Korean J Physiol Pharmacol
Vol 14: 285−289, October, 2010
DOI: 10.4196/kjpp.2010.14.5.285

Antinociception Effect and Mechanisms of *Campanula Punctata* Extract in the Mouse

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In the present study, the antinociceptive profiles of *Campanula punctata* extract were examined in ICR mice. The *Campanula punctata* contain a large dose of saponin. *Campanula punctata* extract administered orally (200 mg/kg) showed an antinociceptive effect as measured by the tail-flick and hot-plate tests. In addition, *Campanula punctata* extract attenuated the writhing numbers in the acetic acid-induced writhing test. Furthermore, the cumulative nociceptive response time for intrathecal (i.t.) injection of substance P (0.7 μg) was diminished by *Campanula punctata* extract. Intraperitoneal (i.p.) pretreatment with yohimbine (α_2-adrenergic receptor antagonist) attenuated antinociceptive effect induced by *Campanula punctata* extract in the writhing test. However, naloxone (opioid receptor antagonist) or methysergide (5-HT serotonergic receptor antagonist) did not affect antinociception induced by *Campanula punctata* extract in the writhing test. Our results suggest that *Campanula punctata* extract shows an antinociceptive property in various pain models. Furthermore, this antinociceptive effect of *Campanula punctata* extract may be mediated by α_2-adrenergic receptor, but not opioidergic and serotonergic receptors.

Key Words: *Campanula punctata*, Anti-nociception, Inflammatory pain, α_2 adrenoceptor

INTRODUCTION

Campanula punctata is one of several genera in the family Campanulaceae with the common name bellflower. It takes its name from their bell-shaped flowers- *Campanula punctata* is Latin for "little bell". The *Campanula punctata* contain a large dose of saponin. The leaves are alternate and often vary in shape on a single plant, with larger, broader leaves at the base of the stem and smaller, narrower leaves higher up; the leaf margin may be either entire or serrated (sometimes both on the same plant). Many species contain white latex in the leaves and stems. The flowers are produced in panicles (sometimes solitary), and have a five-lobed corolla, typically large (2～5 cm or more long), mostly blue to purple, sometimes white or pink. Below the corolla, 5 leaf-like sepals form the calyx. Some species have a small additional leaf-like growth termed an "appendage" between each sepal, and the presence or absence, relative size, and attitude of the appendage is often used to distinguish between closely-related species. The fruit is a capsule containing numerous small seeds [1].

Campanula punctata is cultivated for food and medical purposes. In Korea, people usually gather bellflower roots in spring and autumn to use in seasoned salads or as a cooked potherb. In addition, because it is known to have a mucolytic and antitussive effect, *Campanula punctata* is used in Oriental medicine for the treatment of acute or chronic bronchitis, tonsillitis, and asthma [2].

However, the mechanism and pain of this herb is not clear. Therefore, in this study, we attempted to characterize antinociceptive profiles and mechanisms of *Campanula punctata* extract in various pain models.

Received August 5, 2010, Revised September 10, 2010, Accepted September 14, 2010

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METHODS

These experiments were approved by the University of Hallym Animal Care and Use Committee (Registration Number: Hallym 2009-05-01). All procedures were conducted in accordance with the 'Guide for Care and Use of Laboratory Animals' published by the National Institutes of Health and the ethical guidelines of the International Association for the Study of Pain.

Experimental animals

Male ICR mice (MJ Co., Seoul, Korea) weighing 20～25 g were used for all the experiments. Animals were housed 5 per cage in a room maintained at 22±0.5°C with an alternating 12 hr light-dark cycle. Food and water were available *ad libitum*. The animals were allowed to adapt to the laboratory for at least 2 hr before testing and were only

ABBREVIATIONS: i.t., intrathecal; i.p., Intraperitoneal.

used once. Experiments were performed during the light phase of the cycle (10:00～17:00).

Drugs

Acetic acid, substance P, naloxone, methysergide and yohimbine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). *Campanula punctata* (300 g) was dissolved in 80% ethanol (1,500 ml) and extracted as refluxing for 3 hours, and then the extract was filtered for obtaining A. This process was repeated again once to obtain B from residue. A and B were mixed. This mixture was decompressed and dried for using as *Campanula punctata* extract. *Campanula punctata* extract, naloxone, methysergide and yohimbine were dissolved in saline. All drugs were prepared just before use.

