Biomolecules & Therapeutics, **16**, 118-125(2008)

Psychopharmacological Profile of the Water Extract of *Gardenia jasminoides* and Its Constituents, Genipin and Geniposide, in Mice

Ji Young Choi, Ike Dela Pena, Jong Hyun Choi, Seo Young Yoon, Dong Sool Yim, Yong Soo Lee[1], Kwang Ho Ko[2], Chan Young Shin[3], Jong Hoon Ryu[4], Won Ki Kim[5], and Jae Hoon Cheong*

Uimyung Institute for Neuroscience, Sahmyook University, Seoul 139-742, Korea
[1]Department of Pharmacology, College of Pharmacy, Duksung Women's University, Seoul 132-714, Korea
[2]Department of Pharmacology, College of Pharmacy, Seoul National University, Seoul 139-742, Korea
[3]Department of Pharmacology, College of Medicine, Konkuk University, Seoul 143-701, Korea
[4]Department of Oriental Pharmaceutical Science, College of Pharmacy, KyungHee University, Seoul 130-701, Korea
[5]Department of Neuoscience, College of Medicine, Korea University, Seoul 136-71, Korea

(Received April 28, 2008; Accepted May 29, 2008)

Abstract – *Gardenia jasminoides* (*G. jasminoides*) is traditionally used to treat insomnia, jaundice, emotional disorders, hepatic disease, and inflammatory disease. Previously, we found that geniposide and the water extract of *G. jasminoides* increased Cl^- influx in neuroblastoma. Here we examined the psychopharmacological activities of *G. jasminoides* and its constituents. *G. jasminoides* extract was orally administered at 100 and 200 mg/kg, and genipin and geniposide were intraperitoneally injected at 2, 10, and 20 mg/kg. *G. jasminoides* extract (200 mg/kg) significantly decreased total open field activity but increased rearing activity in the center of the open field, suggesting an increase in exploratory activity. Genipin and geniposide did not change open field activity, but geniposide (20 mg/kg) increased rearing activity in the central area. The extract (200 mg/kg) significantly decreased rotarod and wire-balancing activity, but genipin and geniposide did not. No compounds influenced thiopental-induced sleeping or electroshock-induced seizures. The extract (200 mg/kg) significantly increased staying time in the open arms of the elevated plus maze and the entry ratio into the open arms, and geniposide (20 mg/kg) also increased open arm entry. Electroshock stress decreased open arm activity, but the extract and geniposide (20 mg/kg) significantly reversed that effect. This results indicate that *G. jasminoides* extract and geniposide alleviated anxiety with greater efficacy in stressed animals than normal animals.

Keywords: *Gardenia jasminoides*, geniposide, genipin, anxiety, behavior

INTRODUCTION

Gardenia jasminoides (G. jasminoides) is an important medicinal herb in traditional Korean medicine. The fruit of *G. jasminoides* is widely employed in the treatment of inflammation, hypertension, jaundice, headache, edema and hepatic disorders (Koo *et al*., 2004; Shin *et al*., 2003). Geniposide and genipin are the major iridoid glycoside compounds existing in the fruit of gardenia and are responsible for its pharmacological activities such as hepatoprotective (Cgang, 1988), antithrombotic (Suzuki *et al*., 2001), neuroprotective (Sakura *et al*., 2001), neuritogenic (Yamazaki *et al*., 1996) and anti-inflammatory effects (Koo *et al*., 2004) and protective activity against oxidative damage (Okada *et al*., 2007), Oral administration of the water extracts of *Gardeniae fructus* and geniposide increased social interaction time in mice (Toriizuka *et al*., 2005). These results indicate that *Gardeniae fructus* and geniposide may exhibit anxiolytic activity.

