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Anxiolytic Effects of Total Saponin Fraction from *Ginseng Radix Rubra* on the Elevated Plus-Maze Model in Mice

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Abstract : This study was performed to investigate the anxiolytic effects of total sponin fraction from *Ginseng Radix Rubra* (KRG) in mice using the elevated plus-maze model. The water extract of KRG and ginseng total saponins (GTS) purified from the water extract of KRG were administered orally to mice. One hour after administration of KRG water extract and GTS, mice were tested on the elevated plus-maze. The water extract of KRG 100 mg/kg, and GTS 25 and 50 mg/kg did not increase open arm entries and time spent on open arms. However, GTS 100 mg/kg increased the number of open arm entries and time spent on open arms. On the other hand, as the plus-maze test was affected by changes in locomotor activity, an additional test was carried out with the specific aim of monitoring locomotor activity. The water extract of KRG 100 mg/kg, and GTS 25 and 50 mg/kg did not affect the locomotor activity. However, GTS 100 mg/kg significantly decreased locomotor activity. From this study, we suggest that GTS may play an important role on the anxiolytic effects in the plus-maze model.

Key words : Ginseng total saponin, anxiolytic, elevated plus-maze, *Ginseng radix rubra* locomotion

INTRODUCTION

Anxiety is affecting one-eight of the total population of the world and become a very important area of research interest in psychopharmacology during this decade. Benzodiazepines form the most important group used as anxiolytic agents, but generally cause sedation and drowsiness which is one of the main drawbacks in the clinical use of anxiolytic drugs.

In recent years, various type of herbal medicines have been used as anxiolytic drugs in different parts of the world. The essential oil of *Stachys lavanduifolia* possesses anxiolytic effects with relatively lower sedative activity than diazepam.[1)] *Panax ginseng,* as a folk medicine, has been used for antioxidant, anticancer, antifatigue, and vasodilator in far easter countries. On the other hand, ginseng administration in animals has shown behavioral changes which seem to be related to the regulation of gamma-amino-butyric acid (GABA)ergic neurotransmission. Ginseng saponin prolonged pentobarbital sleeping time and delayed the onset of convulsion in high dose.[2,3)] It was reported that chronic intake of *Panax ginseng* extract stabilized sleep and wakefulness in food-deprived rats.[4)] Ginsenoside Re is a potent inhibitor of neurotransmitter inhibitor, specially, GABA.[5)] We reported that ginsenosides interact with ligand-bindings of $GABA_A$ and $GABA_B$ receptors.[6)]

Therefore, the aims of present study were to investigate the anxiolytic effects of KRG in the elevated plus-maze, a behavioral test for anxiolytic drugs. Furthermore, the effects of KRG and diazepam on the elevated plus-maze were compared to determine whether the behavioral profile of KRG is differed from an established anxiolytic drug, diazepam.

MATERIALS AND METHODS

Animals and drugs

Male ICR mice weighing 20-28 g, purchased from Samtako (Suwon), in a group of 10-15, were used in all experiments. Groups of 10 mice housed in acrylic cages with water and food availability *ad libitum* under an artificial 12-hour light/dark cycle (light on at 07 : 00), and at the constant temperature (22±2°C). The water extract of

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KRG and ginseng total saponins (GTS) purified from the water extract of KRG was kindly provided from the Korea Ginseng & Tobacco Central Research Institute. The water extract of KRG 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 water extract of KRG were 4.37%. GTS characterized saponin mixture quantitatively containing at least 11 glycosides as known 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_2 (3.62%), Rg_3 (1.37%), Ro (2.82%), Ra (2.91%), and other minor ginsenosides and components (35.73%)]. Mice were given a single oral administration of the water extract of KRG (100 mg/kg) and GTS (25, 50 100 mg/kg) 1 hour before their placement on the elevated plus-maze. Diazepam (2 mg/kg) was injected 30 min before their placement on the elevated plus-maze.