Oral administration, and intraperitoneal (i.p.) and intrathecal (i.t.) injections

Oral administration was performed with gage in a volume of 500 μl/25 g body weight. I.p. injection was conducted to unanesthesized mice with volume of 250 μl. The i.t. administration was performed following the method of Hylden and Wilcox [3,4] using a 30-gauge needle connected to a 25 μl Hamilton syringe with polyethylene tubing. The i.t. injection volume was 5 μl and the injection site was verified by injecting a similar volume of 1% methylene blue solution and determining the distribution of the injected dye in the spinal cord. The dye injected i.t. was distributed both rostrally and caudally but with short distance (about 0.5 cm from the injection site) and no dye was found visually in the brain. The success rate for the injections was consistently found to be over 95%, before the experiments were done.

Assessment of antinociception and experimental protocols

All assessments for measuring antinociceptive properties of *Campanula punctata* extract were carried out by blinded observers.

Tail-flick and hot-plate tests

Antinociception was determined by the tail-flick [5] and the hot-plate paw-licking tests [6]. For the measurement of the tail-flick latency, mice were gently held with one hand with the tail positioned in the apparatus (EMDIE Instrument Co., Maidens, VA, USA, Model TF6) and the tail-flick response was elicited by applying radiant heat to the dorsal surface of the tail. The intensity of radiant heat was adjusted so that the animal flicked its tail within 3 to 5 sec. For the hot-plate test, mice were individually placed on the 55°C hot-plate apparatus (Itic Life Science, Woodland Hills, CA, USA, Model 39 Hot Plate) and then, the reaction time starting from the placement of the mouse on the hotplate to the time of licking the front paw was measured. Basal latency for the hot-plate test was approximately 9 sec. Animals were pretreated orally once with vehicle (control) or *Campanula punctata* extract at 200 mg/kg doses 30 min prior to performing the tail-flick or hot-plate tests.

Acetic acid-induced writhing test

For the writhing test [7], 1% acetic acid was injection i.p. and then, the animals were immediately placed in an acrylic observation chamber (20 cm high, 20 cm diameter). The number of writhes was counted during 30 min after the injection of acetic acid. A writhe was defined as a contraction of the abdominal muscles accompanied by an extension of the forelimbs and elongation of the body. Animals were pretreated orally once with vehicle (control) or *Campanula punctata* extract at 200 mg/kg doses 30 min prior to performing the acetic acid-induced writhing test.

Substance P-induced nociceptive behavioral test

Vehicle (control) or 200 mg/kg of *Campanula punctata* extract was pretreated orally 30 min prior to performing i.t. injection of substance P (0.7 μg/5 μl). Immediately after i.t. injection with substance P the mice were placed in an observation chamber (20 cm high, 20 cm diameter) and their nociceptive behavioral responses were recorded during 30 min. The cumulative response time of licking, scratching and biting episodes directed toward the lumbar and caudal region of spinal cord were measured with a stopwatch timer [4].

Pretreatment of antagonists

At first, mice were pretreated i.p. with either saline, naloxone (5 mg/kg), methysergide (5 mg/kg), or yohimbine (5 mg/kg), 10 min before oral administration of vehicle as a control or a fixed dose of *Campanula punctata* extract (200 mg/kg). And then, the writhing response was tested 30 min after the treatment with either vehicle or *Campanula punctata* extract [8-12].

Statistical analysis

Data were presented as the mean±SEM. The statistical significance of differences between groups was assessed with one-way ANOVA with Bonferroni's post-hoc test using GraphPad Prism version 4.0 for Windows Vista (GraphPad Software, San Diego, CA, USA); $p<0.05$ was considered significant.

RESULTS

Effect of Campanula punctata extract on the tail-flick and hot-plate paw-licking responses

As revealed in Fig. 1, oral treatment of *Campanula punctata* extract at the dose of 200 mg/kg increased latencies of the tail-flick and hot-plate paw-licking responses compare to the control group of mice. The sedative effect was manifested, when the mice were treated with *Campanula punctata* extract orally at the dose of 200 mg/kg. However, there were no paralysis and motor changes.

