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Different Effects of Flavonoids in *Scutellaria baicalensis* on Anxious and Sedative Behaviors

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Abstract – The main aim of this study was to characterize the pharmacological profile of flavonoids utilizing behavioral tests and to investigate how the psychopharmacological activities of wogonin, baicalein and oroxylin A are different. Wogonin, baicalein and oroxylin A were intraperitoneally injected as dosages of 2.5, 5, 10 and 20 mg/kg. In the locomotor activity, Rota-rod test, and elevated plus-maze tests, the behavioral parameters were analyzed by automatic systems. Thiopental induced sleeping time was measured. Water extract of *S. baicalensis* didn't exhibit sedative effect. Wogonin and bacalein exhibited anxiolytic activity although it was less potent than buspirone. Wogonin and baicalein decreased locomotor activity at a dose of 10 mg/kg. Wogonin also shortened significantly running time on the rota-rod at doses of 5 and 10 mg/kg. Wogonin and baicalein enhanced sleeping at doses of 5 and 10 mg/kg. These results indicate that wogonin produce anxiolysis with sedation and so did bacalein with mild sedation. On the contrary, oroxylin A enhanced running activity on the rotarod and did't depress locomotor activity. Oroxylin A significantly hindered sleeping rather than helped it at doses of 5 and 10 mg/kg. Oroxylin A didn't produce anxiolysis and instead, produce awakening effect. This study demonstrates that wogonin and bacalein exhibited anxiolytic activity with mild sedation, but oroxylin A didn't produce anxiolysis and instead, produce awakening effect. This result indicates that anxiolytic effect without sedation induced by *Scutellaria baicalensis* is produced by combination of flavonoids.

Keywords □ Scutellaria baicalensis, wogonin, baicalein, oroxylin A, sedation, anxiolysis

INTRODUCTION

Scutellaria baicalensis is one of the most important medicinal herbs in traditional Korean medicine. The root of *S. baicalensis* is widely employed in traditional Korean prescriptions. Flavonoids from this medicinal herb have been shown to possess a broad spectrum of antiviral, antioxidant, anti-inflammatory, and antiallergic actions (Ma *et al.*, 2002; Hui *et al.*, 2002; Shieh et al., 2000; Wakabayash and Yasui, 2000; Zhu, 1998). Wogonin, baicailin, baicalein, and oroxylin A, the major chemical constituents of this herb, are flavone derivatives containing a phenylbenzopyrone nucleus(Hui *et al.*, 2002; Lin and Shieh, 1996). As part of the screening of traditional herbal extracts for benzodiazepine activity, it was reported that several flavonoids isolated from this herb exhibited moderate affinities for the receptor (Dekermendjian *et al.*, 1999). Behavioral studies demonstrated that water extract of *Scutellaria baicalensis* exerted potent anxiolysis in mice without sedative and myorelaxant actions (Jeong *et al.*, 2004). The biological and pharmacological properties of its flavonoids are broad and include anti-inflammatory actions (Lin and Shieh, 1996; Barnard *et al.*, 1993), the reduction of neuronal oxidative metabolism (Oyama *et al.*, 1994), steroid hormone like effects (Miksicek, 1993), and the inhibition of enzymes including protein kinase C and tyrosine kinase (Ferriola *et al.*, 1989; Cushman *et al.*, 1991). Flavonoids, as a class of naturally occurring compounds found in most vascular plants, have been demonstrated by a number of groups to be centrally active, possessing efficacies for a

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number of receptor systems in the central nervous system (Hui *et al.*, 2002).

Flavonoids from *S. baicalensis* may exert pharmacologically and clinically important profiles including anxiolysis, anti-convulsion, muscle-relaxation, and sedation because they bind with benzodiazepine or GABA receptors (Hui *et al.*, 2000). The psychopharmacological properties of flavonoids may be also different although several groups have reported anxiolytic effects of flavonoids (Hui *et al.*, 2002; Paladini *et al.*, 1999; Salgueiro *et al.*, 1997). In previous study, total extract of *Scutellaria baicalensis* partially blocked the suppression of locomotion as well as behavioral changes induced by electro-shock stress (Ryu *et al.*, 2004), but it was not identified which constituent has anti-stress or anxiolytic effect.