Both pharmacological and genetic studies associate deficits in inhibitory neurotransmission in the mammalian forebrain with increased anxiety-related behavior, and γ-amino-butyric acid (GABA), the main inhibitory neurotransmitter in the mammalian central nervous system,

*Corresponding author
Tel: +82-2-3399-1605, Fax: +82-2-3399-1617
E-mail: cheongjh@syu.ac.kr

activates chloride ion channel and cause hyperpolarization of the neuron (Depino *et al*., 2008). The benzodiazepine-GABA system plays an important role in anxiety, benzodiazepine agonists acting at their site in the $GABA_A$ receptor complex produce anxiolytic effect (Argyropoulos *et al*., 2000). Initially, we found that geniposide and water extract of *G. jasminoides* increased Cl^- influx in neuroblastoma cell. Thus, *G. jasminoides* and its constituents may influence psychological activities. In this study, we tested the psychopharmacological activities of *G. jasminoides* and its constituents.

MATERIALS AND METHODS

Animals

The male ICR mice (20-25 g) used in this study were obtained from Hanlim experimental animal Co. (Hwasung, Korea). All animals were maintained on a standard right-dark cycle (6 A.M.-6 P.M.), at ambient temperature (22 ± 2^oC) and humidity ($55 \pm 5\%$). They had free access to food and water throughout the experiments. The animals were stabilized for 1 week in our animal room. The *G. fructus* extract was orally injected at 100 and 200 mg/kg. Genipin and geniposide were intraperitoneally injected at 2, 10 and 20 mg/kg. Animals of control group were intraperitoneally injected with the same volume of saline. Diazepam was intraperitoneally injected to mice in the positive control group.

Materials

We used dried fruit of *G. jasminoides* which was obtained from herbal suppliers in Seoul. Stir-baked Fructus gardeniae was boiled in water three times, and the resulting decoctions pooled. This solution was then clarified by centrifugation and filtration. The resulting primary extract was lyophilized. Geniposide was supplied by Natural product laboratory of Sahmyook university. Geniposide was isolated from the water extract of *G. jasminoides*. Diazepam was purchased from Samjin Pharm. Co. (Seoul, Korea) and thiopental sodium was purchased from Choongwae Pharm. Co. (Seoul, Korea). Genipin and other materials were purchased from Sigma-Aldrich Co. (St. Louis, Mo, USA).

Intracellular Cl^- measurement

Relative changes in intracellular Cl^- concentration ($[Cl^-]$) in IMR-32 human neuroblastoma cells were monitored using a Cl^--sensitive indicator, *N*-(6-methoxyquinolyl) acetoetylester (MQAE) (West and Molly, 1996). Briefly, cells were washed twice and re-suspended at a concentration of 4×10^5 cells/ml in Hank's solution. Cells were incubated with MQAE dye overnight at a final concentration of 5 mM at room temperature to load the dye into the cells. Fluorescence (excitation wavelength set at 365 nm and emission wavelength at 450 nm) was monitored in a well-stirred cuvette. All fluorescence values were corrected for background fluorescence, which was separately determined using a HEPES-buffered KSCN solution containing 5 μM valinomycin to maximally quench the MQAE ion-selective signal (Shumaker *et al*., 1999). In separate experiments the base line was determined by bathing the cells with Cl^--free (KNO_3) solution containing 10 mM tributyltin and 10 mM nigericin.

Behavioral apparatus

The behavioral changes of animals were monitored automatically using a computerized EthoVision system (Noldus IT b.v., Netherlands). In the locomotor activity, rota-rod test, balanced wire test and elevated plus-maze tests, the behavioral parameters were analyzed by automatic systems.

Locomotor activity

The apparatus consisted of 9 black plastic boxes (47×47 cm), and the field was bordered by 42-cm-high side walls. The total moved distance, total movement time and turn angles were monitored for 30 minutes after administration (Kim *et al*., 2003; Noldus *et al*., 2001).

Rota-rod test

Twenty-four hours before the experiment, all mice were habituated to running in a rota-rod at a speed of 60 rpm until they could remain there for 60 seconds without falling. The latency to fall and falling frequency were recorded with a stopwatch (Lee *et al*., 2005; Farkas *et al*., 2005).

