Natural Product Sciences
20(4) : 301-305 (2014)

Anti-nociceptive and Anti-inflammatory Effect of an Ethanol Extract of The Leaf and Stem of *Aralia cordata*

Ji Yeon Jang and Yeon Hee Seong*

College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk 362-763, Republic of Korea

Abstract – The aim of our study is to investigate the anti-nociceptive and anti-inflammatory properties of an ethanol extract of the leaf and stem of *Aralia cordata*. Writhing responses induced by acetic acid, tail immersion test, and formalin-induced paw pain response for nociception and formalin-induced paw edema for inflammation were evaluated in mice. *A. cordata* (50 - 200 mg/kg, p.o.) and ibuprofen (100 mg/kg, p.o.), a positive non-steroidal anti-inflammatory drugs (NSAIDs), inhibited the acetic acid-induced writhing response, but they did not protect the thermal nociception in tail immersion test. However, morphine (5 mg/kg, s.c.) used as positive opioid control alleviated both the acetic acid-induced writhing response and thermal nociception in tail immersion test. In the formalin test, *A. cordata* (50 - 200 mg/kg) and ibuprofen (200 mg/kg) inhibited the second phase response (peripheral inflammatory response), but not the first phase response (central response), whereas morphine inhibited both phase pain responses. Both *A. cordata* (100 mg/kg) and ibuprofen (200 mg/kg) significantly alleviated the formalin-induced increase of paw thickness, the index of inflammation. These results show for the first time that the leaf and stem of *A. cordata* has a significant anti-nociceptive effect that seems to be peripheral, but not central. *A. cordata* also displays an anti-inflammatory activity in an acute inflammation model. The present study supports a possible use of the leaf and stem of *A. cordata* to treat pain and inflammation.
Keywords – Leaf and stem of *Aralia cordata*, Anti-nociception, Anti-inflammation.

Introduction

Tissue injury results in inflammatory nociception. Various inflammatory mediators such as prostaglandins (PGs), leukotrienes, histamine, bradykinin, and platelet-activating factor are involved in initiating and sustaining nociception and inflammatory cascade.[1] The inflammation process involves also vascular permeability, active migration of blood cells, and the passage of plasma constituents into injurious tissue. The human body's natural response to injury results in inflammation-induced pain, swelling, and erythema. Symptoms of inflammation process can be alleviated by the aspirin-like non-steroidal anti-inflammatory drugs (NSAIDs) which inhibit mainly the cyclooxygenase (COX) and reduce synthesis of PGs, and by corticosteroids which prevent the formation of PGs by inhibition of phospholipase A_2 responsible for arachidonic acid release.[1,2] Steroidal and NSAIDs medication can have undesirable side effects including gastric erosions.[3,4] Therefore, nowadays there is a greater interest in natural compounds, such as herbal remedies, which have been used for centuries to reduce pain and inflammation with fewer side effects.

Aralia, which belongs to the family Araliaceae, has long been recognized as therapeutic herbs for antinociceptive, antidiabetic, antioxidant, and anti-inflammatory activities in China, Japan, and Korea. The root of *Aralia cordata* Thunb. (*A. cordata*) has been used as a traditional Chinese medicine for rheumatism, lumbago, and lameness.[5] Many studies of plant-derived therapeutic compounds have isolated several diterpenes, pimaric acid, pimaradienoic acid, abietic acid, and 7-oxosandaracopimaric acid, as the potential anti-inflammatory candidates from the root of *A. cordata* in *in vivo* and *in vitro*.[6-10] Most of the previous studies have focused on the root of *A. cordata*, but the aerial part (leaf and stem) of the plant has been usually discarded. However, a recent study on the leaf and stem of *A. cordata* has reported for its inhibitory activity against COX-1 and COX-2. Diterpenes, triterpenes, and a saponin were isolated as COX-1 and COX-2 inhibitory constituents from an ethanol extract of the leaf and stem of this plant.[11] In addition, we previously have reported a possible therapeutic activity of the leaf and stem of *A.*

