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The Analgesic Effect and Mechanisms of *Dianthus chinensis L* Extract in the mice.

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Abstract - In the present study, the antinociceptive profiles of *Dianthus chinensis L* extract were examined in ICR mice. *Dianthus chinensis L* extract administered orally (200 mg/kg) showed an antinociceptive effect as measured by the tail-flick and hot-plate tests. In addition, *Dianthus chinensis L* 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 *Dianthus chinensis L* extract. Intraperitoneal (i.p.) pretreatment with yohimbine (α_2-adrenergic receptor antagonist) attenuated antinociceptive effect induced by *Dianthus chinensis L* extract in the writhing test. However, naloxone (opioid receptor antagonist) or methysergide (5-HT serotonergic receptor antagonist) did not affect antinociception induced by *Dianthus chinensis L* extract in the writhing test. Our results suggest that *Dianthus chinensis L* extract shows an antinociceptive property in various pain models. Furthermore, this antinociceptive effect of *Dianthus chinensis L* extract may be mediated by α_2-adrenergic receptor, but not opioidergic and serotonergic receptors.

Key words - *Dianthus chinensis L*; Anti-nociception; Inflammatory pain; α_2 adrenoceptor

Introduction

Dianthus chinensis L is a species of Dianthus native to northern China, Korea, Mongolia, and southeastern Russia [Flora of China, 1753]. It is a herbaceous perennial plant growing to 30~50 cm tall. The leaves are green to greyish green, slender, 3~5 cm long and 2~4 mm broad. The flowers are white, pink, or red, 3~4 cm diameter, produced singly or in small clusters from spring to mid-summer [Flora of China, 1753]. It is widely cultivated as an ornamental plant, both in China and elsewhere across temperate regions of the world; numerous cultivars have been selected for garden use [Huxley, 1992].

However, the effect of this herb on pain is unclear. Therefore, in this study, we attempted to characterize antinociceptive profiles and mechanisms of *Dianthus chinensis L* extract in various pain models.

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Materials and 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.

Drugs

Acetic acid, substance P, naloxone, methysergide and yohimbine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). *Dianthus chinensis L* (300 g) was extracted with 80 % ethanol under reflux 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 *Dianthus chinensis L* extract. *Dianthus chinensis L* extract, naloxone, methysergide and yohimbine were dissolved in saline. All drugs were prepared just before use.

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

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 [Hylden and Wilcox, 1980; 1981] 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 *Dianthus chinensis L* extract were carried out by blinded observers.

Tail-flick and hot-plate tests

Antinociception was determined by the tail-flick [D'Amour and Smith, 1941] and the hot-plate paw-licking tests [Eddy and Leimbach, 1953]. 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℃ 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 *Dianthus chinensis L* 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 [Koster et al., 1959], 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 *Dianthus chinensis L* extract at 200 mg/kg doses 30 min prior to performing the acetic acid-induced writhing and formalin tests.

Substance P-induced nociceptive behavioral test

Vehicle (control) or 200 mg/kg of *Dianthus chinensis L* 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 stop-watch timer [Hylden and Wilcox, 1981].

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 *Dianthus chinensis L* extract (200 mg/kg). And then, the writhing response was tested 30 min

after the treatment with either vehicle or *Dianthus chinensis L* extract [Choi et al., 2003; Park et al., 2009; Suh et al., 1996; 1996; 1999].

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 *Dianthus chinensis L* extract on the tail-flick and hot-plate paw-licking responses

As revealed in Fig. 1a and b, oral treatment of *Dianthus chinensis L* 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 *Dianthus chinensis L* extract orally at the dose of 200 mg/kg. However, there were no paralysis and motor changes.

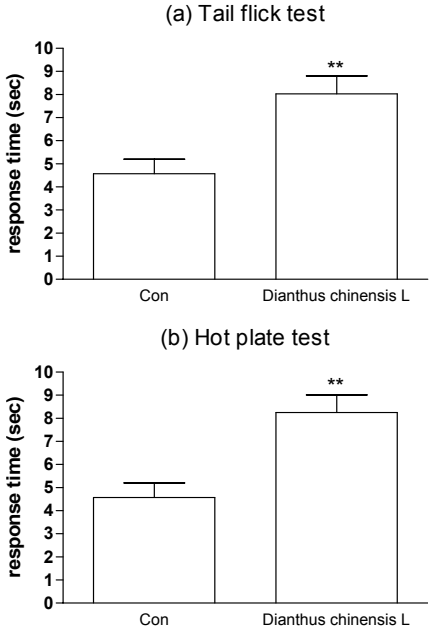

Fig. 1. The antinociceptive effect of *Dianthus chinensis L* extract administered orally in the tail-flick and hot-plate tests. Mice were administered orally with either vehicle or 200 mg/kg of *Dianthus chinensis L* extract and the tail-flick (a) or hot-plate (b) response was measured at 30 min after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

Effect of *Dianthus chinensis L* extract on the nociceptive behavior induced by acetic acid and substance P

