

Full Length Research Paper

# Anti-depressive activity of *Gardeniae Fructus* and geniposide in mouse models of depression

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Accepted 8 September, 2011

We evaluated the anti-depressive activity of ethanolic extracts of *Gardenia jasminoides* Ellis. (GJ<sub>EtOH</sub>), *Gardenia jasminoides* Ellis. var. *grandiflora* Nakai. (GJG<sub>EtOH</sub>) and geniposide with mouse forced swimming test (FST) and tail suspension test (TST). In addition, we investigated the possible mechanisms of action of anti-depressive effect of geniposide (GPO). The results showed that GJ<sub>EtOH</sub> (0.1, 1.0 g/kg, p.o.) and GJG<sub>EtOH</sub> (0.1, 1.0 g/kg, p.o.) significantly reduced the immobility time of mice in both FST and TST, but they did not affect the locomotor activity of mice. Geniposide (10 mg/kg, i.p.) augmented the anti-depressive effect of desipramine (5 mg/kg, i.p.) and fluoxetine (5 mg/kg, i.p.) in the FST, but it did not affect the anti-depressive effect of clorgyline and maprotiline. Geniposide (10 mg/kg, i.p.) increased the levels of serotonin and 5-HIAA in striatum and serotonin in hippocampus. These results suggested that anti-depressive mechanism of GPO may be related to the increasing serotonin level in striatum and hippocampus in mice.

**Key words:** *Gardenia jasminoides* Ellis., *Gardenia jasminoides* Ellis. var. *grandiflora* Nakai., geniposide, forced swimming test, anti-depressive effect, monoamines.

## INTRODUCTION

Gardenias, which are both beautiful and extremely fragrant, are one of the most popular flowers. This plant is native to the subtropical regions of Taiwan, southern China, Vietnam and southern Japan. *Gardenia jasminoides* Ellis. (GJ) is the fruit of Rubiaceae *Gardenia*. In common Chinese folk medicine practice, Fructus *Gardeniae Grandiflorae*, *Gardenia jasminoides* Ellis. var. *grandiflora* Nakai (GJG) can be used as a substitution for Fructus *Gardeniae* (*G. jasminoides* Ellis). *Gardenia* fruit is commonly used to treat depression, anxiety, insomnia, psychosis and other mental disorders in Chinese traditional medicine (Dong et al., 2011). It is known for its antibacterial, antiviral and snake bite (Huang, 2008;

Yang et al., 2010; Ma et al., 2006; Li et al., 2008; Jioufack et al., 2009).

One of the main effectual components of *Gardenia* is geniposide (GPO) (He et al., 2010; Zhou et al., 2005; Kim and Kim, 2007), which is soluble in ethanol (Chen et al., 2009). The crude extracts soaked with ethanol for *Gardenia* fruits were experimented (Yang et al., 2008; Lee et al., 2009). The concentration of GPO in Fructus *Gardeniae Grandiflorae* was found to be higher than that in Fructus *Gardeniae* (Tsai et al., 2002). Therefore, the antidepressant effect of GJ<sub>EtOH</sub>, GJG<sub>EtOH</sub> and GPO on two despair animal models: the mouse tail suspension test (TST) and the mouse forced swimming test (FST) were evaluated. Moreover, whether the effect of GPO in the FST is dependent on its interaction with the 5-HT, DA and NA receptors and the changes in the levels of norepinephrine, serotonin, dopamine and its metabolites in mice hippocampus, striatum and cortex were also

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investigated.

## MATERIALS AND METHODS

### Chemicals

Geniposide were purchased from Medical and Pharmaceutical Industry Technology and Development Center (Taipei, Taiwan).

Desipramine HCl (DES), fluoxetine HCl (FLU), clorgyline (CLO) and maprotiline HCl (MAP) were purchased from SIGMA-ALDRICH Company (Steinheim, Germany).

The monoamine standard: norepinephrine (NE), dopamine (DA), 5-hydroxytryptamine (5-HT), 4-hydroxy-3-methoxyphenylglycol (MHPG), 3, 4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindole-3-acetic acid (5-HIAA) were purchased from SIGMA-ALDRICH Company (Steinheim, Germany).

Ethylenediaminetetraacetic acid (EDTA) was purchased from MERCK Company (Darmstadt, Germany). Sodium dihydrogen phosphate monohydrate ( $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ) was purchased from J.T. BAKER (Phillipsburg, USA) Sodium 1-octane sulfonate was purchased from TCI (Tokyo, Japan). Methanol and ethanol were purchased from UNI-ONWARD Company (Taipei, Taiwan). All other chemicals were of reagent grade or better.

### Plant material and crude extract preparation

Fructus Gardeniae and Fructus Gardeniae Grandiflorae were purchased from the local market in the Changhua County in Taiwan. The materials were identified by Dr. Chao-Lin Kuo, the leader of the School of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, College of Pharmacy, China Medical University, Taichung, Taiwan, where a plant specimen has been deposited.

The fruits were sliced into small pieces, dried in a circulating air stove and ground (3 kg). Ten liters of ethanol were added to 3 kg dried fruits in 20 L flask and decocted for 4 h. This procedure was repeated thrice. The flu extract was filtered and concentrated under reduced pressure at a temperature below 50°C. The remaining solution was dried in a circulating air stove (50°C) and stored under light protection at -20°C until use. The yielding ratios of ethanol extracts of *G. jasminoides* Ellis. ( $\text{GJ}_{\text{EtOH}}$ ) and ethanol extracts of *G. jasminoides* Ellis. var. *grandiflora* Nakai. ( $\text{GJG}_{\text{EtOH}}$ ) were 13.2% (w/w) and 14.0% (w/w), respectively.

### Animals

ICR male albino mice (weighing around 25 g), purchased from BioLASCO Taiwan Co., Ltd. were used in this study. They were maintained at  $22 \pm 1^\circ\text{C}$  with free access to water and food, under a 12:12 h light/dark cycle (lights on at 08:00 h). All manipulations were carried out between 9:00 and 15:00 h, with each animal used only once. All procedures in this study were performed in accordance with the National Institutes of Health (NIH) guide for the care and use of laboratory animals. The experimental protocol was approved by the Committee on Animal Research, China Medical University, under the code 2006-79. The minimum number of animals and duration of observations required to obtain consistent data were employed.

### Drug administration

$\text{GJ}_{\text{EtOH}}$ ,  $\text{GJG}_{\text{EtOH}}$  or saline was administered by oral route, whereas the other drugs, GPO, DES, FLU, CLO and MAP were administered

by intraperitoneal injection route. The aforementioned tested samples were administered to the mice at a volume of 0.1 ml/10 g (body weight).  $\text{GJ}_{\text{EtOH}}$  and  $\text{GJG}_{\text{EtOH}}$  were administered orally 60 min before FST or TST. GPO was administered intraperitoneally 30 min before FST or TST.