*Author for correspondence
Prof. Dr. Yeon Hee Seong, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk 362-763, Korea
Tel: +82-43-261-2968; E-mail: vepharm@cbnu.ac.kr

cordata for preventing dementia and stroke in experimental animal models and isolated oleanolic acid as an active component.[12,13] Plant extracts rich in oleanolic acid have been shown to exhibit anti-nociceptive property.[14]

However, no studies have been done previously on the anti-nociceptive and anti-inflammatory effect of the leaf and stem of *A. cordata* using experimental animals. Thus, the present study represents the first research to evaluate the anti-nociceptive and anti-inflammatory effects of an ethanol extract of the leaf and stem of *A. cordata* employing different pain stimuli and formalin-induced paw inflammation in mice.

Experimental

Preparation of ethanol extract of the leaf and stem of *A. cordata* and reagents – The leaf and stem of *A. cordata* were collected at Keryong Mountain in Daejeon, Korea, from May to July in 2009 and identified by Dr. KiHwan Bae, Chungnam National University, Korea, and voucher specimens were deposited in College of Veterinary Medicine, Chungbuk National University, Korea. *A. cordata* (4 kg) were extracted 3 times with ethanol at room temperature for 3 days. The extract was filtered, and the filtrate was concentrated under reduced pressure using a rotary evaporator to yield an ethanol extract (300 g; yield: 7.5%), which was then stored at room temperature until required. Ibuprofen was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and morphine, from Myungmoon Pharm. Co. (Hwaseong, Korea).

Experimental animals – Male ICR mice (5-week-old, 22 ± 2 g) were supplied by Daehan BioLink Co., Ltd. (Chungbuk, Korea) and housed in an environmentally controlled room at 22 ± 2 ºC with a relative humidity of 55 ± 5%, a 12-h light/dark cycle, and food and water *ad libitum*. The procedures involving experimental animals complied with the regulations for the Care and Use of Laboratory Animals of the Animal Ethics Committee of Chungbuk National University.

Measurement of anti-nociceptive activity using acetic acid-induced writhing test – Acetic acid (0.8%) was intraperitoneally injected to mice 20 min after the administration of *A. cordata* (50, 100, and 200 mg/kg, p.o.), ibuprofen (100 mg/kg, p.o.), or morphine (5 mg/kg, s.c.). Each mouse was then placed in an individual clear plastic observation chamber, and the total number of writhes made by each mouse was counted between 10 and 40 min after acetic acid administration. For scoring purposes, a writhe was indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb.[15]

Measurement of anti-nociceptive activity using tail immersion test – The procedures were similar to those used previously.[16] Briefly, the terminal 3 cm of each mouse's tail was immersed on a water bath set at temperature of 52 ± 2 ºC and the time in seconds taken to flick the tail was recorded. Each mice group served as its own control. Only mice showing a pretreatment reaction time less or equal to 4 s were selected for the study. Immediately after basal latency assessment, *A. cordata* (50, 100, and 200 mg/kg, p.o.), ibuprofen (100 mg/kg, p.o.), and morphine (5 mg/kg, s.c.) were administered and the reaction time was again measured at 15, 30, 45, 60, 90, and 120 min after the administration. Cut-off time was 10 s for tail immersion measurements in order to minimize tissue injury. For each animal, the percentage of maximum possible effect (% of MPE) was calculated using the following formula: [(post drug latency – pre drug latency)/(10 – pre drug latency)] × 100.

Measurement of formalin-induced nociceptive responses – Formalin-induced nociception was measured using a method described previously with a slight modification.[17] Mice received 20 μl of 5% formalin solution in saline via an intraplantar injection of the right hind paw 30 min after the administration of *A. cordata* (50, 100, and 200 mg/kg, p.o.), ibuprofen (200 mg/kg, p.o.), or morphine (5 mg/kg, s.c.). Following the intraplantar injection of formalin, the mice were immediately placed in a glass cylinder (20 cm diameter) and the time in seconds spent licking the injected paw was recorded with a chronometer for both the early neurogenic phase (0 - 5 min) and late inflammatory phase (20 - 30 min) of this model. These behaviors were considered nociceptive responses.

