Full Length Research Paper

Antinociceptive profiles and mechanisms of orally administered *Aster Koraiensis* extract in the mouse

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In the present study, the antinociceptive profiles of *Aster koraiensis* extract (AKE) were examined in ICR mice. AKE administered orally (200 mg/kg) showed an antinociceptive effect as measured by the tail-flick and hot-plate paw-licking tests. In addition, AKE attenuated the writhing numbers in the acetic acid-induced writhing test. Moreover, the cumulative response time of nociceptive behaviors induced by an intraplantar formalin injection was reduced by AKE treatment during the 2nd phases. Furthermore, the cumulative nociceptive response time for intrathecal (i.t.) injection of substance P (0.7 µg) was diminished by AKE. Intraperitoneal (i.p.) pretreatment with yohimbine (α_2-adrenergic receptor antagonist) attenuated antinociceptive effect induced by AKE in the writhing test. However, naloxone (opioid receptor antagonist) or methysergide (5-HT serotonergic receptor antagonist) did not affect antinociception induced by AKE in the writhing test. Our results suggest that AKE shows an antinociceptive property in various pain models. Furthermore, this antinociceptive effect of AKE may be mediated by α_2-adrenergic, but not opioidergic and serotonergic receptors.

Key words: *Aster koraiensis*, anti-nociception, inflammatory pain, α2 adrenoceptor.

INTRODUCTION

Aster koraiensis is a valuable species as a Korean endemic perennial among many plants native to Korea. *A. koraiensis* traditionally has been used for the purpose of food, medicine or health in Korea. *A. koraiensis* is widely distributed in the southern and the central part of Korean peninsula and Jeju island, and it has also been cultivated and marketed as ground cover and bedding plant in Korea (Lee and Han, 1995). This herb has also been utilized as pot plant, vegetable and medicinal plants in traditional Korean medicine for a variety of medical purposes, such as pertussis, chronic bronchitis and pneumonia (Ahn, 1998; Ko and Lee, 1996). However, the effect of this herb on pain is unclear. Therefore, in this study, we attempted to characterize antinociceptive profiles and mechanisms of *A. koraiensis* extract in

various pain models.

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.

Experimental animals

Male ICR mice (MJ Co., Seoul, Korea) weighing 20 to 25 g were used for all the experiments. Animals were housed 5 per cage in a room maintained at 22 ± 0.5℃ with an alternating 12 h light-dark cycle. Food and water were available *ad libitum*. The animals were allowed to adapt to the laboratory for at least 2 h before testing and were only used once. Experiments were performed during the light phase of the cycle (10:00 to 17:00).

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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 (1980, 1980) using a 30-gauge needle connected to a 25 µ 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 *A. koraiensis* extract were carried out by blinded observers.

Tail-flick and hot-plate paw-licking 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 s. For the hot-plate paw-licking 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 paw-licking test was approximately 9 s. Animals were pretreated orally once with vehicle (control) or *A. koraiensis* extract at 200 mg/kg doses 30 min prior to performing the tail-flick or hot-plate paw-licking tests.

Acetic acid-induced writhing and intraplantar formalin tests

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. For the formalin test (Hunskaar et al., 1958), 10 µl of 5% formalin was injected subcutaneously under the plantar surface of the left hindpaw. Following injection of formalin, the animals were immediately placed in an acrylic observation chamber, and the time spent licking, shaking and biting the injected paw was measured with a stop-watch timer and considered as indication of nociception. The early phase of the nociceptive response normally peaked 0 to 5 min, and the last phase 20 to 40 min after formalin injection, representing the direct effect on nociceptors and inflammatory nociceptive responses, respectively (Hunskaar and Hole, 1987). Animals were pretreated orally once with vehicle (control) or *A. koraiensis* 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 *A. koraiensis* 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 *A. koraiensis* extract (200 mg/kg). And then, the writhing response was tested 30 min after the treatment with either vehicle or AKE (Choi et al., 2003; Park et al., 2009; Suh et al., 1996, 1997, 1999).