The doses of the drugs used were decided according to previous literature in order to not to increase locomotor activity (Binfaré et al., 2009; Brocardo et al., 2008; Kaster et al., 2005; Machado et al., 2007, 2009; O'Neill and Conway, 2001; Rodrigues et al., 2002).

### Forced swimming test (FST)

The method used was essentially similar to that described by Porsolt et al. (1977) in their work. The forced swim test was carried out in mice, individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 15 cm of water at  $25 \pm 1^\circ\text{C}$ . The duration of immobility during the final 4 min interval of the swimming test was measured. Immobility was defined as floating and treading water just enough to keep the nose above water. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. The water was changed after each mouse was tested. During the test session, the immobility time was recorded by two observers who had no knowledge of the type of treatment. Decrease in the duration of immobility during the FST was taken as a measure of antidepressant activity (Nisar et al., 2011).

FSTs for  $\text{GJ}_{\text{EtOH}}$  and  $\text{GJG}_{\text{EtOH}}$  oral administration were as followed. Mice were randomly divided into eight groups ( $n = 10$ ). Group I (control) received 0.9% saline water 10 ml/kg (p.o.). Groups II received reference drug: 20 mg/kg (i.p.) of DES. Group III, IV and V received 10, 100 and 1000 mg/kg (p.o.) of  $\text{GJ}_{\text{EtOH}}$ , respectively. Group VI, VII and VIII received 10, 100 and 1000 mg/kg (p.o.) of  $\text{GJG}_{\text{EtOH}}$ , respectively. Treatment groups were blinded to the investigator.

FSTs for GPO intraperitoneal injection administration also were evaluated as followed. Mice were randomly divided into 5 groups ( $n = 10$ ). Group I (control) received 0.9% saline water 10 ml/kg (i.p.). Groups II received reference drug: 20 mg/kg (i.p.) of DES. Group III, IV and V received 2.0, 5.0 and 10 mg/kg (i.p.) of GPO, respectively. Treatment groups were blinded to the investigator.

### Tail suspension test (TST)

The mouse was hung by the tail (clipped 1 cm from the end) for 6 min in a box of dimensions  $50 \times 25 \times 50$  cm, with its head 15 cm above the bottom of the box. Data was recorded only in the final 4 min of the test (Steru et al., 1985). Immobility was scored as a failure to make any struggling movements, attempts to catch the adhesive tape, or body torsions or jerks. During the test session, the immobility time was recorded by two observers who had no knowledge of the type of treatment.

The number of mice and medical treatment of  $\text{GJ}_{\text{EtOH}}$ ,  $\text{GJG}_{\text{EtOH}}$  and GPO in TSTs were the same as in FSTs.

### Open-field behavior

In order to detect any link between immobility in the FST and TST and changes in motor activity, the activity of animals treated with the extracts was analyzed in the mice locomotor activity apparatus (model Tru Scan system, Coulbourn Instruments, USA). The ambulatory behavior was assessed as described previously (Rodrigues et al., 1996). The apparatus consisted of a box measuring  $25.4 \times 25.4 \times 40.64$  cm. The mice were placed in the apparatus for 6 min. During this period, measurements were taken

only in the final 4 min. The apparatus was cleaned after each test session to prevent each mouse from being influenced by the odors present in the urine and faces of the previous mouse.

Mice were randomly divided into five groups ( $n = 10$ ). Group I (control) received 0.9% saline water 10 ml/kg orally. Groups II and III received 100 and 1000 mg/kg (p.o.) of  $GJ_{EtOH}$ , respectively. Group IV and V received 100 and 1000 mg/kg (p.o.) of  $GJG_{EtOH}$ , respectively.

In the same way, mice also were randomly divided into three groups ( $n = 10$ ). Group I (control) received 0.9% saline water 10 ml/kg intraperitoneally. Groups II and III received 5.0 and 10 mg/kg (i.p.) of GPO, respectively.

### Combination of geniposide with DES, FLU, CLO and MAP on immobility time in FST

In a series of activity evaluation experiments, we investigated whether the antidepressant-like effect of GPO in FST were dependent on its interaction with DES (a tricyclic antidepressant, monoamine reuptake inhibitor), FLU (selective 5-HT reuptake inhibitors), CLO (selective MAO-A inhibitor) and MAP (selective noradrenaline reuptake inhibitor). The mice were pretreated with GPO 10 mg/kg (i.p.) or saline, and after 30 min they received DES 5 mg/kg (i.p.), FLU 5 mg/kg (i.p.), CLO 0.5 mg/kg (i.p.), or MAP 20 mg/kg (i.p.) before being tested in FST 30 min later.

The doses of the drugs which do not affect locomotor activity and immobility time were selected on the basis of literature data (Fletcher et al., 2004; D'Aquila et al., 2000) and our preliminary tests.

### Measurement of the levels of monoamines and their metabolites in the cortex, hippocampus and striatum of mice brain

Monoamines and their metabolites were measured according to the method of Renard et al. (2003) in their work. Briefly, mice which were administered GPO 10 mg/kg intraperitoneal injection were killed by cervical dislocation without anesthesia just after the FST. The brain was removed after a rapid dissection of cranium and striatum, and hippocampus and frontal cortex were then isolated. The three brain tissues were weighed, and placed separately in 5 ml of ice-cold homogenizing solution (8.8 mg of ascorbic acid and 122 mg of EDTA in 1000 ml of perchloric acid 0.1 M). After homogenization, the solution was centrifuged at  $10,000 \times g$  for 10 min at  $4^\circ C$ . Twenty micro liters of the resultant supernatant was injected in the high performance liquid chromatography (HPLC) system. The levels of monoamines (NE, DA and 5-HT) and their metabolites (DOPAC, MHPG and 5-HIAA) were measured by HPLC with electrochemical detection in the three brain tissues. The number of mice in each group was 6. The HPLC model waters 610 (waters) was used. The mobile phase [4.2 g/L citric acid monohydrate, 6.8 g/L sodium acetate trihydrate, 0.8 g/L octanesulfonic acid sodium salt, 0.05 g/L tetrasodium EDTA, 0.02% (v/v) dibutyl amine and 7% (v/v) methyl alcohol was delivered at 1.0 ml/min. The reverse-phase column used was a Merk Lichrospher 100 RP-18 endcapped column with a length of 12.5 cm and an internal diameter of 4.0 mm (E. Merck 50734). The compounds were measured at + 0.75 V using a Bioanalytical Systems LC-4C electrochemical detector.