Effect of Campanula punctata extract on the nociceptive behavior induced by acetic acid and substance P

Campanula punctata extract attenuated the acetic acid-induced writhing numbers (Fig. 2A). Treatment with *Campanula punctata* extract at the dose of 200 mg/kg led to 85% decrease in the acetic acid-induced writhing response compare to the control group of mice. In vehicle- treated control mice, i.t. injection of substance P (0.7 μg) caused

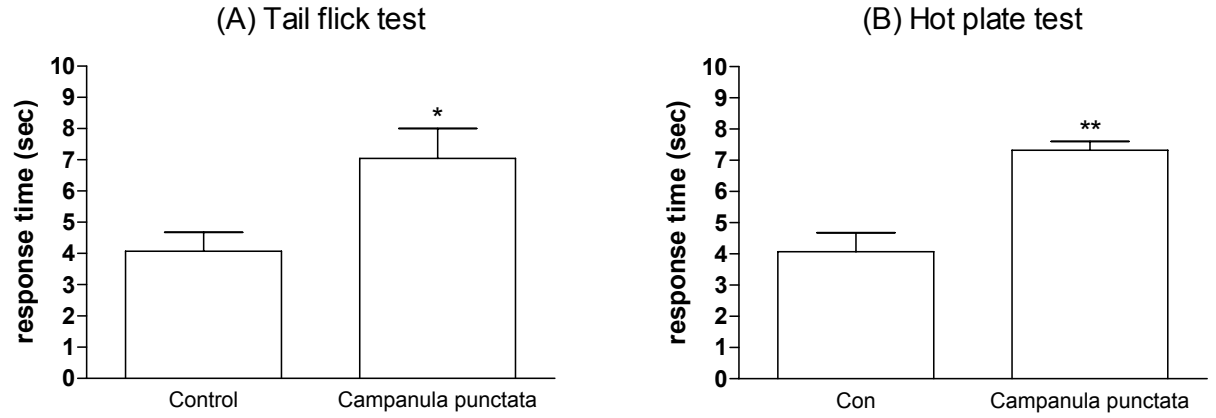

Fig. 1. The antinociceptive effect of *Campanula punctata* extract administered orally in the tail-flick and hot-plate tests. Mice were administered orally with either vehicle or 200 mg/kg of *Campanula punctata* extract and the tail-flick (A) or hot-plate (B) response was measured at 30 min after treatment. The vertical bars denote the standard error of the mean. The number of animal used for each group was 8~10 (*p<0.05, **p<0.01, compared to the vehicle-treated control group of mice).