Screening of traditional medicines has proven invaluable to drug development and discovery. We have been studying to characterize the psychopharmacological properties of flavonoids isolated from *Scutellaria baicalensis*. The main aim of this study was to characterize the pharmacological profile of these flavonoids utilizing behavioral tests and to investigate how the psychopharmacological activities of wogonin, baicalein and oroxylin A are different.

MATERIALS AND METHODS

Animals and materials

The male Sprague-Dawley (SD) rats (8-10 weeks of age) and the male ICR mice (20-25 g) used in this study were obtained from Hanlim experimental animal Co. We used *Scutellaria baicalensis* which was obtained from herbal suppliers in Seoul and wogonin, baicalein and oroxylin A were supplied from National center for standardization of herbal medicine. Buspirone, diazepam, and other materials were purchased from Sigma-Aldrich Co. (St. Louis, Mo, USA). All animals were housed in a temperature (22±2°C) and humidity (55±5%) controlled animal room on a 12 hr/12 hr light/dark (6 A.M.-6 P.M.) schedule. They had free access to food and water throughout the experiments. The animals were divided into six groups after stabilizing them for 1 week in our animal room. Total extract of *Scutellaria baicalensis* was orally injected as dosages of 100 and 200 mg/kg. Wogonin, baicalein and oroxylin A were intraperitoneally injected as dosages of 2.5, 5, 10 and 20 mg/kg. Animals of control group were intraperitoneally injected the same volume of saline as flavonoid or extract solution. Diazepam 5 mg/kg was intraperitoneally injected to mice of positive control group.

Behavioral apparatus

The equipment was located in the animal room allowing the observer to view and observe the animals through a computer outside the room. 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, 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).

Elevated plus-maze test

The Elevated plus-maze box and arms were made of Plastic. 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. The each four arms has 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).

Rotarod evaluation

The rotarod test was used to assess whether constituents of SB caused myorelaxation or gross motor impairment in the animals. Twenty-four hours before the experiment, all mice were habituated to running in a rotarod at a speed of 60 rpm until they could remain there for 60 s without falling. The latency to fall were recorded with a stopwatch (Lee *et al.*, 2005; Farkas *et al.*, 2005).

Thiopental-induced sleep

Male ICR mice weighing 20-25 g were treated subcutanously with thiopental sodium (50 mg/kg) after intraperitoneal (-30 min) administration of the test compound. Time between the loss and recovery of the righting reflex was measured. Animals were observed for 30 min following thiopental injection. If no recovery was seen, the sleep time was taken as 30 min for calculation purposes. A saline-treated control group was also tested each day (Farkas *et al.*, 2005).

Statistical analysis

Data are expressed as the mean±S.E.M.. ANOVA was used to compare the scores among the groups for one variable. This was followed by post hoc comparisons using the Newman-Keuls test.

RESULTS

As shown in Fig. 1A and B, movement distance and moving duration of mice in open field were not significantly changed by treating with total extract of *S. baicalensis* and running time in rota-rod was also not changed. Total extract of *S. baicalensis* didn't produce sedation.

Figure 2 shows that wogonin and baicalein decreased locomotor activity in mice but oroxylin A didn't. Locomotor activities were measured as the total moving time and moved distance. Administration of wogonin 10 mg/kg significantly decreased movement duration and movement time and baicalein 10mg/kg significantly decreased movement duration. This results indicate that wogonin and bacalein may produce sedation.

Motor coordination was evaluated in Rota-rod test. As shown in figure 3, 5 mg and 10 mg/kg of wogonin significantly shortened the running time on the rota-rod, but 5 mg and 10 mg/kg of oroxylin A significantly prolonged the running time. Oroxylin A enhanced motor coordinative activity but wogonin decreased the activity. The results in Rota-rod test also explain that wogonin and bacalein may produce sedation.

Figure 4 shows that adminstration of wogonin 5 and 10 mg/kg and baicalein 10 mg/kg significantly shortened onset time of sleeping induced by thiopental sodium and wogonin 5 and 10 mg/kg significantly prolonged sleeping duration, but oroxylin A 5 mg/kg, 10 mg/kg and 20 mg/kg significantly prolonged sleeping onset and 5 mg/kg and 10 mg/kg significantly shortened sleeping duration. This results also prove that wogonin and bacalein can produce sedation. On the other hand, oroxylin A can produce awakening effect.