### Phytochemical analysis

$GJ_{EtOH}$  and  $GJG_{EtOH}$  were analyzed using HPLC analysis. All the samples were dissolved in methanol and then filtrated through a 0.45  $\mu m$  membrane filter. HPLC analysis was performed on a

Shimadzu LC-10AT series system (Shimadzu Corporation, Kyoto, Japan). Chromatographic separation was carried out on a Synergi 4  $\mu$  Fusion-RP 80 A column (250  $\times$  4.6 mm, Phenomenex) heated to  $25^\circ C$  using a isocratic elution of solvent (A)  $H_2O$  (0.1% formic acid) and solvent (B) methanol (70 : 30) at a flow rate of 1 ml/min. Peaks were detected by using UV detector at 238 nm.

### Statistical analysis

Data were represented as the mean  $\pm$  SEM. Data were analyzed by one-way ANOVA followed by Scheffe's multiple range test. The criterion for statistical significance was  $P < 0.05$ . All statistical analyses were carried out by using SPSS for Windows (SPSS Inc.).

## RESULTS

### Effect of $GJ_{EtOH}$ and $GJG_{EtOH}$ on immobility time in FST and TST

$GJ_{EtOH}$  and  $GJG_{EtOH}$  decreased significantly the immobility time in FSTs, dose range: 0.1 g/kg ( $P < 0.001$ ) – 1.0 g/kg ( $P < 0.01$ ), p.o., (Figure 1). The tricyclic antidepressant DES, used as a positive control, administered by i.p. route also caused a reduction in the immobility time in FST at a dose of 20 mg/kg ( $P < 0.001$ ) (Figure 1).

$GJ_{EtOH}$  and  $GJG_{EtOH}$  also caused a reduction in the immobility time in TSTs, dose range: 0.1 g/kg ( $P < 0.001$ ) – 1.0 g/kg ( $P < 0.001$ ), p.o. (Figure 2). Desipramine produced a reduction of the immobility time in TST at a dose of 20 mg/kg ( $P < 0.001$ ), i.p. (Figure 2).

However,  $GJ_{EtOH}$  or  $GJG_{EtOH}$  did not decrease the immobility time in FST and TST at the dose of 0.01 g/kg, p.o.

### Effect of GPO on immobility time in FST and TST

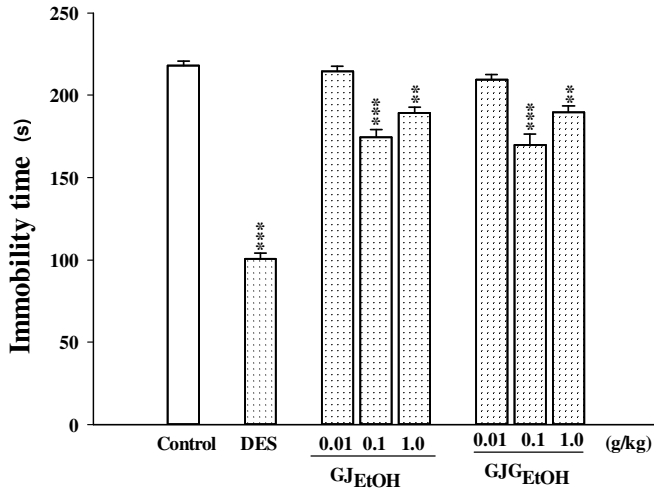
Geniposide decreased significantly the immobility time in FSTs, dose range: 5.0 mg/kg ( $P < 0.01$ ) – 10 mg/kg ( $P < 0.001$ ), i.p. (Figure 3). The tricyclic antidepressant DES, used as a positive control, administered by i.p. route also caused a reduction in the immobility time in FST at a dose of 20 mg/kg ( $P < 0.001$ ) (Figure 3).

Geniposide also caused a reduction in the immobility time in TSTs, dose range: 5.0 mg/kg ( $P < 0.01$ ) – 10 mg/kg ( $P < 0.001$ ), i.p. (Figure 4). Desipramine produced a reduction of the immobility time at a dose of 20 mg/kg ( $P < 0.001$ ), i.p. (Figure 4).

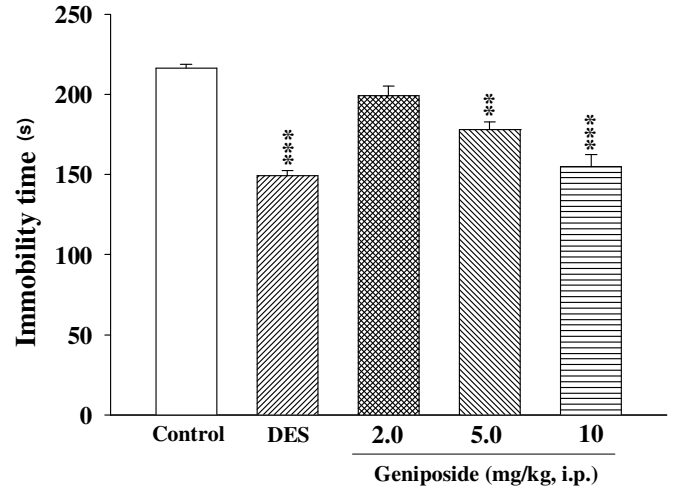
However, GPO did not decrease the immobility time in FST and TST at the dose of 2 mg/kg, i.p.

### Effect of $GJ_{EtOH}$ , $GJG_{EtOH}$ and geniposide on locomotor activity

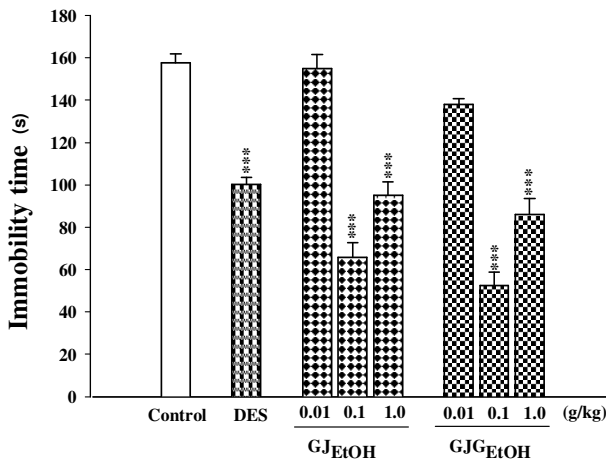
As shown in Figure 5, there was no significant effect on locomotor activity of mice when treated with  $GJ_{EtOH}$  and  $GJG_{EtOH}$ , 0.1 – 1.0 g/kg (p.o.) when compared with



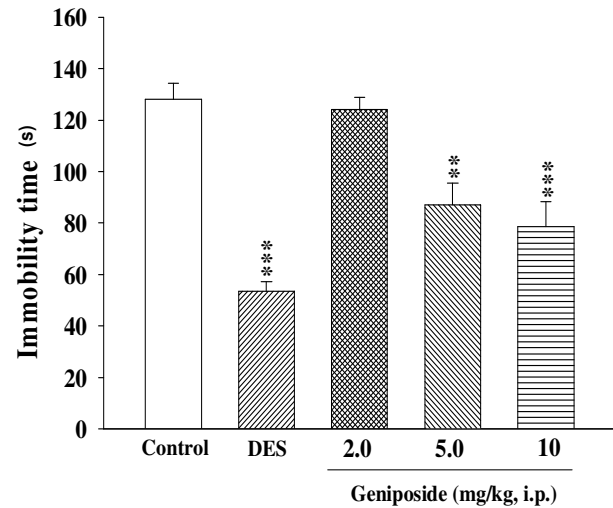
**Figure 1.** Immobility time of GJ<sub>EtOH</sub> and GJG<sub>EtOH</sub> and desipramine (DES) in the forced swimming test. The values are mean ± SEM (n = 10) for each group. \*\*\*P < 0.001 as compared to control group. (One-way ANOVA followed by Scheffe't multiple range test).



