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Anxiolytic-like Effects of *Panax ginseng* on the Elevated Plus-maze Model in Mice

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Abstract – This study was performed to investigate the anxiolytic-like effects *Panax ginseng* in mice using the elevated plus-maze model. Furthermore, the anxiolytic-like effects of *Panax ginseng* were compared to a known active anxiolytic drug, diazepam. Ginseng total saponin (GTS, 100 mg/kg) from red ginseng (RG), sun ginseng (SG) total extract (50 mg/kg), butanol fraction of SG (25 and 50 mg/kg) and ginsenosides (Rb_1, Rg_1, and Rg_5 and Rk mixture) significantly increased the number of open arm entries and the time spent on the open arm, compared with that of control. However, Red ginseng (RG) total extract (100 mg/kg), GTS (25, 50 mg/kg), SG total extract (25 mg/kg) and ginsenosides (Rg_3-R and Rg_3-S) did not increase the number of open arm entries and the time spent on the open arm. On the other hand, butanol fraction of RG (100 mg/kg), total extract of SG (50 mg/kg), butanol fraction of SG (50 mg/kg), ginsenosides (Rb_1, and Rg_5 and Rk mixture) decreased the locomotor activity, in a similar fashion to diazepam. These data support that ginseng has the anxiolytic-like effects and the anxiolytic potential of SG was stronger than that of RG. Ginsenosides Rb_1, Rg_1, and Rg_5 and Rk mixture play important role on the anxiolytic-like effects of *Panax ginseng*. We provide evidence that ginseng and some ginsenosides may be useful for the treatment of anxiety.

Keywords □ *Panax ginseng*, SG, RG, ginsenosides, anxiolytic-like effects, elevated plus-maze, open arm, closed arm, locomotor activity, mouse

INTRODUCTION

Anxiety has become a highly important area of psychopharmacology research during this decade. One-eighth of the world's population is affected by anxiety (Eisenberg *et al.*, 1998). The symptoms of anxiety become quite discomforting and can interfere with a person's ability to function effectively. Benzodiazepines are still the most frequently used drugs for the treatment of generalized anxiety disorder despite of their undesirable side effects such as muscle relaxation, sedation, physical dependence, memory disturbance, and interaction with other drugs (Rang *et al.*, 1995).

On the other hand, there is considerable interest in the development of new anxiolytics. Various types of herbal medicines have been used as anxiolytic drugs in different parts of the world (Beaubrum and Gray, 2000). Various types of herbal medicine have been used as anxiolytic agents in different parts of the world, and several plants have been reported to possess anxiolytic activity. 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 *Albizzia lebbeck* from India are known to have anxiolytic effects (Rex *et al.*, 2002; Friede and Freudenstein, 2002; Heinrich and Gibbons, 2001). *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 (Lee *et al.*, 1990). 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-ben-

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zodiazepine chloride channel complex (Jung and Jin, 1996). Ginseng saponins increased the affinity of specific binding of [^{3}H]-baclofen and [^{3}H]-flunitrazepam in crude synapse membrane from the rat frontal cortex (Kimura et al., 1994).

Of the three kinds of ginseng, white ginseng is air-dried ginseng, red ginseng (RG) is produced by steamed raw ginseng at 98-100°C for 2-3 hours and sun ginseng (SG) is ginseng steamed at 120°C using on autoclave. RG is reportedly more pharmacologically active than white ginseng. The different biological activities of red and white ginseng may result from production of different chemical constituents during steaming treatment. Several investigators have reported new ginsenosides from RG that are not usually found in raw ginseng (Ryoji et al., 1983; Baek et al., 1996). Ginseng saponins, referred to as ginsenosides Rb_1, Rg_1, Rg_3-R, Rg_3-S, and Rg_5 and Rk mixture, are believed to have a pharmacologically important role. Steamed at higher temperature, SG abounds with ginsenosides Rg_3, Rg_5 and Rk_1 which are not found in white ginseng. And then four new acetylated ginsenosides, Rs_4, Rs_5, Rs_6, and Rs_7, and three new dammarane glycosides, Rk_1, Rk_2, and Rk_3, were isolated from SG (Park *et al.*, 2002a; Park *et al.*, 2002b). SG contains these different types of ginsenosides, was reportedly more potent than RG at inducing certain pharmacological effects such as expanding blood vessels and inducing antiplatelet activity (Kim *et al.*, 2000).