The time spent in the open or closed arm for 5 minutes significantly differed between buspirone treated mice and saline treated mice as shown in figure 5. The mice treated buspirone, anxiolytic agent spent more time in the open arm than saline treated mice. Furthermore, buspirone treated mice enter more frequently into open arm. Administration of wogonin 5, 10 and 20 mg/kg and baicalein 10 mg/kg significantly also spent more time in the open arm and entered more frequently into the open arm than the saline treated animals. Wogonin and bacalein

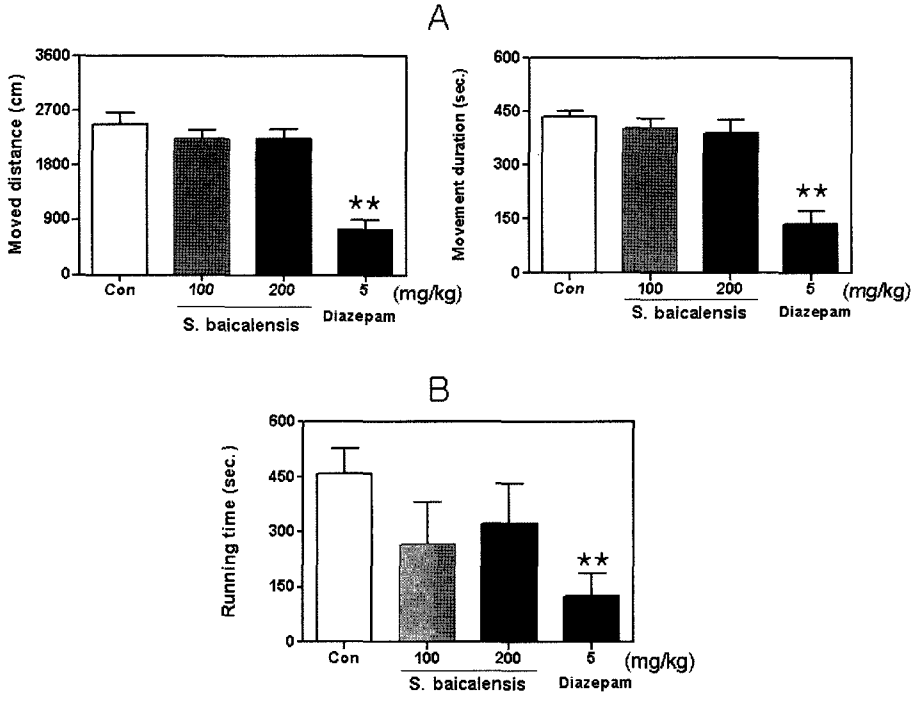

Fig. 1. Effects of *Scutellaria baicalensis* on locomotor activity and rotarod test in ICR mice (n=9). A : Each bar represents mean± SEM of total moved times (right) and distances (left) for 10 minutes. B : Each bar represents mean±SEM of running times to fall on the Rota-rod. S. *Baicalensis* or Diazepam versus Control, ** $p < 0.01$; * $p < 0.05$.