**Figure 3.** Immobility time of geniposide (GPO) in the forced swimming test. The values were mean ± SEM (n = 10). \*\*P < 0.01, \*\*\*P < 0.001 as compared to the control group. (One-way ANOVA followed by Scheffe's test).



**Figure 2.** Immobility time of GJ<sub>EtOH</sub> and GJG<sub>EtOH</sub> and desipramine (DES) in the tail suspension test. The values are mean ± SEM (n = 10) for each group. \*\*\*P < 0.001 as compared to control group. (One-way ANOVA followed by Scheffe't multiple range test).



**Figure 4.** Immobility time of geniposide (GPO) in the tail suspension test. The values were mean ± SEM (n = 10). \*\*P < 0.01, \*\*\*P < 0.001 as compared to the control group. (One-way ANOVA followed by Scheffe's test).

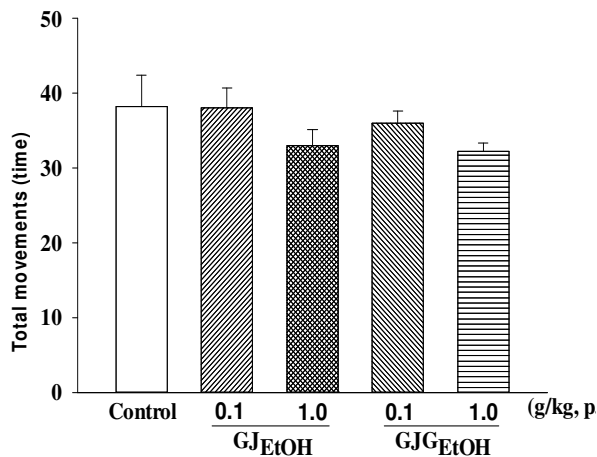
control group.

Also, as shown in **Figure 6**, there was no significant effect on locomotor activity of mice when treated with GPO 5.0 – 10 mg/kg (i.p.) when compared with control group.

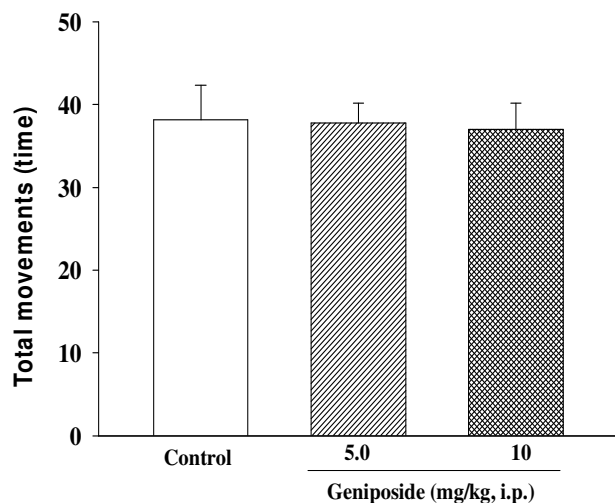
#### Effect of the combination of geniposide with DES, FLU, CLO and MAP on immobility time in FST

The results depicted in **Figure 7, 8, 9 and 10** showed the

effects of treatments on mice with DES 5 mg/kg, FLU 5 mg/kg, CLO 0.5 mg/kg and MAP 20 mg/kg (i.p., a dose that did not affect the immobility time), respectively on the reduction in immobility time elicited by GPO (10 mg/kg, i.p.). Post-hoc analyses indicated that the treatment on mice with DES augmented the antidepressant-like effect of GPO/DES (P < 0.001) and FLU augmented the antidepressant-like effect of GPO/FLU (P < 0.05), but CLO did not affect the antidepressant-like effect of GPO/CLO and MAP did not affect the antidepressant-like effect of GPO/MAP in FST.



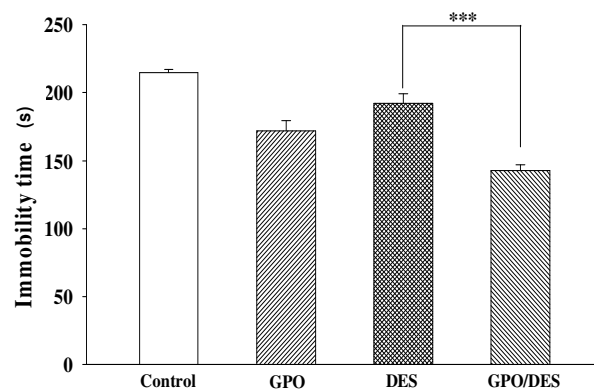
**Figure 5.** Effects of GJ<sub>EIOH</sub> and GJG<sub>EIOH</sub> on the locomotor activity in the mice. The amount of locomotion occurring in the final 4 min of the test is shown. All the test substances were orally administered 60 min before the test. Each column represents the mean  $\pm$  SEM (n = 10).



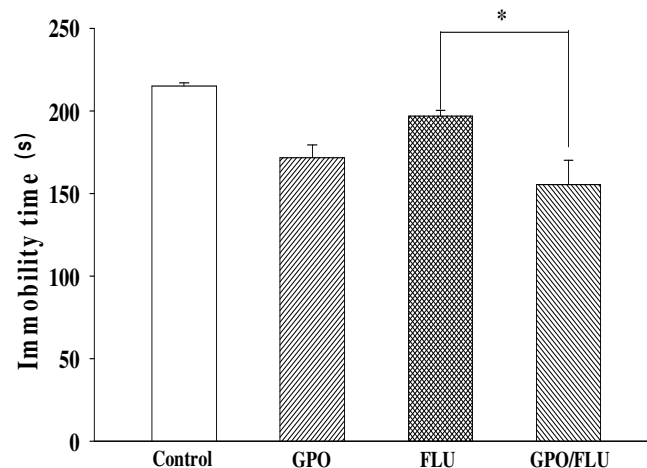
**Figure 6.** Effects of geniposide (GPO) on the locomotor activity in the mice. The amount of locomotion occurring in the final 4 min of the test is shown. All the test substances were intraperitoneally administered 60 min before the test. Each column represents the mean  $\pm$  SEM (n = 10).