Therefore, the present study was undertaken to investigate the anxiolytic-like effects of diverse fractions and compounds of *Panax ginseng*. Furthermore, their effects were compared with that of diazepam to determine whether the behavioral profile of *Panax ginseng* were differed from an established anxiolytic drug, 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 mice were housed in acrylic cages (45 × 60 × 25 cm) with water and food available *ad libitum* under an artificial 12-hour 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 drugs

RG and ginseng total saponin (GTS) purified from the water extract of RG, Water extracts of RG and butanol fractions of RG were provided from the Korea Ginseng & Tobacco Central Research Institute, and water extract of SG and butanol fraction of SG were provided from Ginseng Science LTD, respectively. The water extract of RG was manufactured by the Korea Ginseng & Tobacco Central Research Institute from the roots of a 6-year-old fresh *Panax ginseng*. The yields of for saponin fraction from the RG water extract were 4.4%. The butanol fraction of RG is characterized as saponin mixture quantitatively containing ginsenosides [Rb_1 (12.59%), Rb_2 (6.18%), Rc (6.86%), Rd (3.43%), Re(6.64%), Rf (2.06%), Rg_1 (15.79%), Rg_3 (1.37%)]. Total amount of saponins in RG butanol fraction was 56.29%. In addition, SG was produced by steaming (120°C, 3h) and drying the root of a 6-years old fresh *Panax ginseng* as in the previous report (Kwon et al., 2001). Main composition of SG butanol fraction was ginsenosides-Rb_1 (4.47%), Rb_2 (4.83%), Rc (4.93%), Rg_3 (23.79%), Rk_1 (12.3%), Rg_5 (13.13%). Total amount of saponins in butanol fraction of SG was 63.75%. On the other hand, Total amount of saponins as ginsenosides of SG is 10.0%. Mice were given a single oral administration of the water extract of RG (100 mg/kg), butanol fraction (total crude saponins) of RG (100 mg/kg), water extract of SG (25 and 50 mg/kg) and butanol fraction of SG (25 and 50 mg/kg), 1 hour before their placement on the elevated plus-maze.

Ginsenosides Rb_1, Rg_1, Rg_3-S, Rg_3-R, and Rg_5 and Rk mixture were kindly provided from the Ginseng Science Inc. Rg_3-S, Rg_3-R, and Rg_5 and Rk mixture, which are not usually found in white ginseng, were isolated in the ginseng steamed at higher temperature. Those compound are unique constituents of red and sun ginseng, and more abundant in sun ginseng than red ginseng (Park *et al.*, 2002a; Park *et al.*, 2002b; Kown *et al.*, 2001). Mice were given a single oral administration of each ginsenoside 60 min before their placement on the elevated plus-maze. Diazepam (2 mg/kg, Dae-Won Pharm. Co. Korea) was administered orally 30 min before their placement in the elevated plus maze. All tested ginseng each fraction and ginsenosides were dissolved in 0.9% physiological 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 (Fogg, 1996; Rodgers *et al.*, 1997) Briefly, the plus-maze apparatus was comprised of two open arms (30 × 5 cm) and two closed arms (30 × 5 × 15 cm) that extend from a common cen-

tral 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 above the floor level, as has been validated and described (Pellow and File, 1986). 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 × time spent on open arm/total time in the plus maze); and 2) the number of entries onto both open and closed arms, expressed as a percentage (100 × open/total entries). Four paws into 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.