Measurement of formalin-induced paw edema – The same formalin-injected mice were served for the measurement of paw edema volume. For the measurement of paw edema, the vertical thickness of the injected paw at the metatarsal level was measured with a fine caliper (Vernier caliper 530 series, Mitutoyo, Japan) before and 18 h after formalin injection.[17,18] It was calculated by subtracting the pre-thickness of paw from the post-thickness of paw formalin-injected and expressed as mm thickness.

Statistical analysis – Data were expressed as mean ± S.E.M. and the statistical significance was assessed by the one-way analysis of variance (ANOVA) with subsequent Tukey's tests. P Values of < 0.05 were considered to be significant.

Results

Anti-nociceptive activity. – Anti-nociceptive activity

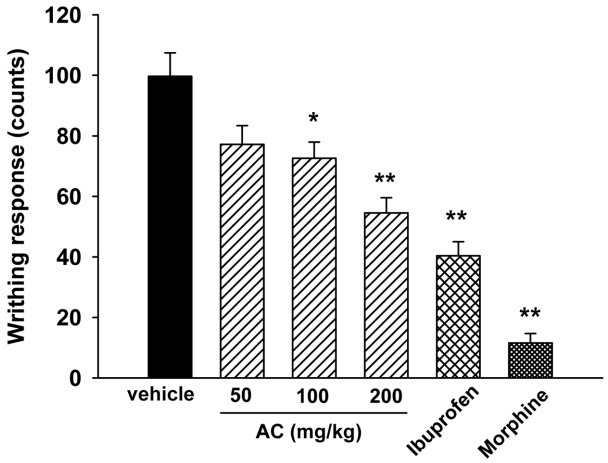

Fig. 1. Inhibitory effect of *A. cordata* (AC) on acetic acid-induced writhing response in mice. The number of writhes produced by 0.8% acetic acid was counted for 30 min. Stretching of the abdomen with simultaneous stretching of at least one hind limb was considered as writhes. Results are expressed as mean ± S.E.M (n = 10 - 12). $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* vehicle.

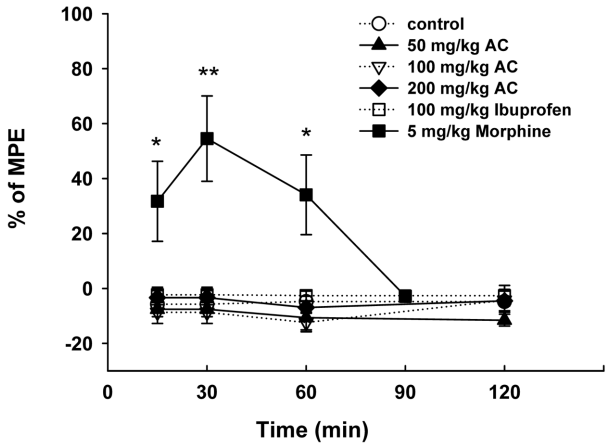

Fig. 2. Inhibitory effect of *A. cordata* (AC) on thermal pain in tail immersion test in mice. For each animal, the percentage of maximum possible effect (% of MPE) was calculated using the following formula: [(post drug latency - pre drug latency)/(10 - pre drug latency)] × 100. Results are expressed as mean ± S.E.M (n = 7). $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* vehicle at the time indicated, respectively.