### Effects of geniposide on the concentration of monoamines and their metabolites in the cortex, hippocampus and striatum of mice brain

The concentrations of NE, MHPG, DA, DOPAC, 5-HT and 5-HIAA in cortex, hippocampus and striatum of mice brain were presented in [Tables 1, 2 and 3](#), respectively. Geniposide 10 mg/kg (i.p.) increased significantly the levels of 5-HT ( $P < 0.01$ ) and 5-HIAA ( $P < 0.01$ ) in striatum as shown in [Table 3](#) and increased the levels of



**Figure 7.** The effects of geniposide (GPO) on the duration of immobility induced by desipramine (DES) in the forced swimming test. The values were mean  $\pm$  SEM (n = 10). \*\*\* $P < 0.001$  as compared to the DES. (One-way ANOVA followed by Scheffe's test)



**Figure 8.** The effects of geniposide (GPO) on the duration of immobility induced by fluoxetine (FLU) in the forced swimming test. The values were mean  $\pm$  SEM (n = 10). \* $P < 0.05$  as compared to the FLU. (One-way ANOVA followed by Scheffe's test).

5-HT ( $P < 0.05$ ) in the hippocampus at [Table 2](#).

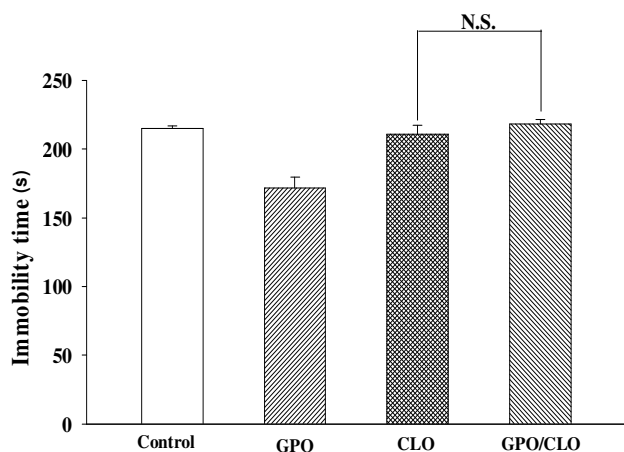
### Phytochemical analysis

By referring to standard, the chromatographic analysis showed that the main component of GJ<sub>EIOH</sub> and GJG<sub>EIOH</sub> was geniposide ([Figure 11](#)).

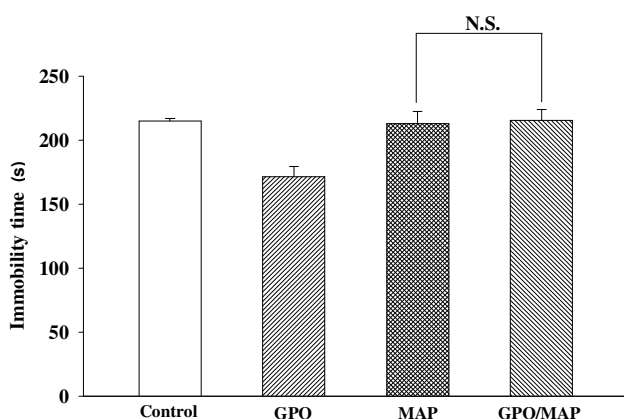
### DISCUSSION

The FST and TST are the most widely accepted models for assessing antidepressant-like activity in mice. The





**Figure 9.** The effects of geniposide (GPO) on the duration of immobility induced by clorgyline (CLO) in the forced swimming test. The values were mean  $\pm$  SEM ( $n = 10$ ). N.S.: Non-significant.



**Figure 10.** The effects of geniposide (GPO) on the duration of immobility induced by maprotiline (MAP) in the forced swimming test. The values were mean  $\pm$  SEM ( $n = 10$ ). N.S.: Non-significant.

immobility taken in these two tests is understood to reflect a state of “behavioral despair” or “failure to adapt to stress” (Wilner, 1997; Borsini and Meli, 1998). These two tests are based on the fact that animals will develop an immobile posture when subjected to the short-term, inescapable stress of being suspended by their tail or being dropped into water. These two models are widespread in the laboratory largely due to their ease-of-use, consistency across laboratories and their ability to detect a broad spectrum of antidepressant agents. Most clinically active antidepressants are effective in the FST and TST. Antidepressants can also be distinguished from stimulants, because stimulants cause marked motor stimulation, in contrast to antidepressants, which do not (Borsini and Meli, 1998).

*G. jasminoides* Ellis. and *G. jasminoides* Ellis. var.

*grandiflora* Nakai. used as a natural food colorant, also contained GPO (Watanabe et al., 1998; Nishizawa et al., 1998; Tsm et al., 1994; Wu et al., 2010a; Zhu et al., 2009). Geniposide can improve cognitive ability of Vascular Dementia in rats (Li et al., 2009). The protective effects of geniposide (10, 20 and 40 mg/kg, i.p., 10 days) on the dysfunction of memory were observed by Morris water maze experiment. It was concluded that GPO can improve the learning and memory impairment induced by ethanol in mice, and significantly decrease the escaping latency and swimming distance (Wu et al., 2010b). In addition, the quantity of GPO in GJG was more than that in GJ (Tsai et al., 2002). Therefore, Fructus Gardeniae Grandiflorae may be used as a new resource for the Gardeniae fruits in traditional Chinese medicine (Bussmann et al., 2010).

Using HPLC analysis, we found that the main component of  $GJ_{EtOH}$  and  $GJG_{EtOH}$  was GPO, which is the active component in Gardeniae Fructus. Yueju-Wan, which contains GPO, possesses antidepressant activity (Wei et al., 2008). This information suggests that the antidepressant activity of  $GJ_{EtOH}$  and  $GJG_{EtOH}$  may be related to the GPO. The data presented has demonstrated that  $GJ_{EtOH}$  and  $GJG_{EtOH}$  can significantly reduce the immobility time in the FST and TST, indicating significant antidepressant effects in the two animal model tests. In addition, the antidepressant effects of  $GJ_{EtOH}$  and  $GJG_{EtOH}$  cannot be attributed to the increase in motor activity because they did not induce hyper-locomotion in the mice. These results suggest that  $GJ_{EtOH}$  and  $GJG_{EtOH}$  possess the antidepressant effect.