Dianthus chinensis L extract attenuated the acetic acid-induced writhing numbers (Fig. 2a). Treatment with *Dianthus chinensis L* 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

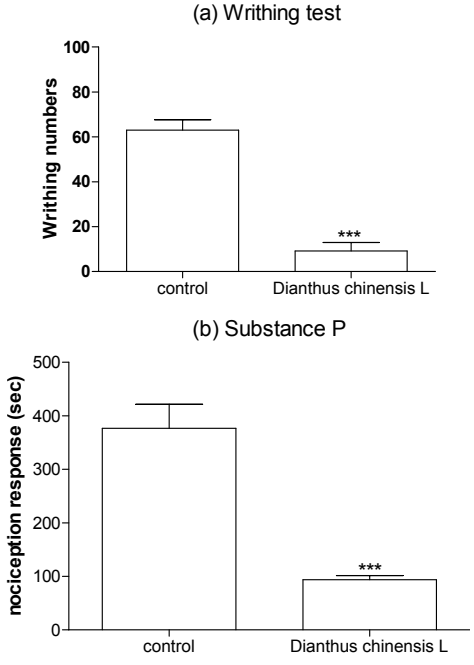

Fig. 2. Effect of *Dianthus chinensis L* extract on the nociceptive response induced by various pain models. *Dianthus chinensis L* extract (200 mg/kg) was administered orally and then, 0.25 ml of 1% acetic acid solution was injected intraperitoneally 30 min after treatment. The number of writhing was counted for 30 min following acetic acid injection (a). *Dianthus chinensis L* 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).

control mice, i.t. injection of substance P (0.7 μg) caused acute, immediate behavioral responses, i.e., licking, scratching and biting the lumbar or caudal region, which lasted about 30 min. As shown in Figs. 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 *Dianthus chinensis L* extract

We examined the possible involvement of opioidergic, serotonergic and adrenergic system in the *Dianthus chinensis L* extract-induced antinociception. The pretreatment with naloxone (opioid receptor antagonist, Fig. 3a) or methysergide (serotonergic receptor antagonist, Fig. 3b) did not affect *Dianthus chinensis L* extract-induced antinociception. However, the blockade of α_2-adrenergic receptor with systemic pre-administration of yohimbine abolished the *Dianthus chinensis L* 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 *Dianthus chinensis L* 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 [Ref. Chapman et al., 1985]). 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. Our results demonstrate that *Dianthus chinensis L* extract causes to prolong the tailflick and hot-plate response latencies, indicating the increase of nociceptive threshold.

We also examined the effect of *Dianthus chinensis L* 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 [Koster et al., 1959 for review, see Vyklicky, 1979]. In the present study, we clearly showed the antinoci-

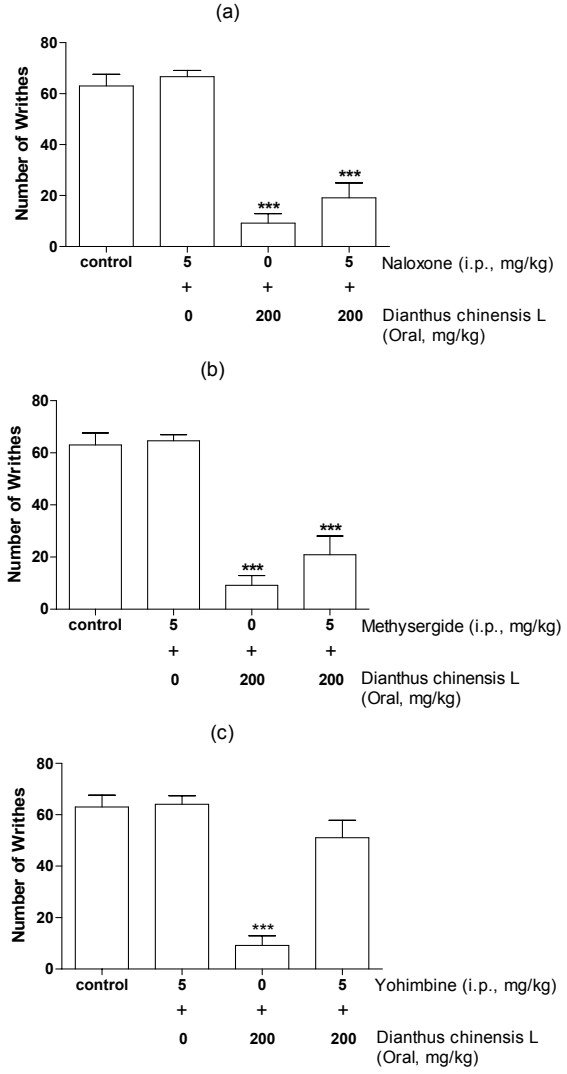

Fig. 3. Effect of naloxone (a), methysergide (b) and yohimbine (c) injected intraperitoneally (i.p.) on inhibition of the writhing response induced by *Dianthus chinensis L* extract administered orally. Naloxone, methysergide, or yohimbine (5 mg/kg) was pretreated intraperitoneally for 10 min, before oral administration of vehicle or *Dianthus chinensis L* extract (200 mg/kg). *Dianthus chinensis L* 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).