Drugs

Acetic acid, formalin, substance P, naloxone, methysergide and yohimbine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). *A. koraiensis* (300 g) was dissolved in 80% ethanol (1,500 ml) and extracted as refluxing for 3 h, 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 extract of *A. koraiensis*. *A. koraiensis* extract, naloxone, methysergide and yohimbine were dissolved in saline. All drugs were prepared just before use.

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 *A. koraiensis* extract on the tail-flick and hot-plate paw-licking responses

As revealed in Figure 1a and b, oral treatment of *A. koraiensis* 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 AKE orally at the dose of 200 mg/kg. However, there were no paralysis and motor changes.

Effect of *A. koraiensis* extract on the nociceptive behavior induced by acetic acid, formalin, substance P

A. koraiensis extract attenuated the acetic acid-induced
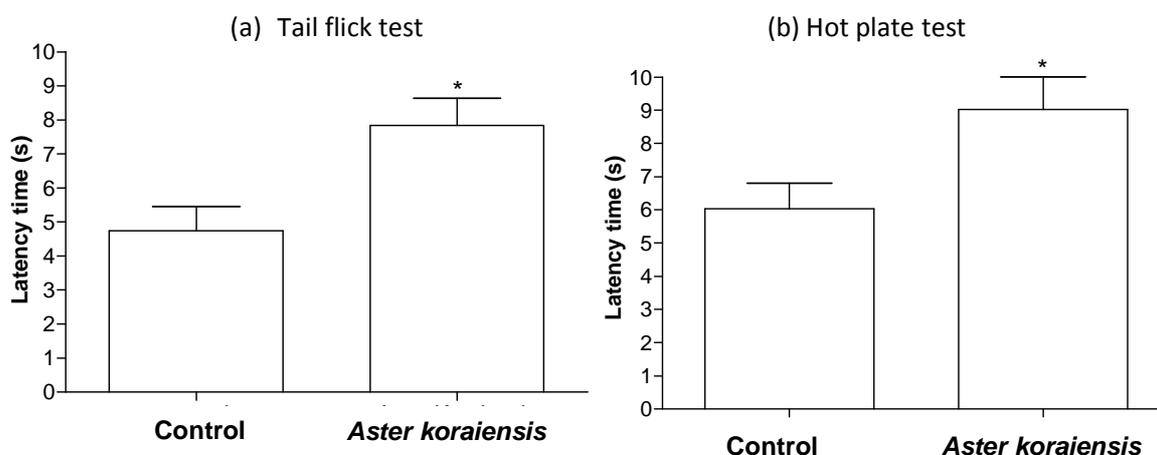
Figure 1. The antinociceptive effect of *Aster koraiensis* extract administered orally in the tail-flick and hot-plate paw-licking tests. Mice were administered orally with either vehicle or 200 mg/kg of AKE and the tail-flick (a) or hot-plate (b) response was measured at 30 min after treatment. The vertical bars denote the standard error of the mean. The number of animal used for each group was 8–10. *$P < 0.05$ compared to the vehicle-treated control group of mice.

writhing numbers (Figure 2a). Treatment with AKE at the dose of 200 mg/kg led to 70% decrease in the acetic acid-induced writhing response compare to the control group of mice. In vehicle-treated control group, injection of 5% formalin caused acute, immediate nociceptive formalin responses (that is, licking/flinching and biting the injected paw) that lasted for 5 min (1st phase response). The 2nd phase nociceptive responses began about 20 min after formalin administration and lasted for about 20 min (20 to 40 min after formalin injection). In AKE-treated mice, the nociceptive behaviors induced by intraplantar injection of formalin were decreased as compared with control group of mice during the only 2nd phases (Figure 2b). Treatment with AKE at the dose of 200 mg/kg, did not affected in the 1st phase of formalin test over the control group of mice. However, the effect of AKE led to 60% decrease in the 2nd phase of formalin test over the control group of mice. In vehicle-treated control mice, i.t. injection of substance P (0.7 μg) caused acute, immediate behavioral responses, that is, licking, scratching and biting the lumbar or caudal region, which lasted about 30 min. As shown in Figure 2c, cumulative nociceptive response times for i.t. administration of substance P was significantly diminished by 76%.