Balanced wire test

Like the rota-rod test, rats were habituated to grasp horizontal wires (5 mm diameter, 150 cm length, elevated 80 cm above the floor) with their forepaws and tails. The balanced wire test was carried out for 20 min, and the latency time to fall and falling frequency were recorded (Lee *et al*., 2006).

Thiopental-induced sleep

Male ICR mice were treated subcutaneously with thiopental sodium (50 mg/kg) 30 min after intraperitoneal administration of the test materials. The time between the loss and recovery of the righting reflex was measured. Animals were observed for 30 min following thio-

pentalinjection. If no recovery was seen, sleep time was taken as 30 min for calculation purposes (Farkas *et al*., 2005).

Anticonvulsive activity test (electroshock model)

A constant current stimulator was used to evoke seizure, as determined by overt hind-limb extension. Animals were given electroshocks for 1 sec to determine the current-convulsion relationship. If an animal showed convulsion, the next animal was electrically shocked in 3 mA decrements of the current intensity. If this did not induce a convulsion in the animal, the next animal was electro shocked with 3 mA increments of the current intensity. This resulted in an establishment of a current-convulsion relationship. For each treatment group, 20-30 pups were prepared and the animals sacrificed immediately following determination of the electroshock seizure threshold (Park *et al*., 2007).

Induction of anxiety by electro-shock stress

The mice were exposed to electroshocks with an intensity of 0.5 mA (1 sec duration; 20 sec inter-shock interval) for 5 minutes (Lee *et al*., 2006). As soon as the final stress was loded, the animals were placed in the central square after measuring stress related activity and allowed to explore the maze freely for 5 minutes.

Elevated plus-maze test

The apparatus consisted of two open arms (30 × 6 cm in mice), alternating at right angles, with two arms enclosed by high walls of 20 cm. Each four arms has a delimited central area of 6 × 6 cm. The whole apparatus was placed 50 cm above the floor. Animals were placed in the central square after measuring stress related activity and allowed to explore the maze freely for 5 minutes. The parameters measured were the times spent in open and closed areas (Kim *et al*., 2003; Noldus *et al*., 2001).

Statistical analysis

Data are expressed as the mean ± S.E.M.. ANOVA was used to compare the scores among groups for one variable. This was followed by post hoc comparisons using the Newman-Keuls test. Differences with $p<0.05$ were considered to be statistically significant.

RESULTS

Cl^- ion inlux in neuroblastoma cells

Treatment with geniposide dramatically and dose-dependently increased intracellular Cl^- influx (Fig. 1), as did *G. jasminoides* extract, but genipin did not.

The locomotor activities

Locomotor activity included the total distance, duration of movement, total degrees of turn angles, and rearing frequency in the total and central areas (Fig. 2 and 3). Diazepam significantly decreased locomotor activity. *G. jasminoides* extract decreased the distance, movement duration, and total turn angle degree in the total area, but increased rearing frequency in the central area, with less efficacy than diazepam. Genipin and geniposide did not significantly affect locomotor activity.

Activities on the rota-rod and the balanced wire

Motor coordination was evaluated using the rota-rod test and the balanced wire. Diazepam significantly shortened the running time on the rota-rod and the balanced wire and