Measurement of anxiolytic effects

One of the best-established devices for observing anxiety-like behavior in rodents is the elevated plus-maze model. This apparatus was elevated above a table (38 cm above the floor) and consisted of two arms that intersect to form the shape of plus sign. The sides of one arm (30×5cm) were closed and the sides of the other arm were opened (30×5×15cm). An animals is placed at the intersection of the arms (5×5cm) faced to closed arm or open arm, and the amount of time it chose to spend in each arm was measured. An entries into an arm was defined as the animal placing all four paws over the line marking that area. The number of entries and the time spent in the open and closed arms were recorded during a 5-min test period as described.[7] The percentage of open arm entries (100×open/total entries) was calculated for each animals[8]. The behavioral experiments took place under quiet conditions, and low light (50 lux) and carried out between 13 : 00-17 : 00. Between each trial, the maze was wiped clean with a damp sponge and dried with paper towels.

Locomotor activity

Immediately after the elevated plus-maze test, the locomotor activity of the mice was measured with a tilting-typed ambulometer (AMB-10; O'Hara Co., Ltd., Tokyo, Japan). Each mouse was placed in an tilting-typed cage (20cm in diameter and 18cm in height) 5 min after administration and the ambulatory activity was measured for 60 min. Each mouse was tested individually.

Statistics

All data are expressed mean±S.E.M. Statistical analysis was performed using one-way ANOVA. In the case of significant variation, the individual values are compared by Duncan's test.

RESULTS AND DISCUSSION

In the elevated plus-maze, the behavior observed confirmed the anxiolytic activity of diazepam as reported pre-

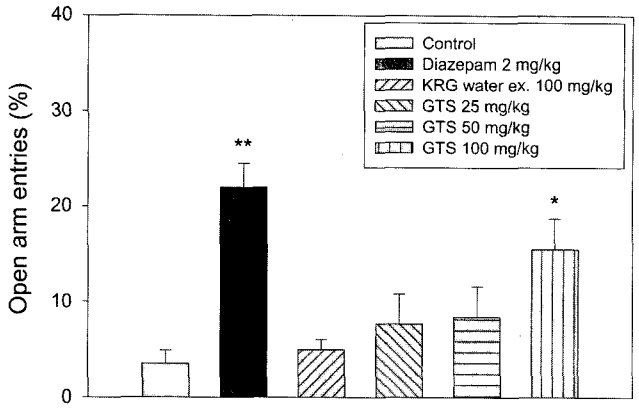

Fig. 1. Effects of diazepam, KRG water extract and GTS on the percentage of open arm entries. Diazepam was injected 30 min prior to test. KRG water extract and GTS were administered orally to mice 1 hour prior to test. Data are presented as mean values (±S.E.M) from group of at least 10 mice. $^{*}P<0.05$, $^{**}P<0.01$, compared with that of vehicle-treated control.

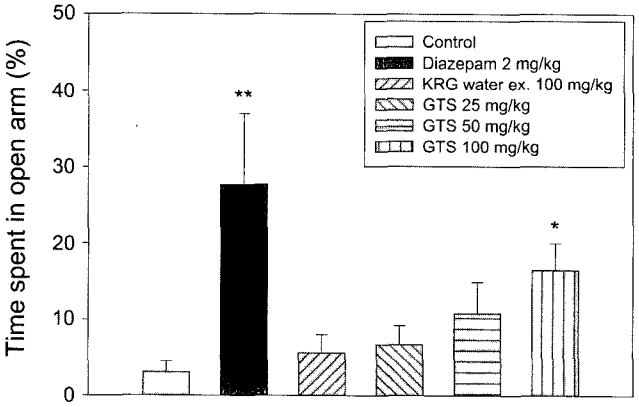

Fig. 2. Effects of diazepam, KRG water extract and GTS on the percentage of spent time in open arm. Diazepam was injected 30 min prior to test. KRG water extract and GTS were administered orally to mice 1 hour prior to test. Data are presented as mean values (±S.E.M) from group of at least 10 mice. $^{*}P<0.05$, $^{**}P<0.01$, compared with that of vehicle-treated control.