Effect of opioidergic, serotonergic and adrenergic system on the inhibition of writhing response induced by *A. oraiensis* extract

We examined the possible involvement of opioidergic, serotonergic and adrenergic system in the *Aster koraiensis* extract-induced antinociception. The pretreatment with naloxone (opioid receptor antagonist (Figure 3a), or methysergide (serotonergic receptor

antagonist (Figure 3b) did not affect AKE-induced antinociception. However, the blockade of α_2-adrenergic receptor with systemic pre-administration of yohimbine abolished the AKE-induced inhibition of the writhing response (Figure 3c). The treatment of naloxone, methysergide or yohimbine itself did not affect the writhing response (Figure 3).

DISCUSSION

In the present study, we found that *A. koraiensis* 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 AKE causes to prolong the tail-flick and hot-plate response latencies, indicating the increase of nociceptive threshold.

We also examined the effect of AKE on the acetic acid-induced writhing and intraplantar formalin 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; Vyklicky, 1979). In the present study, we clearly showed the antinociceptive effect of AKE in an acetic acid-induced writhing test. Moreover, in the formalin test, we showed that AKE had an antinociceptive
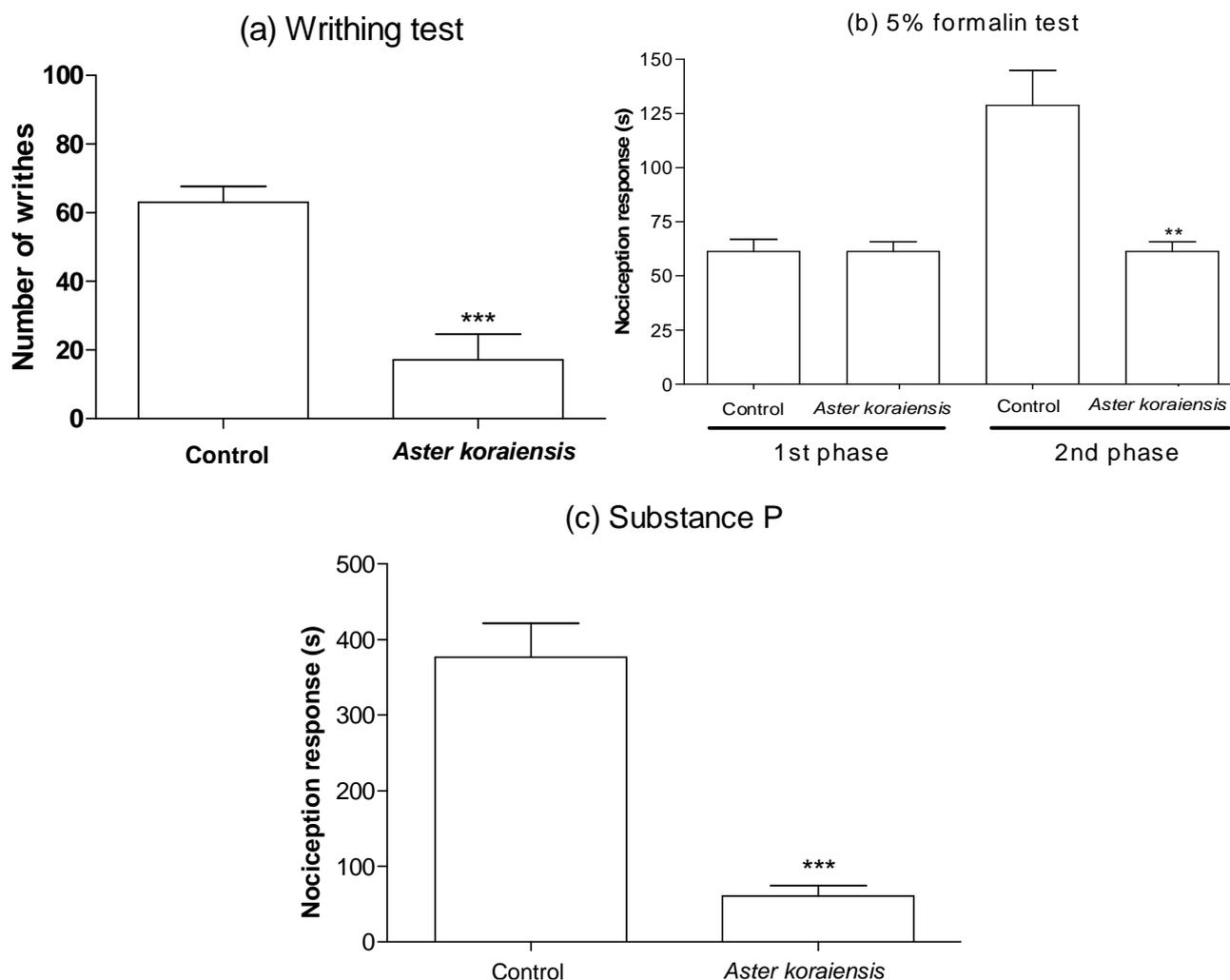
Figure 2. Effect of *Aster koraiensis* extract on the nociceptive response induced by various pain models. AKE (200 mg/kg) was administered orally and then, 0.25 ml of 1% acetic acid solution was injected intraperitoneally 30 min after treatment. The number of writhing was counted for 30 min following acetic acid injection (a). Animal were pretreated orally with AKE (200 mg/kg) for 30 min prior to the formalin (5%, 10 μl) injection subcutaneously into the plantar aspect of the left side hindpaw. The cumulative response time of licking, biting and shaking the injected paw was measured during the period of 0-5 min (1st phase) and 20-40 min (2nd phase) (b). AKE (200 mg/kg) was administered orally for 30 min prior to the substance P (0.7 μg per 5 μl) injection intrathecally (c). 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 (**$p < 0.01$; ***$p < 0.001$, compared with control group).