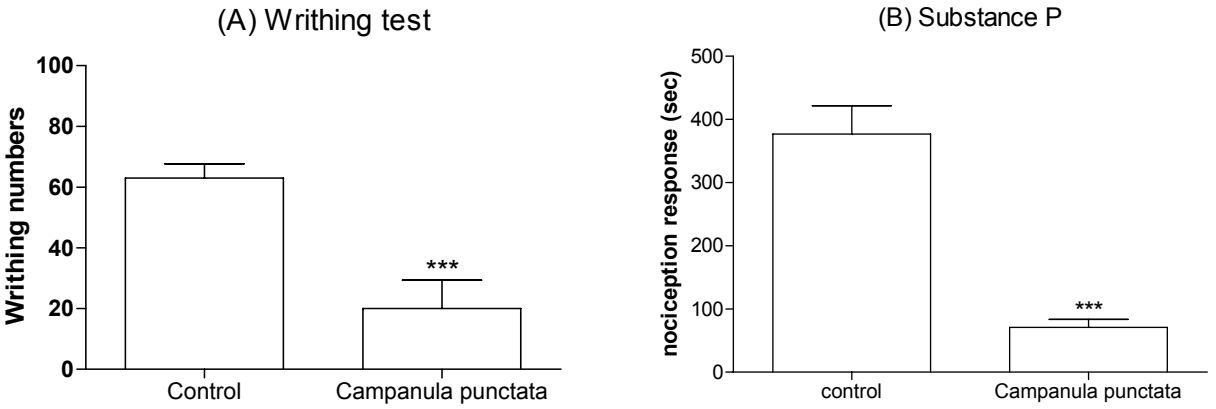

Fig. 2. Effect of *Campanula punctata* extract on the nociceptive response induced by various pain models. *Campanula punctata* extract (200 mg/kg) was administered orally and then, 0.25 ml of 1% acetic acid solution was injected intraperitoneally 30 min after treatment. The number of writhing was counted for 30 min following acetic acid injection (A). *Campanula punctata* extract (200 mg/kg) was administered orally for 30 min prior to the substance P (0.7 μg per 5 μl) injection intrathecally (B). The cumulative response time of licking, scratching and biting episodes was measured for 30 min. The vertical bars indicate the standard error of the mean. The number of animal used for each group was 8~10 (***p<0.001, compared with control group).

acute, immediate behavioral responses, i.e., licking, scratching and biting the lumbar or caudal region, which lasted about 30 min. As shown in Fig. 2B, cumulative nociceptive response times for i.t. administration of substance P was significantly diminished by 80%.

Effect of opioidergic, serotonergic and adrenergic system on the inhibition of writhing response induced by Campanula punctata extract

We examined the possible involvement of opioidergic, serotonergic and adrenergic system in the *Campanula punctata* extract-induced antinociception. The pretreatment with naloxone (opioid receptor antagonist, Fig. 3A) or methysergide (serotonergic receptor antagonist, Fig. 3B) did not affect *Campanula punctata* extract-induced antinociception. However, the blockade of α_2-adrenergic receptor with systemic pre-administration of yohimbine abolished the *Campanula punctata* extract-induced inhibition of the writhing response (Fig. 3C). The treatment of naloxone, methysergide or yohimbine itself did not affect the writhing response (Fig. 3).

DISCUSSION

In the present study, we found that *Campanula punctata* extract administered orally produces antinociception in various pain models. The tail-flick response is believed to be a spinally mediated reflex and the paw-licking hotplate response is a more complex supraspinally organized behavior (for review, see [13]). Moreover, Grumbach (1966) has shown that the effectiveness of analgesic agents in the tail-flick pain model is highly correlated with relief of human pain [14]. Our results demonstrate that *Campanula punctata* extract causes to prolong the tailflick and hot-plate response latencies, indicating the increase of nociceptive threshold.