viously.[9] In order to determine the effective dose on the elevated plus-maze, various doses of the water extract of KRG (data not shown). KRG water extract at 100 mg/kg, and GTS 25 and 50 mg/kg did not increase the percentage of open arm entries and percentage of the time spent in open arm (Fig. 1 and 2). However, GTS 100 mg/kg significantly increased the percentage of open arm entries and percentage of the time spent in open arm. In a similar fashion, diazepam increased the percentage of open arm entries and percentage of the time spent in open arm. Therefore, saponin fraction from KRG may play an important role on the anxiolytic effect of KRG. On the other hand, the effect of the water extract of KRG and GTS on locomotor activity was measured for 60 min. Locomotor activity was not decrease in animals administered orally with KRG water extract (100 mg/kg) and GTS (25, 50 mg/kg), compared with that of saline treated controls (Fig. 3). Anxiolytic effects of diazepam 2 mg/kg were measured as control. Administrations of diazepam 2 mg/kg and GTS 100 mg/kg significantly suppressed locomotor activity, respectively.

In the present study, we used the elevated plus-maze model of anxiety to evaluate the anxiolytic effects of the water extract KRG and GTS. As expected, diazepam produced significant increases in the number of open arm entries and the time spent in open arm. These increases were accompanied by statistically significant changes in motor activity, as was indicated by locomotor activity counts. There are evidences that GTS inhibits psychostimulants-induced hyperactivity in mice.[10,11] These data

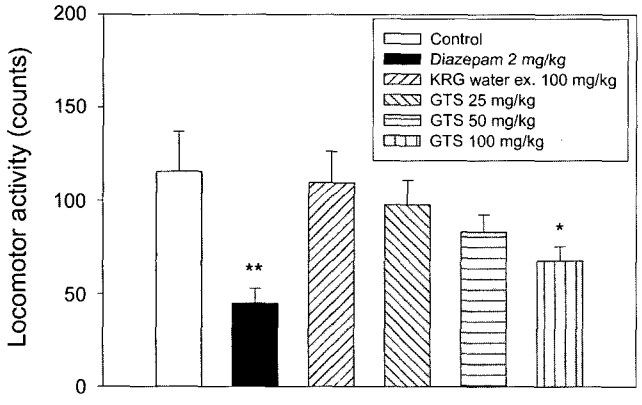

Fig. 3. Effects of diazepam, KRG water extract and GTS on spontaneous locomotor activity. The locomotor activity counts (mean±S.E.M) were measured for 1 hour period with a tilting-typed ambulometer. Data are presented as mean values (±SEM) from group of at least 10 mice. $^{*}P<0.05$, $^{**}P<0.01$, compared with that of vehicle-treated control.

were consistent with the results of numerous previous studies, which have shown that diazepam and other benzodiazepines produce robust anxiolytic effects in a variety of anxiolytic screening procedures, including conflicting models,[12] elevated plus-maze model procedures,[8] other non-punishment procedures,[13,14] and drug discrimination models.[15] The anxiolytic effect of GTS 100 mg/kg was accompanied by a decrease in the locomotor activity producing slight sedation at the utilized doses (100 mg/kg). It is well known that many drugs such as benzodiazepines possess anxiolytic and sedative effects.[16]

We reported that ginsenosides interact with ligand-bindings of $GABA_A$ and $GABA_B$ receptors. Especially, ginsenosides enhance the specific [^{3}H]flunitrazepam binding without non-specific binding and increased the affinity of [^{3}H]flunitrazepam binding[6]. The mechanism of action is still unclear. However, we suggest that GTS interacts with $GABA_A$-benzodiazepine receptor complex.

The results of this study demonstrate that GTS from KRG possesses anxiolytic effects.

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