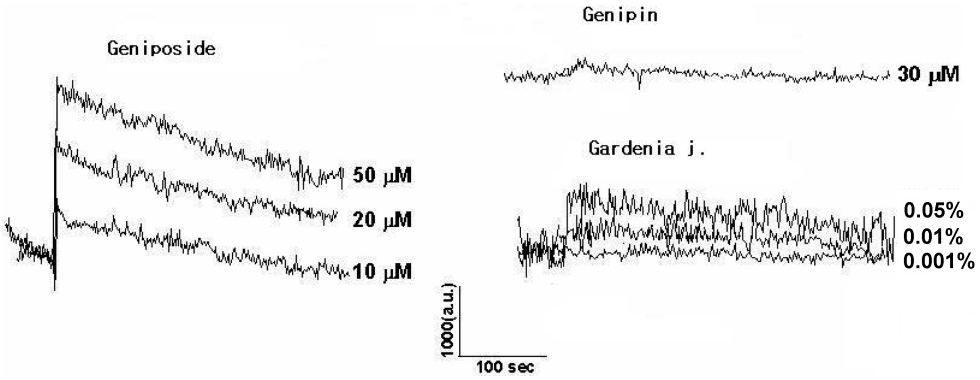

Fig. 1. Effects of *G. jasminoides* extract, genipin and geniposide on $[Cl^-]_i$ in neuroblastoma cells. Fluorescence was monitored in the excitation wavelength at 365 nm and the emission wavelength at 450 nm using the Cl^--sensitive indicator, *N*-(6-methoxyquinolyl) acetoetylester (MQAE). Contents of influx Cl^- ion was expressed as a peak (a.u.).

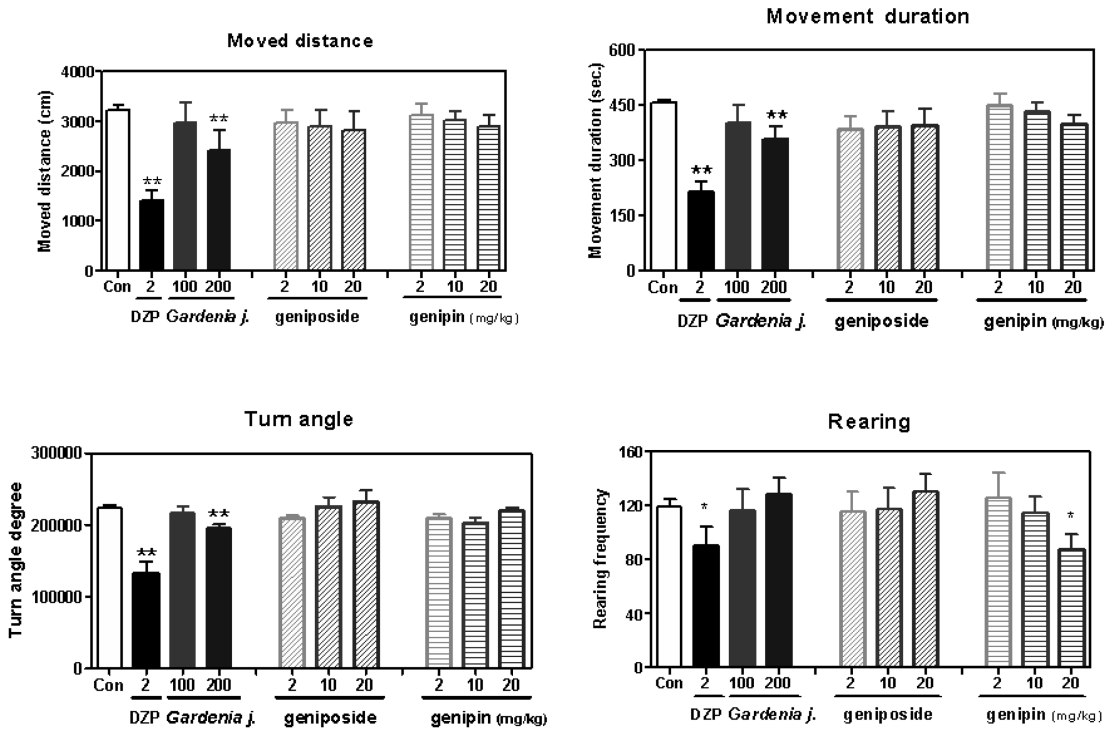

Fig. 2. Effects of *G. jasminoides* extract, genipin and geniposide on locomotor activity (total area) in mice (n=9). Each bar represents the mean±S.E.M of the moved distances, movement durations, turn angle degrees or rearing frequencies for 10 minutes. (**p*<0.05, ***p*<0.01 versus control group). DZP : diazepam.