It was indicated by Toriizuka et al. (2005) that Gardeniae Fructus and GPO played a role in the anxiolytic effect of Kamishoyosan, a formula used to treat menopausal psychotic syndromes in women. When the antidepressant potential of genipin, aglycone of GPO extracted from the fruit of *G. jasminoides* Ellis. and their possible mechanism was investigated by Tian et al. (2010), it was found that when compared with FLU, genipin exerts antidepressant-like effects significantly. At least, one possible mechanism for GPO is the regulation of the 5-HT and NE levels in the hippocampus. Otherwise, the precise mechanisms by which GPO produced antidepressant-like effect could not be completely understood.

However, according to our results, the antidepressant-like effect of GPO was additive to the treatment of animals with DES (a tricyclic antidepressant, monoamine reuptake inhibitor), FLU (a selective serotonin reuptake inhibitor), CLO (a selective MAO-A inhibitor) and MAP (selective noradrenaline reuptake inhibitor) when tested in FST. These suggested that GPO might produce antidepressant-like effect by interaction with 5-HT reuptake inhibitor of mice. From the studies of the monoamines in mouse frontal cortex, hippocampus and striatum, we found that GPO (10 mg/kg, i.p.) significantly increased the levels of 5-HT and 5-HIAA in

**Table 1.** Effects of geniposide on the concentrations of monoamines and their metabolites in the cortex of mice brain.

Group	Cortex(ng/g tissue)					
	NE	MHPG	DA	DOPAC	5-HT	5-HIAA
Normal	476.05 ± 38.88	166.12 ± 3.06	42.32 ± 6.45	33.98 ± 6.74	723.36 ± 39.77	162.78 ± 8.03
Control versus FST	287.11 ± 17.75 <sup>#</sup>	161.66 ± 14.12	25.75 ± 3.47 <sup>#</sup>	27.28 ± 5.05	455.71 ± 40.71 <sup>#</sup>	100.14 ± 3.54 <sup>#</sup>
Geniposide 10 mg/kg	318.65 ± 17.09	136.15 ± 7.70	28.40 ± 2.20	30.94 ± 3.43	658.23 ± 58.10	142.25 ± 13.52
Fluoxetine 5 mg/kg	324.00 ± 41.21	148.18 ± 15.57	24.72 ± 1.14	29.45 ± 1.86	464.96 ± 14.01	129.81 ± 23.49
Desipramine 5 mg/kg	251.35 ± 22.78	104.46 ± 13.04	22.93 ± 3.05	23.97 ± 4.39	463.76 ± 54.66	100.07 ± 9.12

Values were the means ± SEM (n = 6). <sup>#</sup>P < 0.05 as compared to the normal group.

**Table 2.** Effects of geniposide on the concentrations of monoamines and their metabolites in the hippocampus of mice brain.

Group	Hippocampus(ng/g tissue)					
	NE	MHPG	DA	DOPAC	5-HT	5-HIAA
Normal	351.52 ± 15.54	150.87 ± 5.99	28.99 ± 1.33	31.36 ± 2.83	664.71 ± 40.27	201.73 ± 20.61
Control versus FST	277.06 ± 4.83 <sup>#</sup>	113.45 ± 8.65 <sup>#</sup>	20.30 ± 1.01 <sup>#</sup>	21.30 ± 1.77 <sup>#</sup>	482.20 ± 44.27 <sup>#</sup>	141.00 ± 10.77 <sup>#</sup>
Geniposide 10 mg/kg	305.01 ± 2.90	119.30 ± 7.22	22.25 ± 1.59	21.62 ± 1.11	647.11 ± 13.62*	159.76 ± 6.61
Fluoxetine 5 mg/kg	285.05 ± 15.12	102.58 ± 7.49	27.15 ± 2.10	22.81 ± 1.15	521.66 ± 16.18	164.98 ± 9.35
Desipramine 5 mg/kg	288.35 ± 10.18	115.41 ± 10.01	22.25 ± 1.70	22.59 ± 1.03	470.36 ± 49.04	184.69 ± 13.21

Values were the means ± SEM (n = 6). <sup>#</sup>P < 0.05 as compared to the normal group. \*P < 0.05 as compared to the control group. One-way ANOVA followed by Scheffe's test).

**Table 3.** Effects of geniposide on the concentrations of monoamines and their metabolites in the striatum of mice brain.

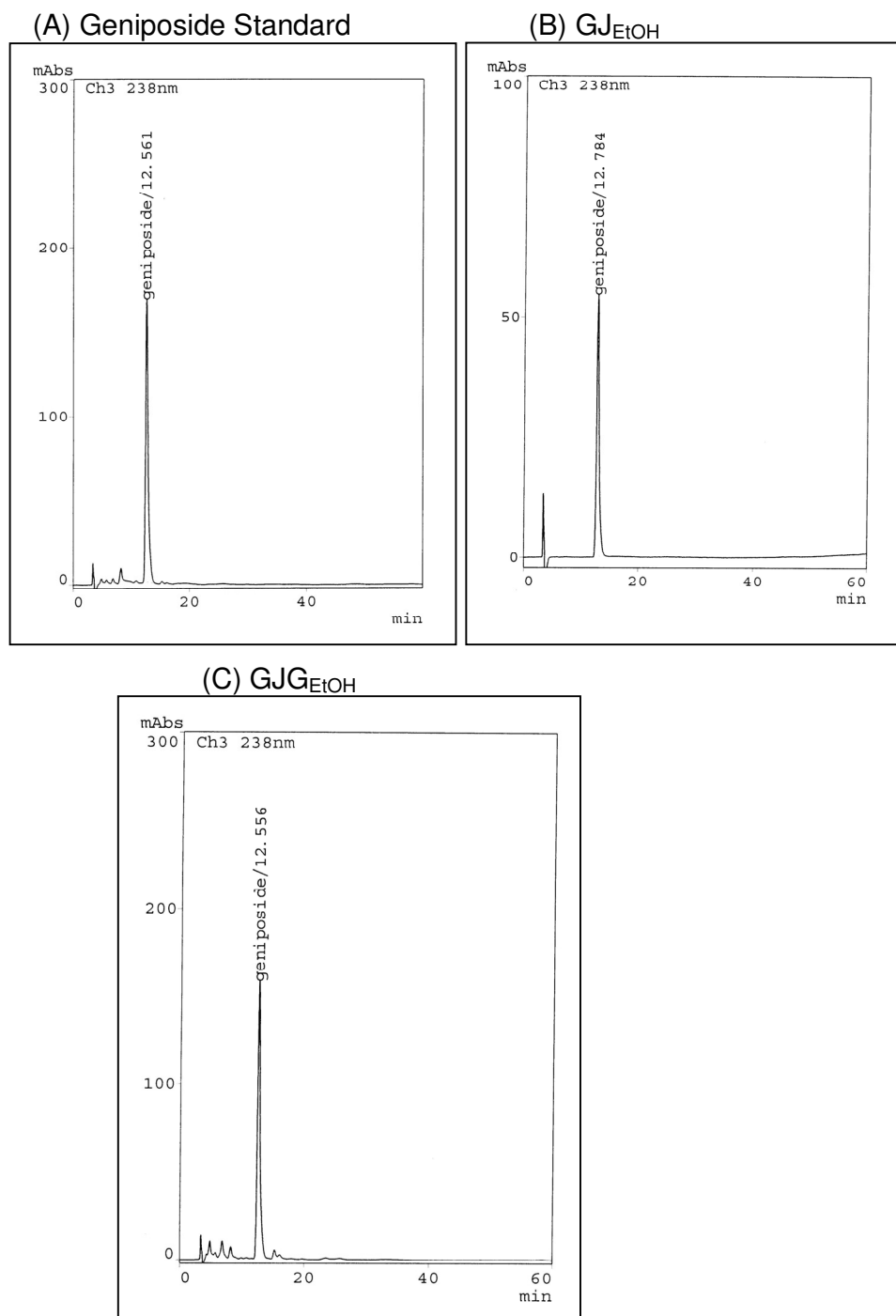
Group	Striatum(ng/g tissue)					
	NE	MHPG	DA	DOPAC	5-HT	5-HIAA
Normal	380.58 ± 7.53	106.20 ± 3.51	3107.21 ± 207.50	1777.85 ± 72.40	898.14 ± 51.76	210.02 ± 16.59
Control versus FST	284.46 ± 13.19 <sup>#</sup>	78.38 ± 5.67 <sup>#</sup>	2046.36 ± 265.43 <sup>#</sup>	1265.61 ± 133.58	586.25 ± 40.97 <sup>#</sup>	138.08 ± 15.25 <sup>#</sup>
Geniposide 10 mg/kg	352.91 ± 10.91	103.32 ± 4.60	2631.06 ± 187.17	1631.17 ± 122.92	944.35 ± 56.29**	235.18 ± 15.86**
Fluoxetine 5 mg/kg	322.82 ± 18.40	104.31 ± 6.70	2396.05 ± 179.70	1596.79 ± 137.97	692.24 ± 46.23	149.93 ± 6.75
Desipramine 5 mg/kg	296.06 ± 16.71	84.21 ± 6.33	2079.87 ± 213.16	1170.06 ± 79.42	617.06 ± 33.70	143.21 ± 15.02