ceptive effect of *Dianthus chinensis L* 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 [Hylden and Wilcox, 1981; Cumberbatch et al., 1994]. We found in the present study that *Dianthus chinensis L* extract was also effective in attenuating substance P-induced nociceptive responses. These results suggest furthermore that *Dianthus chinensis L* 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 [Schmauss and Yaksh, 1984; Yaksh, 1979; 1984]. 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 [Yaksh, 1979; Jensen and Yaksh, 1984; Wigdor and Wilcox, 1987]. We observed in the present study that α_2-adrenergic receptor, but not opioidergic and serotonergic receptors, appear to be involved in orally administered *Dianthus chinensis L* extract-induced antinociception.

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

Acknowledgements

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Literature Cited

Chapman CR, Casey KL, Dubner R, Foley KM, Gracely RH, Reading AE. 1985. Pain measurement: an overview. Pain 22: 1-31.

Choi SS, Han KJ, Lee JK, Lee HK, Han EJ, Kim DH, Suh HW. 2003. Antinociceptive mechanisms of orally administered decursinol in the mouse. Life sciences 13(73): 471-485.

Cumberbatch MJ, Herrero JH, Headley PM. 1994. Exposure of rat spinal neurons to NMDA, AMPA and kainate produces only short-term enhancements of responses to noxious and non-noxious stimuli. Neuroscience Letters 181: 98-102.

D'Amour FE, Smith DL. 1941. A method for determining loss of pain sensation. Journal of Pharmacology and Experimental Therapeutics 72: 74-9.

Eddy NB, Leimbach D. 1953. Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines. Journal of Pharmacology and Experimental Therapeutics 107: 385-93.

Flora of Chinna. 1753. Dianthus chinensis Linnaeus, Flora of Chinna. vol. 6, 104

Grumbach L. 1966. The prediction of analgesic activity in man by animal testing. In: Knighton RS, Dumke PR, editors. Pain. Boston: Little Brown and Co.; 163-82.

Huxley A. 1992. New RHS Dictionary of Gardening. Macmillan, ISBN 0-333-47494-5.

Hylden JL, Wilcox GL. 1980. Intrathecal morphine in mice: a new technique. European Journal of Pharmacology 67: 313-316.

Hylden JL, Wilcox GL. 1981. Intrathecal substance P elicits a caudally- directed biting and scratching behavior in mice. Brain Reserch 217: 212-215.

Jensen TS, Yaksh TL. 1984. Spinal monoamine and opiate systems partly mediate the antinociceptive effects produced by glutamate at brainstem sites. Brain Research 321: 287-297.

Koster R, Anderson M, Beer EJ. 1959. Acetic acid for analgesic screening. Federal Proceeding 18: 412.

Park SH, Sim YB, Choi SM, Seo YJ, Kwon MS, Lee JK, Suh HW. 2009. Antinociceptive Profiles and Mechanisms of Orally Administered Vanillin in the Mice. Arch Pharm Res. 32(11): 1643-1649.

Schmauss C, Yaksh TL. 1984. In vivo studies on spinal opiate receptor systems mediating antinociception. II. Pharmacological profiles suggesting a differential association of mu, delta and kappa receptors with visceral chemical and cutaneous thermal stimuli in the rat. Journal of Pharmacology and Experimental Therapeutics 228: 1-12.

Suh HW, Song DK, Son KH, Wie MB, Lee KH, Jung KY, Do JC, Kim YH. 1996. Antinociceptive mechanisms of dipsacus

saponin C administered intracerebroventricularly in the mouse. General Pharmacology 27: 1167-1172.

Suh HW, Song DK, Kim YH. 1997. Differntial effects of adenosine receptor antagonist injected intrathecally on antinociception induced by morphine and beta-endorphin administered intracerebroventricularly in the mouse. Neuropeptides 31: 339-344.

Suh HW, Chung KM, Kim YH, Huh SO, Song DK. 1999. Effect of histamine receptor antagonists injected intrathecally on antinociception induced by opioids administered intracerebroventricularly in the mouse. Neuropeptides 33: 121-129.

Wigdor S, Wilcox GL. 1987. Central and systemic morphine-induced antinociception in mice: contribution of descending serotonergic and noradrenergic pathways. Journal of pharmacology and Experimental Therapeutics 242: 90-95.

Yaksh TL. 1979. Direct evidence that spinal serotonin and noradrenaline terminals mediate the spinal antinpciceptive effects of morphine in the periaqueductal gray. Brain Resesrch 160: 180-185.

Yaksh TL. 1984. Multiple opioid receptor systems in brain and spinal cord: Part I. European Journal of Anaesthesiology 1: 171-199.

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