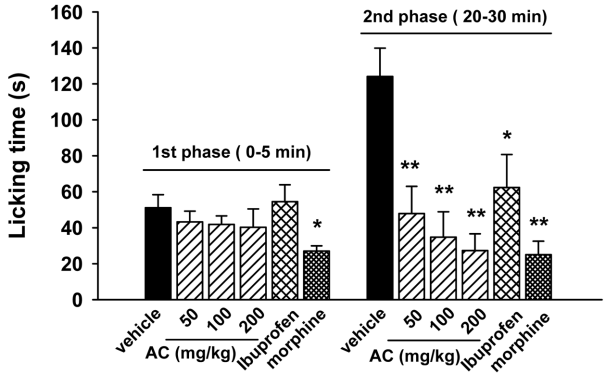

Fig. 3. Inhibitory effect of *A. cordata* (AC) on formalin-induced nociceptive response in mice. Licking of the injected paw at 0-5 min (1st phase) and 20-30 min (2nd phase) after formalin injection time was measured in seconds. Results are expressed as mean ± S.E.M (n = 8). $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* vehicle.

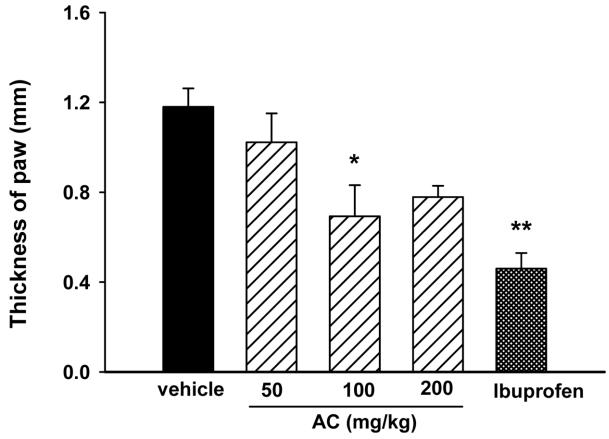

Fig. 4. Inhibitory effect of *A. cordata* (AC) on formalin-induced paw edema in mice. Vertical thickness of the injected paw at the metatarsal level was measured using Caliper to measure a 18 h paw edema induced by 5% formalin. Baseline paw measurement was taken immediately prior to the intraplantar injection of formalin. The change of paw thickness was calculated by subtracting the pre thickness of paw from the post thickness of paw injected. Results are expressed as mean ± S.E.M (n = 8). $^{*}P < 0.05$ *vs.* vehicle.

was evaluated by acetic acid writhing test, tail immersion test and formalin test. The effect of an ethanol extract of *A. cordata* on acetic acid-induced writhing responses in mice is shown in Fig. 1. It was found that the three doses orally assayed caused a dose-dependent inhibition on the writhing responses induced by acetic acid. *A. cordata* (100 and 200 mg/kg) significantly reduced the acetic acid-induced writhes to 72.6 ± 5.3 and 54.5 ± 5.0, respectively, when compared with vehicle-control (99.7 ± 7.7). Ibuprofen (100 mg/kg), an NSAIDS, and morphine (5 mg/kg), an opioid drug, also produced significant inhibitions on the acetic acid-induced writhes showing 40.4 ± 4.6 and 11.6 ± 3.1, respectively (Fig. 1).

As shown in Fig. 2, the pretreatment of mice with this ethanol extract of *A. cordata* and ibuprofen did not alter the tail immersion responses to the nociceptive stimuli. In contrast, the reference opioid drug morphine (5 mg/kg) remarkably increased the latency of escape from the hot water at 15, 30, and 60 min after its administration indicating anti-nociceptive activity.

The results depicted in Fig. 3 reveal that *A. cordata* (50, 100, and 200 mg/kg) exhibited a dose-dependent inhibition

on the second inflammatory (20 - 30 min) phase of formalin-induced licking showing 47.9 ± 15.1, 34.8 ± 14.1, and 27.3 ± 9.4 s of licking time, respectively, compared with that of vehicle-control (124.1 ± 15.8 s). A similar inhibition on the inflammatory nociception was shown by ibuprofen (200 mg/kg). However, neither *A. cordata* nor ibuprofen could inhibit the first phase of neurogenic nociception. Meanwhile, morphine (5 mg/kg) significantly inhibited both the neurogenic and the second inflammatory nociception induced by intraplantar injection of formalin.