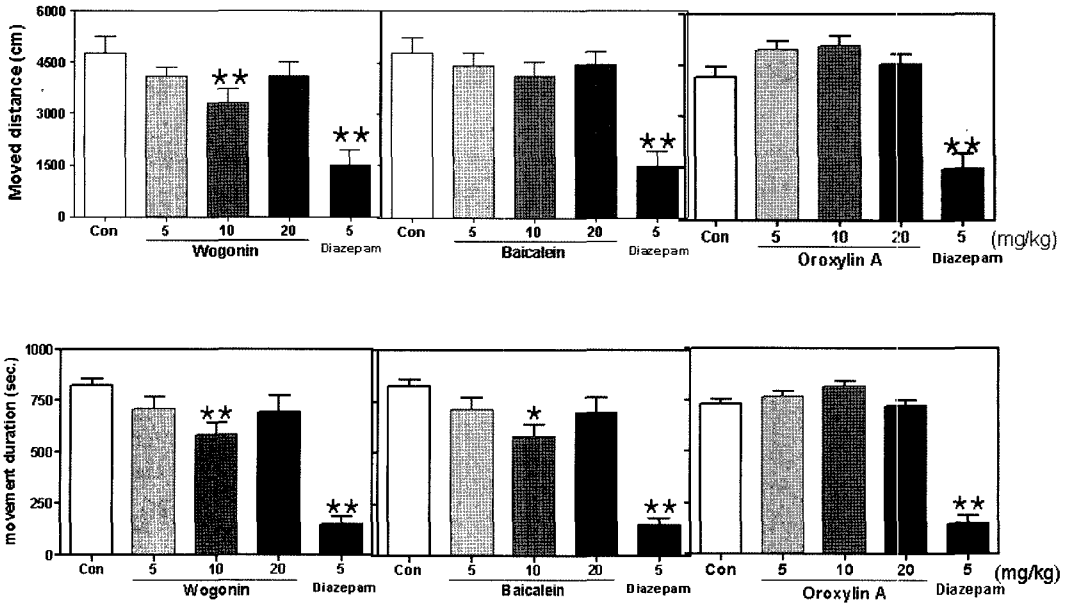

Fig. 2. Effects of *S. baicalensis* flavonoids on locomotor activity in ICR mice (n=9). Each bar represents mean±SEM of total moved distances (upper) and times (lower) for 20 minutes. Wogonin, Baicalein, Oroxylin A or Diazepam versus Control, ** $p < 0.01$; * $p < 0.05$.

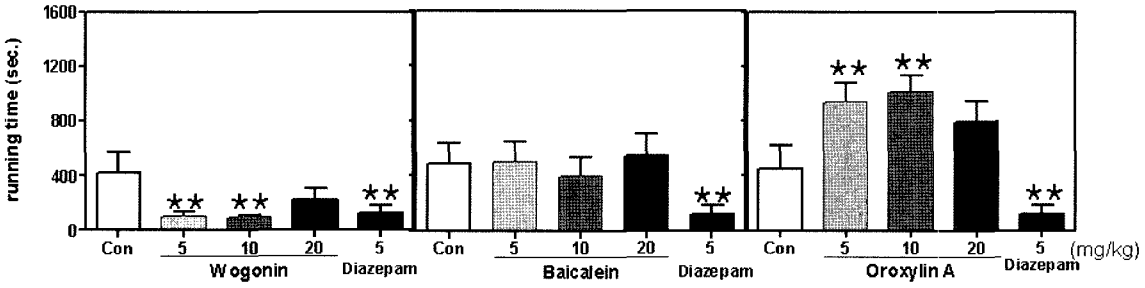

Fig. 3. Effects of *S. baicalensis* flavonoids in ICR mice rotarod test (n=10). Each bar represents mean±SEM of running times to fall on the Rota-rod. Wogonin, Baicalein, Oroxylin A or Diazepam versus Control, ** $p < 0.01$; * $p < 0.05$.

exhibited anxiolytic activity although it was less potent than buspirone.

DISCUSSION

Water extract of *S. baicalensis* have been shown to possess a broad spectrum of antiviral, antioxidant, anti-inflammatory, anxiolytic and antiallergic actions (Jeong *et al.*, 2004; Ma *et al.*, 2002; Hui *et al.*, 2002; Shieh *et al.*, 2000). The total flavonoids from the stems and leaves of *S. baicalensis* could enhance and improve learning and memory abilities in experimental animals, and reduce the neuronal pathologic alterations induced by some reagents in mice (Shang *et al.*, 2006; Shang *et al.*, 2005). Behavioral studies demonstrated that *S. baicalensis* and flavonoids from this medicinal herb exerted potent anxiolysis in mice without sedative and myorelaxant actions (Jeong *et al.*, 2004; Michael *et al.*, 2003). As part of the screening of traditional herbal extracts for benzodiazepine binding site activity, it was reported that several flavonoids isolated from this herb exhibited moderate affinities for the receptor (Hui *et al.*, 2000). In previous study, we also identified the fact that water extract of *S. baicalensis* have anxiolytic effect without sedation (Jeong *et al.*, 2004).

The present study describes the pharmacological characterization of active flavonoids, such as oroxylin A, baicaleien and wogonin isolated from *S. baicalensis*. Wogonin and bacalein exhibited anxiolytic activity although it was less potent than buspirone. Wogonin and baicalein decreased locomotor activity at a dose of 10 mg/kg. Wogonin also shortened significantly running time on the rota-rod at doses of 2, 5 and 10 mg/kg.