Values were the means ± SEM (n = 6). <sup>#</sup>P < 0.05 as compared to the normal group. \*\*P < 0.01 as compared to the control group. (One-way ANOVA followed by Scheffe's test).

the striatum and increased the levels of 5-HT in the hippocampus. Therefore, the

antidepressant-like mechanism of GPO might be mediated by increasing the serotonin level in

striatum and hippocampus on mice. In summary, our results suggested that GJ<sub>E10H</sub> and



**Figure 11.** Fingerprints of geniposide standard, GJ<sub>EtOH</sub> and GJG<sub>EtOH</sub>: (A) Geniposide Standard, (B) GJ<sub>EtOH</sub> and (C) GJG<sub>EtOH</sub>.

GJG<sub>EtOH</sub> exerted antidepressant effect in experimental animal models. The GPO appears to be the major active constituent of GJ<sub>EtOH</sub> and GJG<sub>EtOH</sub>. Further research elucidating the action mechanism of these effects using GPO will give an insight into the usefulness of this herb in the treatment of depression.

#### ACKNOWLEDGEMENTS

This study is supported in part by the National Science Council, Taiwan (NSC 100-2320-B-039-013), Taiwan Department of Health Clinical Trial and Research Center of Excellence (DOH100-TD-B-111-004) and China