Anti-inflammatory activity – Intraplantar injection of formalin elicits significant inflammation (i.e. edema) in the center of the sole of the hind paw.[17] Therefore, to confirm whether the apparent attenuation of formalin-induced nociceptive behavior during the second phase reflected the effect of *A. cordata* on the peripheral inflammation produced by formalin injection, the thickness change of the hind paw edema was compared between the vehicle-treated and *A. cordata*-administered mice. As shown in Fig. 4, *A. cordata* ameliorated formalin-induced paw edema. The formalin-induced paw thickness was significantly reduced by 100 mg/kg *A. cordata* by about 41.5%, showing 0.69 ± 0.14 mm of paw thickness in comparison with that of vehicle-control (1.18 ± 0.08 mm). Ibuprofen (200 mg/kg, p.o.) also inhibited the formalin-induced paw edema (0.46 ± 0.07 mm, thickness).

Discussion

In the present study, the first test performed was the acetic acid-induced writhing in mice, which has been described as a typical experimental model for the study of inflammatory pain and used to evaluate analgesics or anti-inflammatory drugs.[19] The local irritation provoked by intraperitoneal injection of chemical substances, such as phenylquinone or acetic acid, triggers the liberation of a variety of mediators such as bradykinin, substance P and PGs as well as some cytokines such as TNF-α, IL-1β, and IL-8.[20,21] Such mediators activate chemosensitive nociceptors that contribute to the development of this type of inflammatory pain, which is known to be sensitive to NSAIDs like ibuprofen or indomethacin. *A. cordata* as well as ibuprofen used as reference NSAID drug were able to reduce the acetic acid-induced writhing response, suggesting a mechanism involved in antinociceptive effect by the inhibition of mediator release of the inflammatory process.

In order to elucidate a possible central involvement in the anti-nociceptive effect of *A. cordata*, the tail immersion test was conducted. This test using thermal stimuli is employed to investigate the analgesic activity mediated by central mechanisms, like morphine, while peripheral compounds are inactive on this kind of painful stimulus.[22] While morphine increased the latency of the tail immersion, *A. cordata* as well as ibuprofen failed to increase the tail immersion latency, which indicates a negligible effect of *A. cordata* on nociception produced by thermal stimulation, which is associated with central neurotransmission.[23]

In order to obtain more specific evidence on the antinociceptive activity of *A. cordata*, the formalin test was performed. The formalin test is a well-established and frequently used model to study mechanisms of pain and to evaluate the analgesic action of various endogenous and exogenous substances. Unlike traditional reflex tests of nociception (e.g. tail-flick, hot-plate), pain produced by the hind paw injection of formalin results from persistent tissue damage and, thus, more closely resembles clinical pain conditions.[24] The intraplantar injection of a formalin solution produces a reproducible syndrome of nociceptive behaviors, which appear in two distinct phases. The first phase begins at the time of injection and lasts for about 10 min. The subsequent (second) phase starts at 10 min postinjection and has a duration of about 50 min. The first phase is characterized by neurogenic pain caused by a direct chemical stimulation of nociceptors. The second phase is characterized by inflammatory pain triggered by a combination of stimuli, including inflammation of the peripheral tissues and mechanisms of central sensitization.[25] Substance P is involved in the first phase whereas histamine, serotonin, PGs and bradykinin are involved in the second one.[26,27] It is notable that the first phase nociception produced by formalin is quite resistant to the NSAIDs, such as ibuprofen (results presented here), acetyl salicylic acid, and indomethacin. However, these drugs can attenuate the second phase of formalin-induced licking.[26,28] Moreover, it has been established that morphine is able to inhibit both phases of the formalin test.[29] In the present study, the first and the second phase responses were observed 0 - 5 min and 20 - 30 min after formalin injection, respectively. Pre-treatment orally with *A. cordata* showed a significant and dose-dependent inhibitory effect on the licking activity in the second phase, but not that in the first phase. Similarly, ibuprofen produced marked inhibition on inflammatory nociception of the second phase only. However, morphine inhibited both phases of formalin test. Taken together, it is suggested that *A. cordata* could inhibit the inflammatory pain, possibly through the inhibition of PGs synthesis,[11] but not the neurogenic pain.