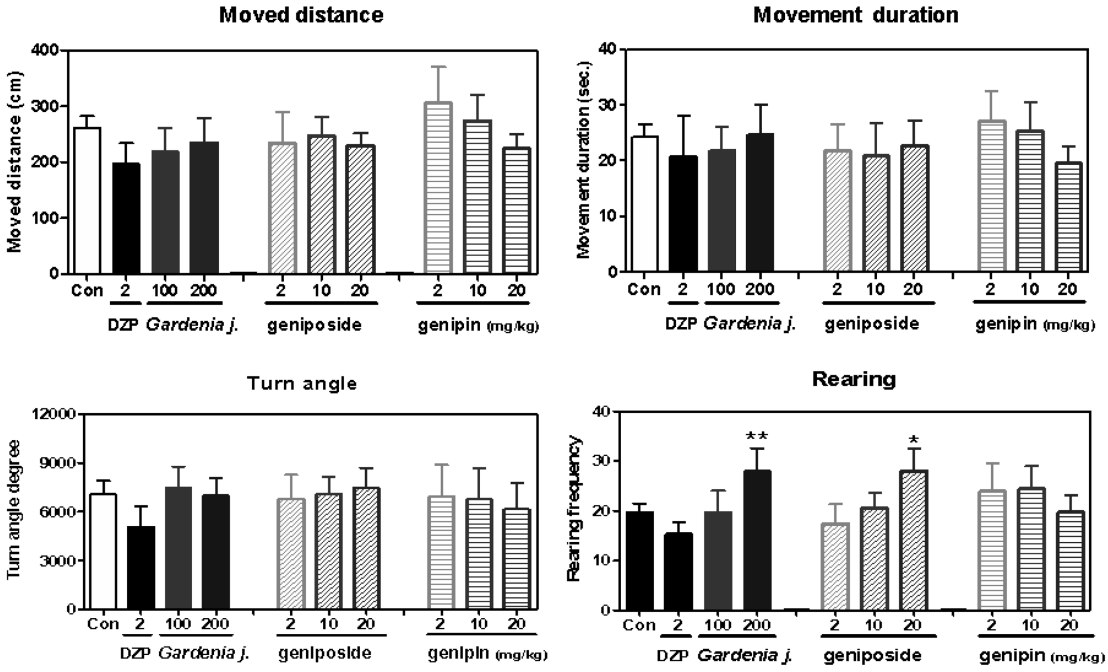

Fig. 3. Effects of *G. jasminoides* extract, genipin and geniposide on locomotor activity (central area) in mice (n=9). Each bar represents the mean±S.E.M of the moved distances, movement durations, turn angle degrees or rearing frequencies for 10 minutes. (**p*<0.05, ***p*<0.01 versus control group). DZP : diazepam.