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±SEM. 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 effect of RG and GTS

Behavior observed in the elevated plus-maze confirmed the anxiolytic activity of diazepam reported previously (Rex *et al.*, 1996; Pellow *et al.*, 1985; Dalvi and Rodgers, 1999; Fernandes *et al.*, 1999). As the positive control, diazepam 2 mg/kg increased open arm entries and time spent on open arms (Fig. 1).

The GTS (100 mg/kg) increased the percentage of open arm

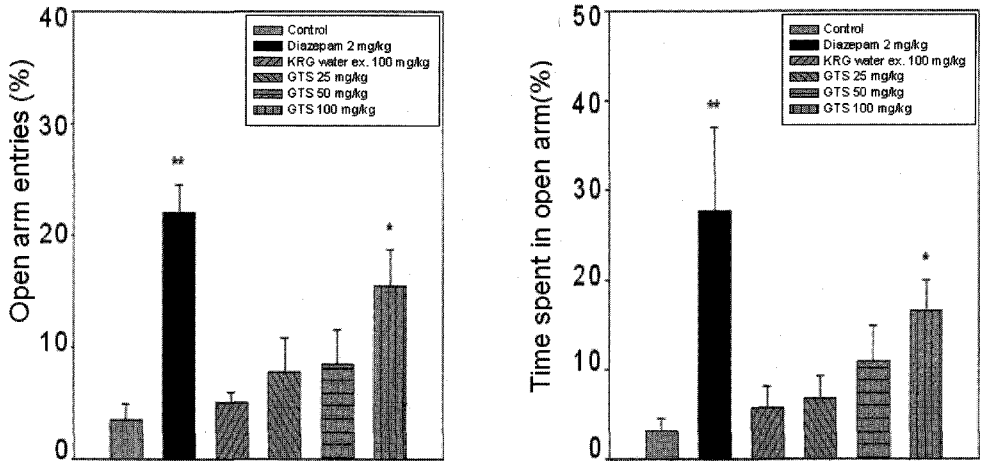

Fig. 1. Effects of diazepam, RG (KRG) and GTS on the percentage of open arm entries and time spent in the elevated plus-maze. Data were expressed as mean±SEM from group of at least 10 mice. *P<0.05, **P<0.01, compared with that of vehicle-treated control (one way ANOVA following by Dunnett's test).

entries and percentage of time spent on open arms, but the GTS (25 and 50 mg/kg) did not (Fig. 1), compared with that of saline group. On the other hand, the KRG water extract (100 mg/kg) did not increase the percentage of time spent on open arms or the percentage of open arm entries (Fig. 1). There were anxiolytic-like effects only in GTS.

Anxiolytic-like effect of RG and SG

The RG butanol fraction (100 mg/kg) increased the percentage of open arm entries and percentage of time spent on open arms, but the RG total extract (100 mg/kg) and RG butanol fraction (25 and 50 mg/kg) did not (Fig. 2), compared with that of saline group. On the other hand, the SG total extract (50 mg/kg) and the SG butanol fraction (25 and 50 mg/kg) increased the percentage of open arm entries and percentage of time spent on open arms, while the SG butanol fraction (25 mg/kg) did not increase the percentage of time spent on open arms or the percentage of open arm entries (Fig. 3). There were anxiolytic-like effects in RG and SG both. The anxiolytic potential of SG was stronger than that of RG in the elevated plus-maze model.

Anxiolytic-like effect of ginsenosides

Ginsenosides Rb_1 (10, 25, and 50 mg/kg), Rg_1 (10, 25, and 50 mg/kg), and the Rg_5 and Rk mixture (25 and 50 mg/kg) increased the percentage of open arm entries, compared with that of vehicle treated-control animals, respectively (Fig. 4, 5, 7). On the other hand, ginsenosides Rb_1 (25 and 50 mg/kg), Rg_1 (50 mg/kg), and the Rg_5 and Rk mixture (50 mg/kg) increased