A. cordata (100 mg/kg) showed a significant inhibition of formalin-induced rat paw edema, although the highest dose (200 mg/kg) used failed to produce a significant inhibition. The formalin injection into rat paw produces localized inflammation and pain.[17] It is suggested that *A. cordata* might be less sensitive to inflammation than pain response. Inhibition of formalin-induced paw edema in mice is one of the most suitable tests to evaluate anti-proliferative activity and to screen anti-arthritic and anti-inflammatory agents.[30] Inhibition of PGs in the inflammatory exudate is considered the mechanism for the anti-inflammatory effects of NSAIDs.[31] We have demonstrated the inhibitory effect of the leaf and stem of *A. cordata* on COX-1 and COX-2 and isolated diterpenes, triterpenes, and a saponin as active constituents of the pharmacological activity in a study.[11] In addition, we previously isolated oleanolic acid as an active component of the leaf and stem of *A. cordata* for preventing dementia and stroke in experimental animal models.[12,13] Anti-inflammatory activity of oleanolic acid has been well defined in *in vivo* and *in vitro*.[32,33] Thus, in conclusion, it is suggested that the inhibition of PGs synthesis of these active compounds including oleanolic acid might be responsible for the anti-nociceptive and anti-inflammatory effect of the ethanol extract of the leaf and stem of *A. cordata*.

In summary, the present study demonstrates for the first time that the leaf and stem of *A. cordata* induces significant peripheral anti-nociceptive effects in several model of nociception (acetic acid-induced writhing, formalin and tail immersion). The leaf and stem of *A. cordata* also shows an anti-inflammatory effect in acute inflammation model (formalin-induced paw edema). These results provide a quantitative basis for explaining the traditional folk-medicine use of the leaf and stem of *A. cordata* to treat pain and inflammation.

Acknowledgements

This work was supported by the research grant of the Chungbuk National University in 2013.