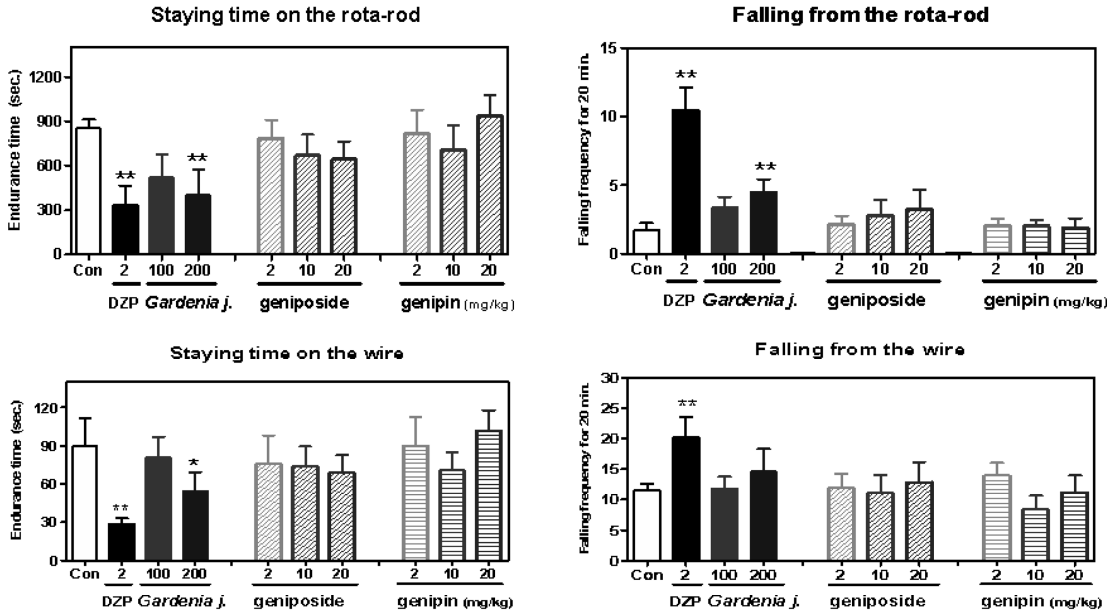

Fig. 4. Effects of *G. jasminoides* extract, genipin and geniposide on activity on the rotarod or wire in mice (n=10). Each bar represents the mean±S.E.M of the endurance times or falling frequencies for 20 minutes. (*p<0.05, **p<0.01 versus vehicle control group). DZP : diazepam.

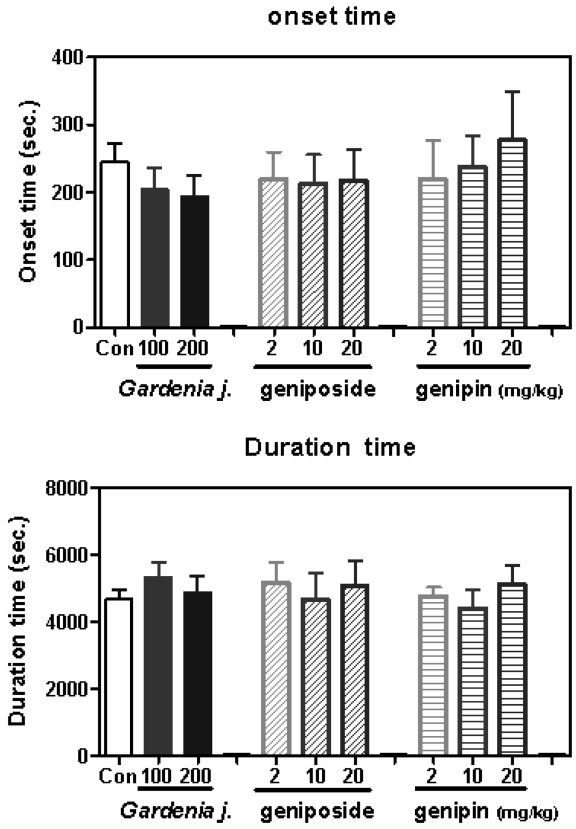

Fig. 5. Effects of *G. jasminoides* extract, genipin and geniposide on thiopental induced sleep in mice (n=10). Each bar represents the mean±S.E.M of the onset times or the duration times. (*p<0.05, **p<0.01 versus vehicle control group).

increased falling frequencies from both (Fig. 4). The extract also significantly decreased running time on the rota-rod and the wire and increased falling frequency from the rotating rod, but with less efficacy than diazepam. Geniposide and genipin did not affect these activities.