## REFERENCES

- Binfaré RW, Rosa AO, Lobato KR, Santos ARS, Rodrigues ALS (2009). Ascorbic acid administration produces an antidepressant-like effect: evidence for the involvement of monoaminergic neurotransmission. *Prog Neuropsychopharmacol. Biol. Psychiatry* 33(3): 530-540.
- Borsini F, Meli A (1998). Is the forced swimming test a suitable model for revealing antidepressant activity? *Psychopharmacol. (Berl)* 94(2): 147-160.
- Brocardo PS, Budni J, Kaster MP, Santos ARS, Rodrigues ALS (2008). Folic acid administration produces an antidepressant-like effect in mice: evidence for the involvement of the serotonergic and noradrenergic systems. *Neuropharmacol.* 54(2): 464-473.
- Bussmann RW, Glenn A, Sharon D (2010). Healing the body and soul: Traditional remedies for "magical" ailments, nervous system and psychosomatic disorders in Northern Peru. *Afr. J. Pharm. Pharmacol.* 4(9): 580-629.
- Chen Y, Zhao C, Zhang H, Cai L (2009). Study on efficiency of different concentration of alcohol for extraction of biological chemicals from gardenia. *Acta. Academiae Medicinae Zunyi* 5: 439-441.
- D'Aquila PS, Peana AT, Carboni V, Serra G (2000). Different effect of desipramine on locomotor activity in quinpirole-treated rats after repeated restraint and chronic mild stress. *J. Psychopharmacol.* 14: 347-352.
- Dong WR, Liu HY, Ding YG, Wang HP, Liu SM (2011). Interpretation of a TCM theory about clearing and discharging the triple burner damp-heat of *Gardenia jasminoides* Ellis. *China J. Trad. Chin. Med. Pharm.* 1: 33-35.
- Fletcher PJ, Sinyard J, Salsali M, Baker GB (2004). Fluoxetine, but not sertraline or citalopram, potentiates the locomotor stimulant effect of cocaine: possible pharmacokinetic effects. *Psychopharmacol.* 174(3): 406-413.
- He B, Tian J, Li CH, Liu Y (2010). Content variation of 4 mainly components in *Fructus Gardeniae* of different maturity and different parts. *Chin. J. Pharmaceu. Anal.* 5: 801-805.
- Huang CP (2008). Inspection on bacteriostasis effect of the extracts of *Gardenia jasminoides* Ellis. *China Pharmacist*, 11:1341-1342.
- Jiofack T, Fokunang C, Guedje N, Kemeuze V, Fongnzossie E, Nkongmeneck BA, Mapongmetsem PM, Tsabang N (2009). Ethnobotanical uses of some plants of two ethnoecological regions of Cameroon. *Afr. J. Pharm. Pharmacol.*, 3(13): 664-684.
- Kaster MP, Santos ARS, Rodrigues ALS (2005). Involvement of 5-HT1A receptors in the antidepressant-like effect of adenosine in the mouse forced swimming test. *BrainRes Bull.* 67(1-2): 53-61.
- Kim CY, Kim JW (2007). Preparative isolation and purification of geniposide from *Gardenia* fruits by centrifugal partition chromatography. *Phytochem. Anal.*, 18: 115-117.
- Lee JH, Lee DU, Jeong CS (2009). *Gardenia jasminoides* Ellis ethanol extract and its constituents reduce the risks of gastritis and reverse gastric lesions in rats. *Food Chem. Toxicol.*, 47(6): 1127-1131.
- Li BL, Chen YH, Yang X, Fang ZY, Yuan BX (2008). Extraction technology of gardenia oil and its effect on central nerve system. *J. Fourth Mil. Med. Univ.*, 29(23): 2152-2155.
- Li LJ, Li HX, Wu XT, Yang B, Zhou D (2009). Effect of geniposide on vascular dementia in rats. *Sichuan Da Xue Xue Bao Yi Xue Ban.* 40(4): 604-607.
- Ma FY, Zhi G, Fan HJ (2006). Experimental study of inhibition of *Gardenia jasminoides* Ellis and others of traditional Chinese herbs on Cocksackie virus B3 in vitro. *J. Xinxiang Med. Coll.*, 23(1): 33-35.
- Machado DG, Bettio LE, Cunha MP, Capra JC, Dalmarco JB, Pizzolatti MG, Rodrigues ALS (2009). Antidepressant-like effect of the extract of *Rosmarinus officinalis* in mice: involvement of the monoaminergic system. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 33(4): 642-650.
- Machado DG, Kaster MP, Binfaré RW, Dias M, Santos ARS, Pizzolatti MG, Brighente IM, Rodrigues AL (2007). Antidepressant-like effect of the extract from leaves of *Schinus molle* L. in mice: evidence for the involvement of the monoaminergic system. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 31(2):421-428.
- Nisar M, Khan S, Dar A, Rehman W, Khan R, Jan I (2011). Antidepressant screening and flavonoids isolation from *Eremostachys laciniata* (L) Bunge. *Afr. J. Biotechnol.*, 10(9): 1696-1699.
- Nishizawa M, Anetai M., Yamagishi T (1998). Progress and application of chemical evaluation of crude drugs. *J. Food Drug Anal.*, 6(4): 637-677.
- O'Neill MF, Conway MW (2001). Role of 5-HT1A and 5-HT1B receptors in the mediation of behavior in the forced swimtest in mice. *Neuropsychopharmacol.*, 24:391-398.
- Porsolt RD, Bertin A, Jalfre M (1977). Behavioral despair in mice: a primary screening test for antidepressants. *Arch. Int. Pharmacodyn. Ther.*, 229(2): 327-336.
- Renard CE, Dailly E, David DJ, Hascot M, Bourin M (2003). Neurochemical changes following the mouse forced swimming test but not the tail suspension test. *Fund. Clin. Pharmacol.*, 17: 449-455.
- Rodrigues AL, Rocha JB, Mello CF, Souza DO (1996). Effect of perinatal lead exposure on rat behaviour in open-field and two-way avoidance tasks. *Pharmacol. Toxicol.*, 79(3): 150-156.
- Rodrigues ALS, Silva GL, Matteussi AS, Fernandes ES, Miguel OG, Yunes RA, Calixto JB, Santos ARS (2002). Involvement of monoaminergic system in the antidepressant-like effect of the hydroalcoholic extract of *Siphocampylus verticillatus*. *Life Sci.*, 70(12):1347-1358.
- Steru L, Chermat R, Simon P (1985). The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacol.*, 85(3): 367-370.
- Tian JS, Cui YL, Hu LM, Gao S, Ch W, Dong TJ, Liu LP (2010). Antidepressant-like effect of genipin in mice. *Neurosci. Lett.*, 479(3): 236-239.
- Toriizuka K, Kamiki H, Ohmura N, Fujii M, Hori Y, Fukumura M, Hirai Y, Isoda S, Nemoto Y, Ida Y (2005). Anxiolytic effect of *Gardeniae Fructus*-extract containing active ingredient from Kamishoyosan (KSS), a Japanese traditional Kampo medicine. *Life Sci.*, 77(24): 3010-3020.
- Tsai TR, Tseng TY, Chen CF, Tsai TH (2002). Identification and determination of geniposide contained in *Gardenia jasminoides* and in two preparations of mixed traditional Chinese medicines. *J. Chromatogr. A.*, 961(1): 83-88.
- Tsm TH, Westly J, Lee TF, Chen CF (1994). Identification and determination of geniposide, genipin, gardenoside and geniposidic acid from herbs by HPLC/Photodiode-Array Detection. *J. Liq. Chrom. Rel. Technol.*, 17(10): 2199-2205.
- Watanabe T, Yamamoto A, Nagai S, Terabe S (1998). Separation and determination of yellow *Gardenia* pigments for food and iridoid constituents in *Gardenia* fruits by micellar electrokinetic chromatography. *Food Sci. Technol. Int.*, 4(1): 54-58.
- Wei XH., Cheng XM, Shen JS, Wang ZT (2008). Antidepressant effect of Yueju-Wan ethanol extract and its fractions in mice models of despair. *J. Ethnopharmacol.*, 117(2): 339-344.
- Wilner P (1997). Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacol. (Berl)*, 134(4): 319-329.
- Wu HJ, Wang QB, Tan ZY, Tang JF., Lu YB (2010a). Determination of geniposide contents in gardenia from different areas with HPLC. *J. Trad. Chin. Med. Univ. Human*, 5: 35-37.
- Wu HX, Zhang DS, Shen LX (2010b). The protective effects of geniposide on memory impaired mice induced by acute alcoholic intoxication. *J. Hebei North Univ. (Medical Edition)*, 27(2): 18-20.
- Yang C, Hao Z, Xi T, Zhao C, Le C, Ying L, Lin J, Hong-Xiang Y., Chu C (2008). Antioxidant potential of crocins and ethanol extracts of *Gardenia jasminoides* Ellis and *Crocus sativus* L.: A relationship investigation between antioxidant activity and crocin contents. *Food Chem.*, 109(3): 484-492.
- ~~Yang CG, Fan MS, Sun JL, Tu HW (2010). The research development of chemical constituents, pharmacological action and in vivo processing for *Fructus Gardeniae*. *Modern Chin. Med.*, 12(9): 7-12.~~
- Zhou TT, Fan GR, Hong ZY, Chai Y F, Wu YT (2005). Large-scale isolation and purification of geniposide from the fruit of *Gardenia jasminoides* Ellis by high-speed counter-current chromatography. *J. Chromatogr. A.*, 1100(1): 76-80.

Zhu DC, Huang ZG, Xia Q, Cheng G, Sun HL, Song SZ, Wei XL, Ge CL and Liu G (2009). The preliminary research of geniposide extraction from Gardenia. *Lishizhen Med. Materia Medica Res.*, 7: 1684-1685