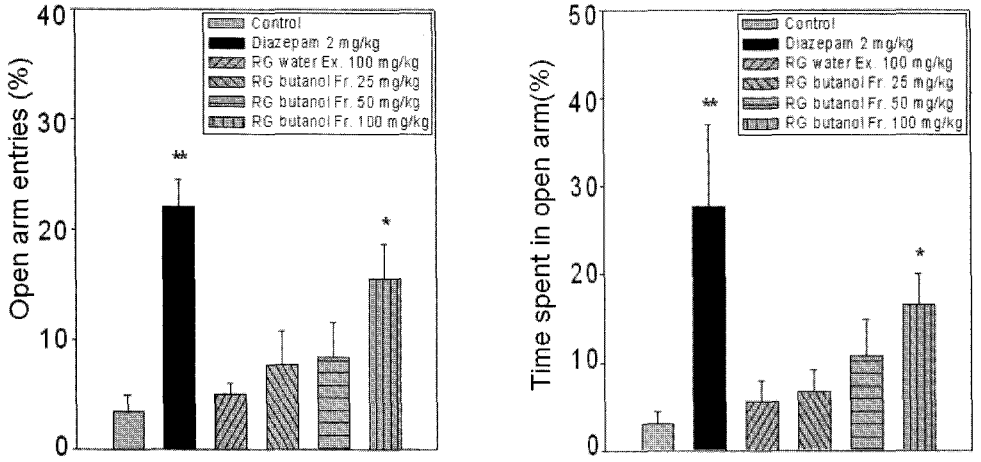

Fig. 2. Effects of diazepam, RG water extract (ex.) and RG butanol fraction (fr.) on the percentage of open arm entries and time spent in the elevated plus-maze. *P < 0.05, **P < 0.01, compared with that of vehicle-treated control.

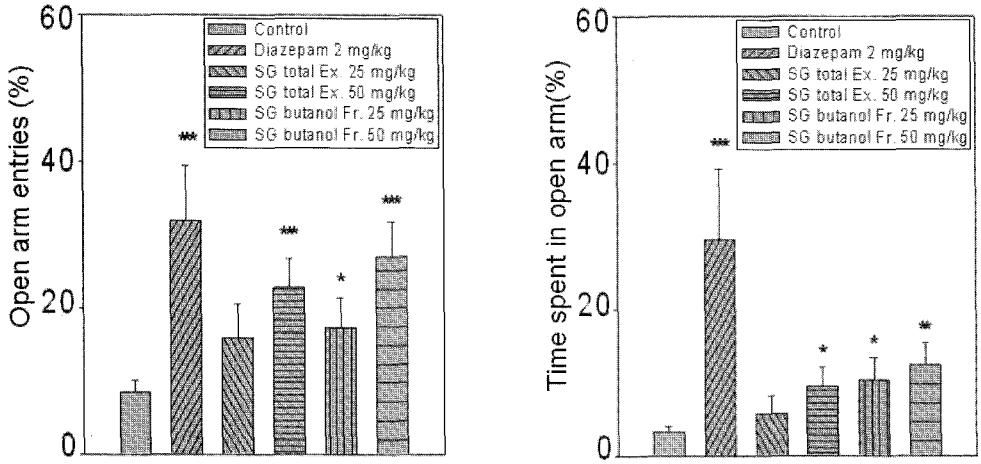

Fig. 3. Effects of diazepam, SG water extract (ex.) and SG butanol fraction (fr.) on the percentage of open arm entries and time spent in the elevated plus-maze. *P < 0.05, **P < 0.01, ***P < 0.005, compared with that of vehicle-treated control.