Thiopental-induced sleeping

Administration of *G. jasminoides* extract, geniposide, and genipin did not significantly change thiopental-

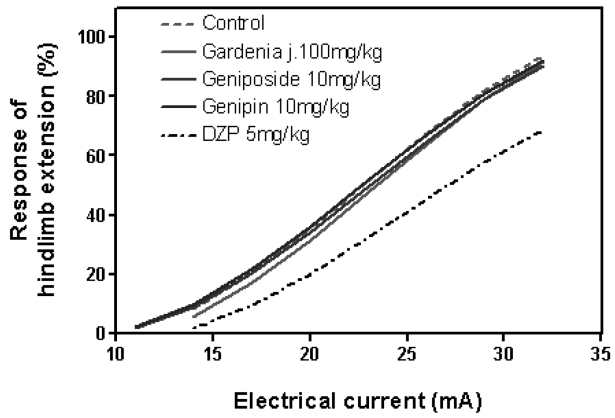

Fig. 6. Effects of *G. jasminoides* extract, genipin and geniposide on electroshock convulsion in mice. Each line represents the mean±S.E.M of % of animals with hind-limb extension by currents (pulse width-0.5 ms, frequency-100 pulses/sec. shock duration-1 sec.). DZP : diazepam.

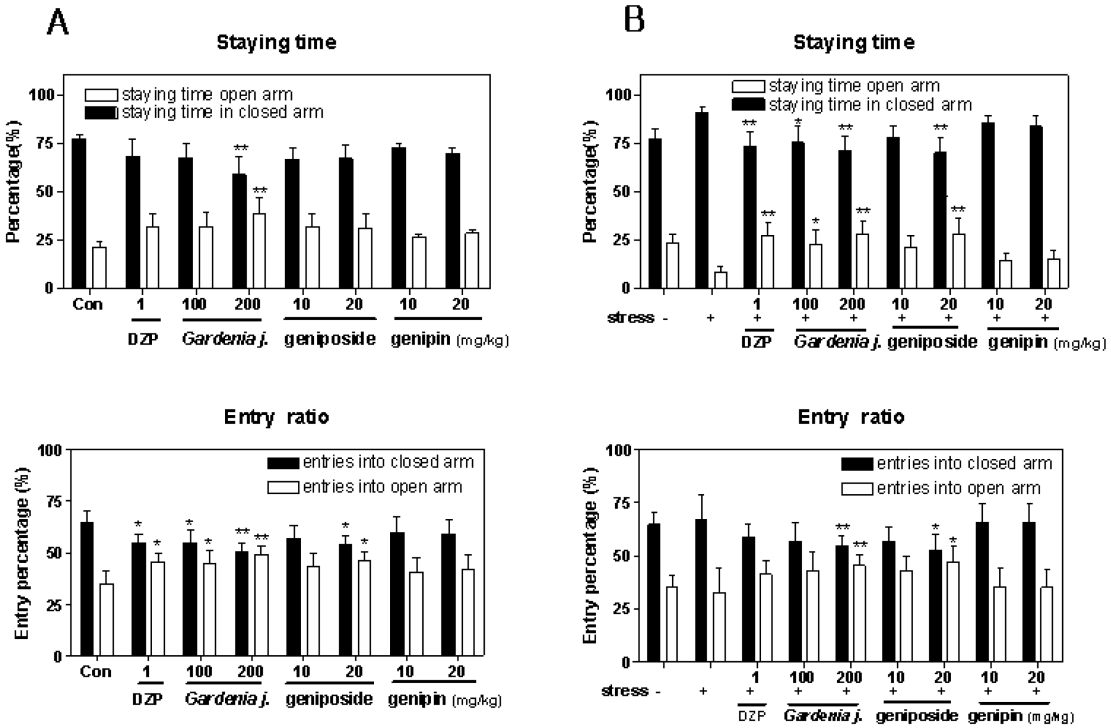

Fig. 7. Effects of *G. jasminoides* extract, genipin and geniposide on activity on elevated plus maze in mice (n=10). A : Activity on elevated plus maze in normal mice. B : Activity on elevated plus maze in mice exposed to electroshock stress. Each bar represents the mean±S.E.M of % of the time spent in arms and frequency entered into arms. (*p<0.05, **p<0.01 versus control group).
DZP : diazepam.

induced sleeping or the seizure response induced by electro-shock (Fig. 5 and 6).