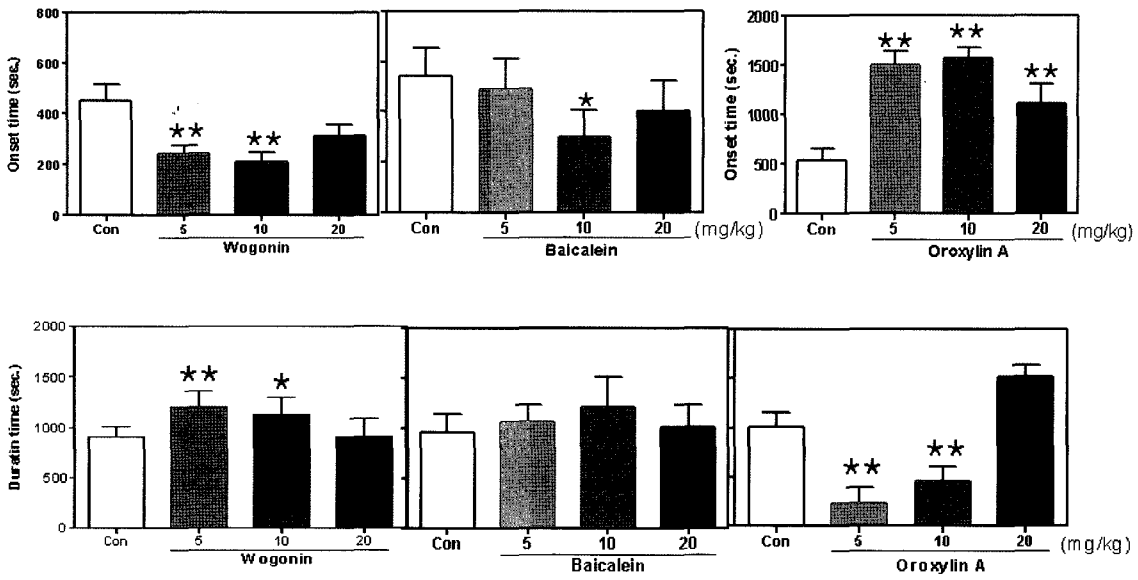

Fig. 4. Effects of *S. baicalensis* flavonoids on thiopental induced sleeping in ICR mice (n=10). Each bar represents mean±SEM of onset times (upper) and durations of sleeping induced by thiopental. Wogonin, Baicalein or Oroxylin A versus Control, ** $p < 0.01$; * $p < 0.05$.

