Nah S. Y., Oh S., *Pharmacol. Res.*, **43**, 473—479 (2001).