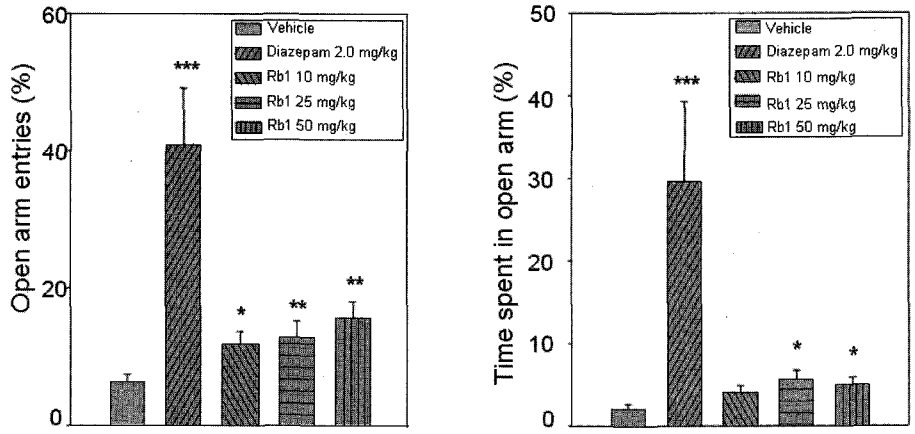

Fig. 4. Effects of diazepam and ginsenoside Rb_1 on the percentage of open arm entries and time spent in the elevated plus-maze. *P < 0.05, **P < 0.01, ***P < 0.005, compared with that of vehicle-treated control.

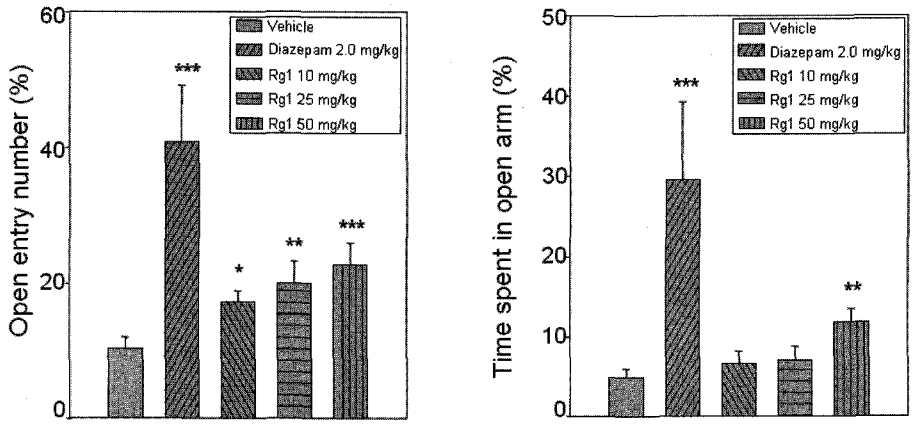

Fig. 5. Effects of diazepam and ginsenoside Rg_1 on the percentage of open arm entries and time spent in the elevated plus-maze. *P < 0.05, **P < 0.01, ***P < 0.005, compared with that of vehicle-treated control.

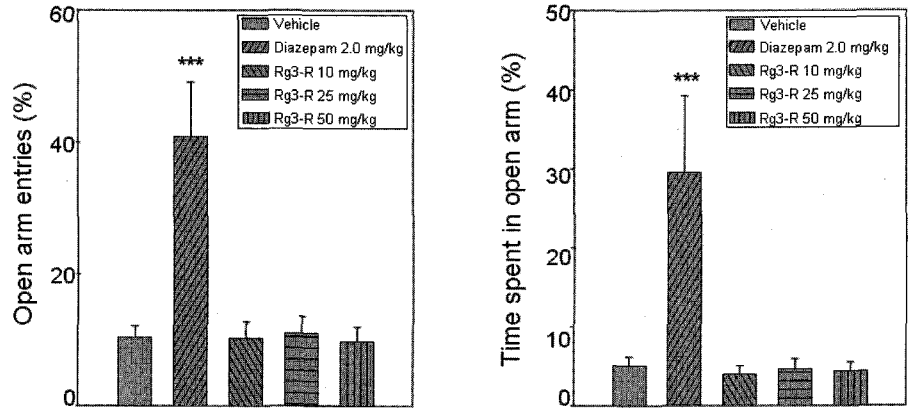

Fig. 6. Effects of diazepam and ginsenoside Rg_3-R on the percentage of open arm entries and time spent in the elevated plus-maze. ***P < 0.005, compared with that of vehicle-treated control.

percentage of time spent on open arms (Fig. 4, 5, 7). However, ginsenosides Rg_3-S and Rg_3-R did not increase the percentage of open arm entries and the percentage of time spent on open arms (Fig. 6, 8).