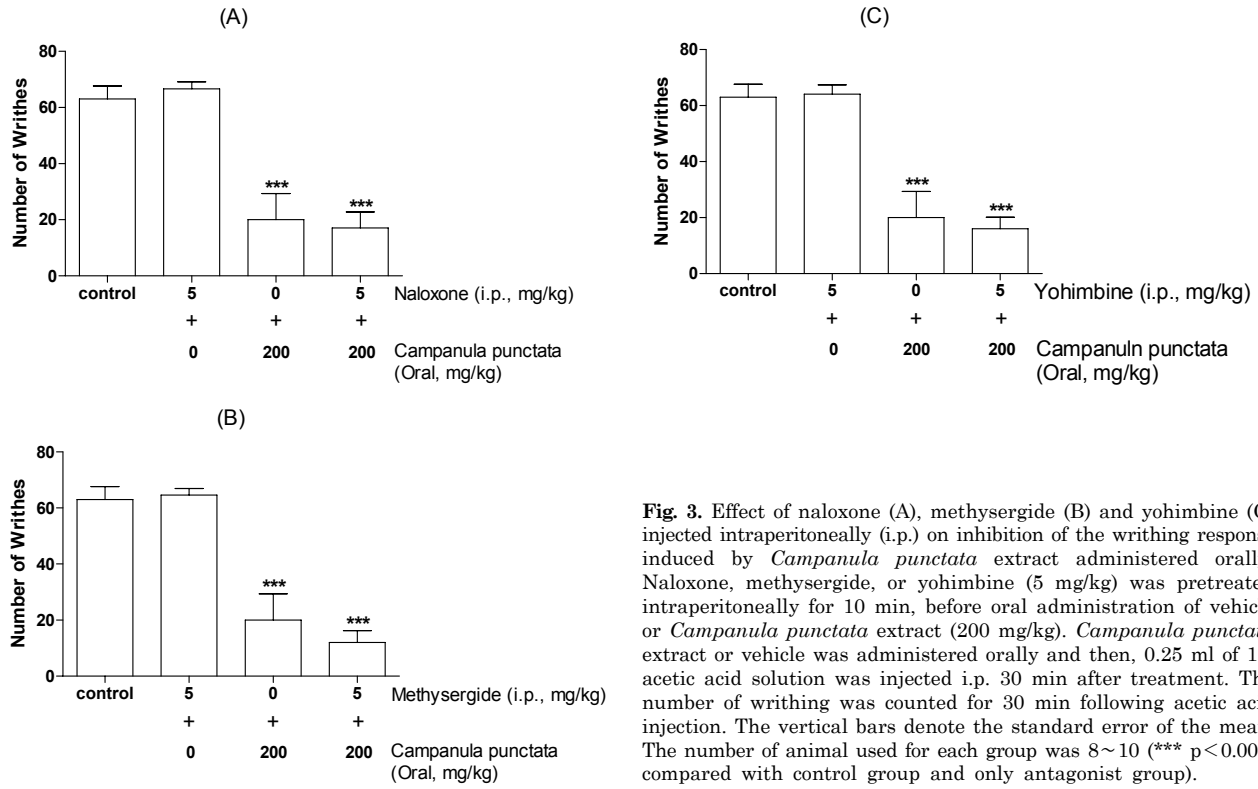

Fig. 3. Effect of naloxone (A), methysergide (B) and yohimbine (C) injected intraperitoneally (i.p.) on inhibition of the writhing response induced by *Campanula punctata* extract administered orally. Naloxone, methysergide, or yohimbine (5 mg/kg) was pretreated intraperitoneally for 10 min, before oral administration of vehicle or *Campanula punctata* extract (200 mg/kg). *Campanula punctata* extract or vehicle was administered orally and then, 0.25 ml of 1% acetic acid solution was injected i.p. 30 min after treatment. The number of writhing was counted for 30 min following acetic acid injection. The vertical bars denote the standard error of the mean. The number of animal used for each group was 8∼10 (*** $p<0.001$, compared with control group and only antagonist group).

We also examined the effect of *Campanula punctata* extract on the acetic acid-induced writhing test. I.p. injection of acetic acid can produce the peritoneal inflammation (acute peritonitis), which cause a response characterized by contraction of the abdominal muscles accompanying an extension of the forelimbs and elongation of the body. This writhing response is considered as a visceral inflammatory pain model [6, for review, see 15]. In the present study, we clearly showed the antinociceptive effect of *Campanula punctata* extract in an acetic acid-induced writhing test. Furthermore, it has been reported that i.t. injection of substance P in mice can also elicit nociceptive responses, consisting of biting, scratching and licking the caudal parts of the body [4,16]. We found in the present study that *Campanula punctata* extract was also effective in attenuating substance P-induced nociceptive responses. These results suggest furthermore that *Campanula punctata* extract may exert their antinociceptive effect via the central sites, possibly spinally mediated mechanisms.

The roles of opioid, serotonergic and adrenergic receptors in the regulation of modulation of nociceptive processing have been demonstrated in many previous studies. For example, it is well known that opioid receptors are involved in the antinociception [17-19]. Also, it has been reported that blockade of the spinal serotonergic or noradrenergic receptors by spinal injection of methysergide or yohimbine antagonize the antinociception induced by morphine administered supraspinally [18,20,21]. We observed in the present study that α_2-adrenergic receptor, but not opioidergic and serotonergic receptors, appear to be involved in orally administered *Campanula punctata* extract-induced antinociception.

In conclusion, our results suggest that *Campanula punctata* extract shows an antinociceptive property in various pain models. Furthermore, this antinociceptive effect of *Campanula punctata* extract may be mediated by α_2-adrenergic receptor, but not opioidergic and serotonergic receptors.

ACKNOWLEDGEMENTS

This work was supported by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0094072).

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