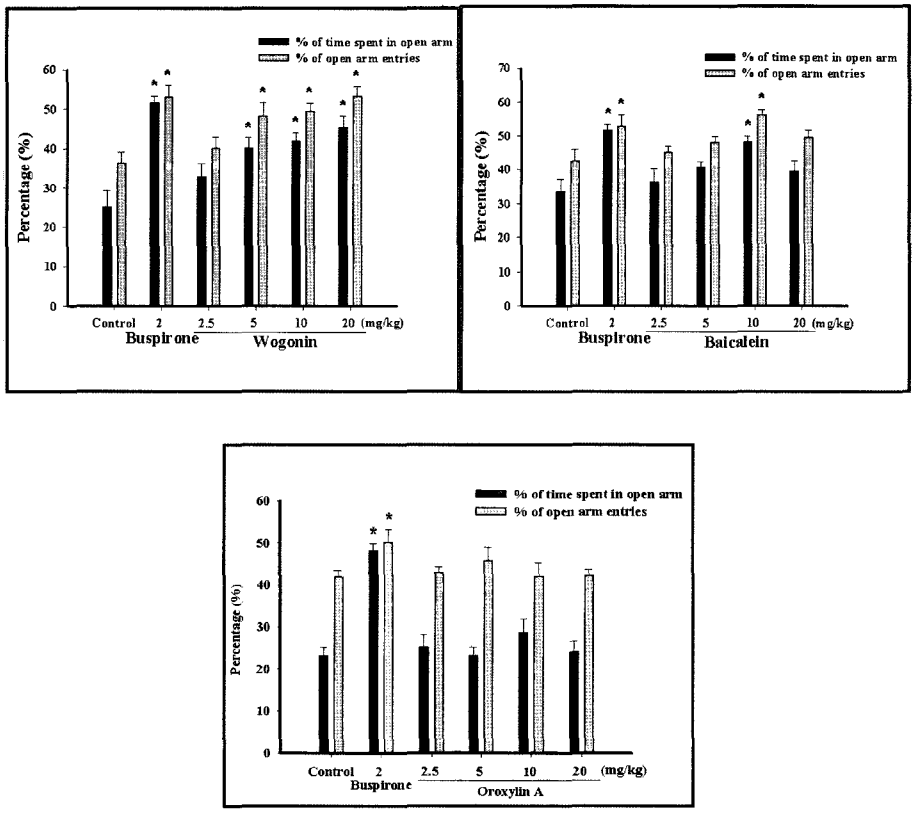

Fig. 5. Effects of *S. baicalensis* flavonoids in mice elevated plus-maze test (n=10). Each bar represents mean±SEM percentage of entries and time spent in open arms. Wogonin, Baicalein, Oroxylin A, Buspirone versus Control, ** $p < 0.01$; * $p < 0.05$.

Wogonin and baicallein enhanced sleeping time at doses of 5 and 10 mg/kg. These results indicate that wogonin produce anxiolysis with sedation and so does bacalein with mild sedation. On the contrary, oroxylin A enhanced running activity on the rota-rod and did't depress locomotor activity. Oroxylin A significantly hindered the sleeping rather than helped it at doses

of 5 and 10 mg/kg. Oroxylin A didn't produce anxiolysis and instead, produce awakening effect. This result corresponds with the other reports that oroxylin A exhibits the pharmacological profile with opposition to the diazepam-induced anxiolytic, myorelaxant and motor incoordination effects, but not the sedative and anticonvulsant effects elicited by this benzodiazepines (Shang *et al.*, 2006).

The effects of flavonoids didn't exhibit dose dependent profile in this study, and instead, they did a bell-shaped dose-response relationship which was showed in another studies (Louis et al., 2006; Hui et al., 2002). One possible cause of such a bell-shaped response is the change of specificity or selectivity on GABA or benzodiazepine receptors (Michael *et al.*, 2003; Hui *et al.*, 2002; Wang *et al.*, 1999).

S. baicalensis contains the various flavonoids such as wogonin, baicailin, baicalein, and oroxylin A which possess different pharmacological spectrum. In the previous study, the anxiolytic effect of *S. baicalensis* was identified (Jeong *et al.*, 2004). In this study, wogonin and baicalein exhibited anxiolytic activity although oroxylin A didn't. This result indicates that the anxiolytic effect of *S. baicalensis* was caused by wogonin and baicalein. Wogonin and baicalein produce sedation but the total extract of *S. baicalensis* didn't. The reason can be explain by oroxylin A. which produced the awakening effect.

The recent discovery of its anxiolytic activity suggests a new mechanism of action, involving interaction with the benzodiazepine binding site of the $GABA_A$ receptor and modulation of this receptor activity. Ligand binding at the benzodiazepine binding site on the GABAA receptor complex are known to exert such pharmacological actions as anxiolysis, anticonvulsion, muscle relaxation and sedation (Wang *et al.*, 1999). The $GABA_A$ receptor is a member of the ligand-gated ion channel superfamily, GABA being the major inhibitory transmitter in the CNS. Binding of GABA to the $GABA_A$ receptor activates a chloride ion flux through the channel, and ligands for the benzodiazepine binding site modulate the inhibitory effects of GABA (Wang *et al.*, 1999). Such benzodiazepine binding site ligands are classified as positive allosteric mdulators, antagonists, or negative allosteric modulators according to their spectrum of intrinsic efficacy towards the $GABA_A$ receptor (Gardner *et al.*, 1993). Positive allosteric modulators increase the frequency of chloride channel openings without altering the channel conductance or duration of opening. Therapeutically, they are used as anxiolytic, anti-convulsant, sedative-hypnotic, and muscle relaxant drugs. Many *in vitro* data indicate positive modulatory efficacies of wogonin and baicalein for the GABAA receptor via interaction with the benzodiazepine binding site and negative efficacy of oroxylin A (Michael *et al.*, 2003; Hui *et al.*, 2002; Hui *et al.*, 2003). Thus reports coincide with behavioral data of this study.

In summary, our study demonstrated that wogonin and bacalein exhibited anxiolytic activity with mild sedation, but oroxylin A didn't produce anxiolysis and instead, produced awakening effect. This result indicates that anxiolytic effect without sedation induced by *Scutellaria baicalensis* was produced by combination of flavonoids.

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