Activities in plus maze

Mice treated with *G. jasminoides* extract (200 mg/kg) spent more time in the open arms than saline-treated mice and entered more frequently into the open arms (Fig. 7A). Administration of diazepam (1 mg/kg) or geniposide (20 mg/kg) also significantly increased open arm entry frequency. Electroshock stress shortened the staying time in the open arms, a sign of anxiety (Fig. 7B). However, stressed mice treated with *G. jasminoides* extract (100 mg/kg and 200 mg/kg) and geniposide (20 mg/kg) significantly spent more time in the open arms and showed increased open arm entry ratios compared to the stressed mice treated with saline only. Thus, *G. jasminoides* extract and geniposide showed greater anxiolytic activity in stressed animals than the normal animals.

DISCUSSION

G. jasminoides extract decreased movement in the open field but increased exploratory activity, such as rearing frequency in the central area. The extract also decreased rota-rod and balance wire activity but did not influence sleeping or convulsions. Thus, *G. jasminoides* extract may induce calmness or myorelaxation without excessive sedation or anti-convulsiveness, or blunting exploratory activity or curiosity. Most anxiolytic drugs, such as diazepam, have sedative and myorelaxing effects (Argyropoulos *et al*., 2000).

In this study, we show similar effects for *G. jasminoides* extract. Calming and exploratory behavior were used as parameters of anxiety level (Depino *et al*., 2008; Aron *et al*., 1971). The elevated plus maze test is used to evaluate anxiety-related behaviors and it involves a conflict between the rodent's desire to explore a novel environment and anxiogenic elements, such as elevation and an unfamiliar, brightly illuminated area (Lister, 1987). Increases in open-arm activity (i.e. percentage of entries made into and percentage of time spent on the open arms) indicate anxiety reduction. The anxiolytic activity of *G. jasminoides* extract was more effective in animals stressed by electroshock than the normal animals. Geniposide induced behavioral changes in the elevated plus maze test only, but dramatically increased Cl^-influx (only

at a high dose). Thus, Cl^- influx by *G. jasminoides* extract and geniposide played a role in their anxiolytic activity, as suggested by other reports (Toriizuka *et al*., 2005).

Ligand binding at the benzodiazepine binding site on the $GABA_A$ receptor complex produces anxiolysis, anticonvulsion, muscle relaxation, and sedation (Wang *et al*., 1999). Binding of GABA to the $GABA_A$ receptor activates chloride ion influx through the channel, and ligands for the benzodiazepine binding site modulate the inhibitory effects of GABA (Wang *et al*., 1999). Classical benzodiazepine anxiolytics (e.g., diazepam) are the most widely prescribed drugs for the treatment of anxiety disorders (Handley, 1994). In this study, diazepam exhibited stronger sedative and myorelaxing effects than *G. jasminoides* extract, but *G. jasminoides* extract had stronger anxiolytic activity than diazepam. The stimulation of $GABA_A$/benzodiazepine receptors in the basolateral amygdala by local administration of midazolam and muscimol impaired inhibitory avoidance acquisition, but did not change escape behavior in the elevated T maze (Duzzioni *et al*., 2008). Benzodiazepine agonist microinjection into the Acb nucleus does not alter open field behavior or other anxiety models, such as the light-dark box and Vogel conflict test, because $GABA_B$ receptors may be more important in fear/anxiety regulation than $GABA_A$ receptors (Lopes *et al*., 2007). However, the mechanism of anxiolytic action of *G. jasminoides* extract and geniposide requires further study.

In summary, we showed that *G. jasminoides* extract and geniposide alleviate anxiety, especially stress-induced anxiety. Moreover, the anxiolytic activity of *G. jasminoides* extract and geniposide is related to Cl^- influx.

ACKNOWLEDGEMENTS

This research was supported by a grant (No. 07092 Hanyakjae332) from SHM program of Korean Food and Drug Administration.

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