Effects of RG and GTS on spontaneous locomotor activity

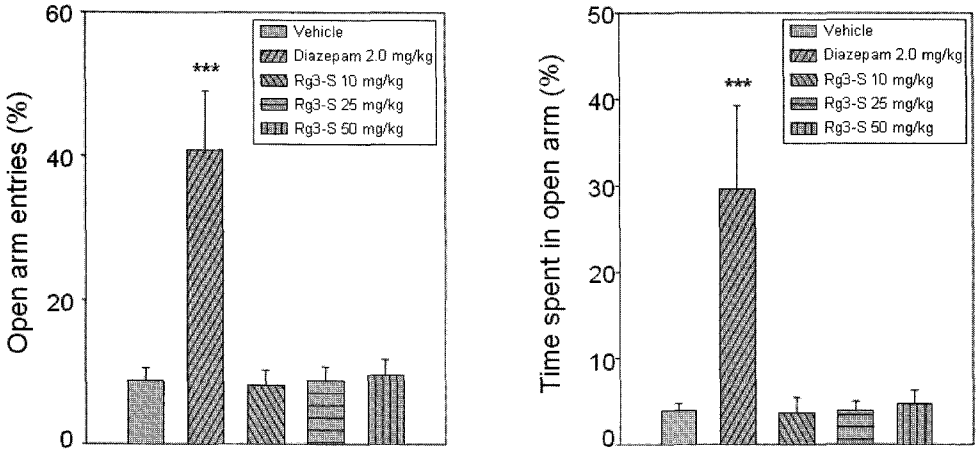

Fig. 7. Effects of diazepam and ginsenoside Rg_3-S on the percentage of open arm entries and time spent in open arms on the elevated plus maze. ***P < 0.005, compared with that of the vehicle-treated control.

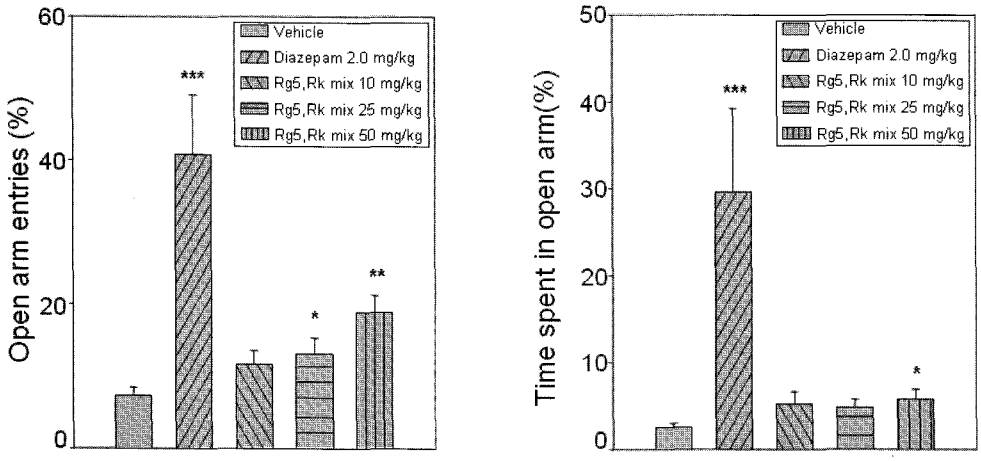

Fig. 8. Effects of diazepam and ginsenoside Rg_5 and Rk mixture on the percentage of open arm entries and time spent in the elevated plus-maze. *P < 0.05, **P < 0.01, ***P < 0.005, compared with that of vehicle-treated control.