effect in a dose-dependent manner during the only 2nd phases. It is widely agreed that the nociceptive behaviors manifested during the acute 1st phase may be caused by the direct effect on peripheral nociceptors activating primary afferent fiber. It is followed by the tonic 2nd phase, which may be resulted from the tonic inflammatory nociceptive response (Hunskaar et al., 1985; Hunskaar and Hole, 1987; Choi et al., 2001; Chung et al., 2001; Puig and Sorkin, 1989) have reported that peripherally acting drugs such as aspirin and glucocorticoid only inhibit the 2nd phase in the formalin test. In contrast, aminipyrine and mefenamic acid, which act on both central and peripheral sites, inhibit nociceptive behaviors manifested during the both phases. Therefore, AKE may be, at least, a centrally acting compound, because oral treatment with AKE inhibited the only 2nd phase of formalin test. Furthermore, it has been reported that i.t. injection of substance P in mice can also(Hylden and Wilcox, 1981; Cumberbatch et al., 1994). We found in the present study that AKE was also effective in attenuating substance P-induced nociceptive responses. These results suggest furthermore that AKE may exert their antinociceptive effect via the central sites, possibly spinally mediated mechanisms.
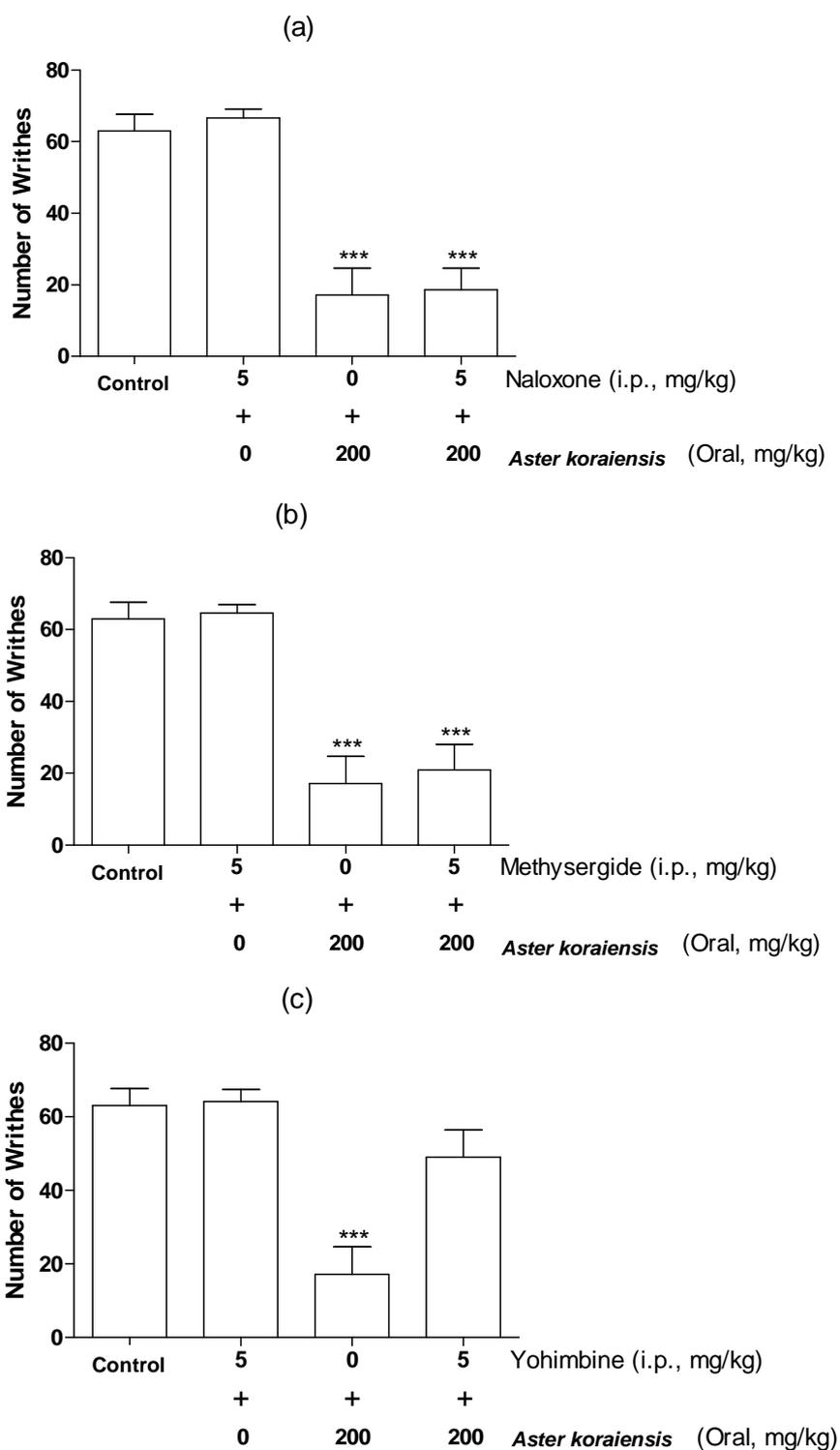
(a)

(b)

(c)

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

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 elicit nociceptive responses, consisting of biting, scratching and licking the caudal parts of the body

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 AKE-induced antinociception.

In conclusion, our results suggest that AKE shows an antinociceptive property in various pain models related to thermal, inflammation, peripheral and central nerves pains. Furthermore, this antinociceptive effect of AKE may be mediated by α_2-adrenergic, but not opioidergic and serotonergic receptors.

ACKNOWLEDGEMENT

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