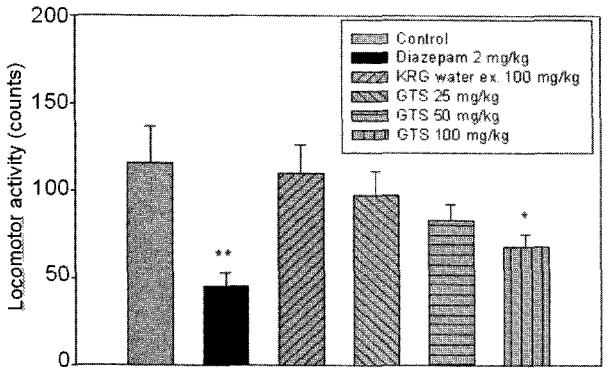

Fig. 9. Effects of diazepam, RG (KRG) and GTS on locomotor activity in mice. Data are expressed as mean values (±SEM) from groups of at least 10 mice. *P < 0.05, **P < 0.01, compared with that of the vehicle-treated control.

Figure 9 shows the cumulative locomotor activity during the 60 min test period. Locomotor activity was significantly decreased by diazepam. Locomotor activity was also decreased in animals pretreated with ginseng, compared to vehicle treated animals. Similar to the effect induced by diazepam and the GTS (100 mg/kg) induced decreases in locomotor activity following administration at the anxiolytic-inducing dose. However, the RG water extract (100mg/kg), GTS (25 and 50 mg/kg) did not decrease locomotor activity.

Effects of RG and SG on spontaneous locomotor activity

Similar to the effect induced by diazepam, the RG butanol fraction (100 mg/kg), the SG total extract (50 mg/kg), and the SG butanol fraction (50 mg/kg) induced decreases in locomotor activity following administration at the anxiolytic-inducing dose. However, the RG total extract, the SG total extract (25

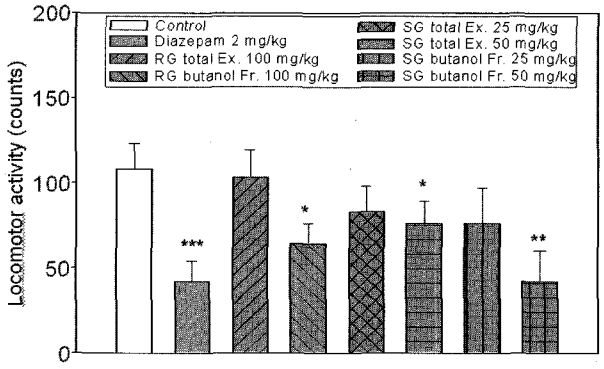

Fig. 10. Effects of diazepam, RG and SG on locomotor activity in mice. *P < 0.05, **P < 0.01, ***P < 0.005, compared with that of the vehicle-treated control.

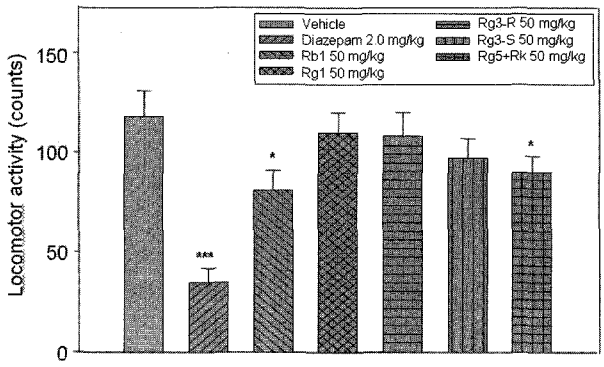

Fig. 11. Effects of diazepam, ginsenosides Rb_1, Rg_1, Rg_3-R, Rg_3-S, and Rg_5 and Rk mixture on locomotor activity in mice. *P < 0.05, **P < 0.01, ***P < 0.005, compared with that of the vehicle-treated control.

mg/kg) and SG butanol fraction (25 mg/kg) did not decrease locomotor activity.

Effects of ginsenosides on spontaneous locomotor activity

Locomotor activity was decreased in animals pretreated with ginsenoside Rb_1 (50 mg/kg) and Rg_5 and Rk mixture (50 mg/kg), compared with that in the vehicle-treated group. However, ginsenoside Rg_1 (50 mg/kg) did not decrease locomotor activity (Fig. 11). 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 (Dawson and Tricklebank, 1995; Kulkarni and Reddy, 1996). 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 (Soderpalm *et al.*, 1989). In the present study, a single acute administration of diazepam caused anxiolytic behavior. Compared with the control, diazepam increased the number of entries onto the open arms and prolonged the time spent on the arms. The present results confirm previous reports of others (Rex *et al.*, 1996; Pellow *et al.*, 1985; Dalvi and Rodgers, 1999; Fernandes *et al.*, 1999).

In order to determine the effective doses of ginseng in the elevated plus-maze model, various doses of the total extracts of RG and SG (data not shown) were administered. The RG total extract (100 mg/kg) did not increase the percentage of open arm entries or the time spent on open arms in this experiment. However, the low dose (25 mg/kg) of the SG total extract administered induced anxiolytic-like effects as compared to RG. Moreover, the anxiolytic potential of the ginseng saponin fraction was stronger than that of the total extract of ginseng. In agreement with these results, we previously reported that SG, which contains these different types of ginsenosides, was more potent than RG at inducing some pharmacological effects, such as expanding blood vessels and inducing anti-platelet activity (Kim *et al.*, 2000). Therefore, ginseng saponin probably plays an important role in inducing the anxiolyic-like effects in the plus-maze model. Saponins containing the *Albizzia. lebbeck* fraction possessed nootropic and anxiolytic activity (Une *et al.*, 2001). The anxiolytic effects of drugs such as benzodiazepines are accompanied by decreased locomotor activity and sedation (Treit, 1985). We previously reported that ginseng saponins inhibited psychostimulant-induced hyperactivity (Tokuyama *et al.*, 1992; Kim *et al.*, 1995a; Kim *et al.*, 1995b). Development of new anxiolytics that do not induce sedative effects and/or inhibit locomotion would be highly useful.

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 of the $GABA_A$ and $GABA_B$ receptors. In particular, ginsenosides enhance specific [^{3}H]-flunitrazepam binding and increase the affinity of [^{3}H]-flunitrazepam binding (Kimura *et al.*, 1994). In addition, the level of [^{3}H]-muscimol binding was strongly elevated in almost all regions of the frontal cortex after administration of ginsenoside Rc, but was decreased after ginsenoside Rg_1 (Kim *et al.*, 2001). Ginseng saponins administered at high dose prolonged the pentobarbital sleeping time and delayed the onset of convulsions in behavioral studies (Jung and Jin, 1996). 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.

Our study demonstrates that ginseng has anxiolytic-like effects, and SG induced greater anxiolytic-like effects than RG in the elevated plus-maze model. Ginseng saponins, referred to as ginsenosides, are believed to be pharmacologically important. Accordingly we were interested in the anxiolytic-like effects of ginsenosides such as Rb_1, Rg_1, Rg_3-R, Rg_3-S, and Rg_5 and Rk mixture from ginseng saponins. In this study, we found that Rb_1, Rg_1, and Rg_5 and Rk mixture increased the percentage of open arm entries and time spent in open arms showing anxiolytic effects in this model. The anxiolytic effects of drugs such as benzodiazepines are accompanied by decreased locomotor activity and sedation (Treit, 1985). We previously reported that ginsenosides Rb_1 and Rg_1 inhibited psychostimulant-induced hyperactivity (Tokuyama, 1992; Kim *et al.*, 1998a; Kim *et al.*, 1998b; Kim *et al.*, 1999). In agreement with previous reports, ginsenoside Rb_1 inhibited locomotor activity in this experiment. However, ginsenoside Rg_1 inhibited locomotor activity to a less extend than diazepam, thus has a better profile for an anxiolytic medicine. There is considerable interest in the development of new anxiolytics that do not induce sedative effects and do not inhibit locomotion.

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

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