References

(1) Maslinska, D.; Gajewski, M. *Folia Neuropathol*. **1998**, *36*, 199-204.
(2) Di Rosa, M. In Future Trends in Inflammation: Effect of Non-steroidal Anti-inflammatory Drugs on Leukocyte Migration; Velo, G. P.; Willoughby, P. A. Ed; Piccin Medical Books; Padova, 1974, pp 143-152.
(3) Chan, F. K. *Nat. Clin. Pract. Gastroenterol. Hepatol.* **2006**, *3*, 563-573.
(4) Takeuchi, K. *World J. Gastroenterol*. **2012**, *18*, 2147-2160.
(5) Perry, L. M. Medicinal Plants of East and Southeast Asia; MIT Press: Massachusetts, 1980; p 41.
(6) Cho, J. H.; Lee, J. Y.; Sim, S. S.; Whang, W. K.; Kim, C. J. *Pulm. Pharmacol. Ther.* **2010** *23*, 190-199.
(7) Kang, O. H.; Chae, H. S.; Choi, J. G.; Oh, Y. C.; Lee, Y.S.; Kim, J. H.; Seung, M. J.; Jang, H. J.; Bae, K. H.; Lee, J. H.; Shin, D. W.; Kwon, D. Y. *Eur. J. Pharmacol*. **2008**, *601*, 179-185.
(8) Kim, T. D.; Lee, J. Y.; Cho, B. J.; Park, T. W.; Kim, C. J. *Arch. Pharm. Res.* **2010**, *33*, 509-514.
(9) Suh, S. J.; Kwak, C. H.; Chung, T. W.; Park, S. J.; Cheeeei, M.; Park, S. S.; Seo, C. S.; Son, J. K.; Chang, Y. C.; Park, Y. G.; Lee, Y. C.; Chang, H. W.; Kim, C. H. *Chem. Biol. Interact*. **2012**, *199*, 112-119.
(10) Tanabe, H.; Yasui, T.; Kotani, H.; Nagatsu, A.; Makishima, M.; Amagaya, S.; Inoue, M. *Bioorg. Med. Chem*. **2014**, *22*, 3204-3212.
(11) Lee, I. S.; Jin, W.; Zhang, X.; Hung, T. M.; Song, K. S.; Seong, Y. H.; Bae, K. *Arch. Pharm. Res.* **2006**, *29*, 548-555.
(12) Cho, S. O.; Ban, J. Y.; Kim, J. Y.; Jeong, H. Y.; Lee, I. S.; Song, K. S.; Bae, K.; Seong, Y. H. *J. Pharmacol. Sci*. **2009**, *111*, 22-32.
(13) Cho, S. O.; Ban, J. Y.; Kim, J. Y.; Ju, H. S.; Lee, I. S.; Song, K. S.; Bae, K.; Seong, Y. H. *Arch. Pharm. Res.* **2009**, *32*, 923-932.
(14) Choi, J.; Lee, K. T.; Choi, M. Y.; Nam, J. H.; Jung, H. J.; Park, S. K.; Park, H. J. *Biol. Pharm. Bull.* **2005**, *28*, 1915-1918.
(15) Hosseinzadeh, H.; Younesi, H. M. *BMC Pharmacol.* **2002**, *2*, 7.
(16) Gomes, N. M.; Rezende, C. M.; Fontes, S. P.; Matheus, M. E.; Fernandes, P. D. *J. Ethnopharmacol.* **2007**, *109*, 486-492.
(17) Fu, K. Y; Light, A. R.; Maixner, W. *J. Pain* **2001**, *2*, 2-11.
(18) Cho, I. H.; Chung, Y. M.; Park, C.K.; Park, S.H.; Li, H.; Kim, D.; Piao, Z. G.; Choi, S. Y.; Lee, S. J.; Park, K.; Kim, J. S.; Jung, S. J.; Oh, S. B. *Brain Res*. **2006**, *1072*, 208-214.
(19) Vinegar, R.; Truax, J. F.; Selph, J. L.; Johnston, P. R. In Anti-inflammatory Drugs: Antagonism of Pain and Hyperalgesia; Vane, J. R.; Ferreira, S. H. Ed; Springer-Verlag Berlin Heidelberg; Berlin, 1979, pp 209-222.
(20) Ikeda, Y.; Ueno, A.; Naraba, H.; Oh-ishi, S. *Life Sci*. **2001**, *69*, 2911-2919.
(21) Ribeiro, R. A.; Vale, M. L.; Thomazzi, S. M.; Paschoalato, A. B.; Poole, S.; Ferreira, S. H.; Cunha, F. Q. *Eur. J. Pharmacol.* **2000**, *387*, 111-118.
(22) Walker, K.; Fox, A. J.; Urban, L. A. *Mol. Med. Today* **1999**, *5*, 319-321.
(23) Pinheiro, B. G.; Silva, A. S .B.; Souza, G. E. P.; Figueiredo, J. G.; Cunha, F. Q.; Lahlou, S.; da Silva, J. K. R.; Maia, J. G. S.; Sousa, P. J. C. *J. Ethnopharmacol.* **2011**, *138*, 479-486.
(24) Zhao, C. S.; Tao, Y. X.; Tall, J. M.; Donovan, D. M.; Meyer, R. A.; Raja, S. N. *Exp. Neurol.* **2003**, *184*, 839-845.
(25) Puig, S.; Sorkin, L. S. *Pain* **1996**, *64*, 345-355.
(26) Hunskaar, S.; Hole, K. *Pain* **1987**, *30*, 103-114.
(27) Murray, C. W.; Porreca, F.; Cowan, A. *J. Pharmacol. Methods* **1988**, *20*, 175-186.
(28) Malmberg, A. B.; Yaksh, T. L. *J. Pharmacol. Exp. Ther*. **1992**, *263*, 136-146.
(29) Sevostianova, N.; Zvartau, E.; Bespalov, A.; Danysz, W. *Eur. J. Pharmacol.* **2003**, *462*, 109-113.
(30) Greenwald, R. A. *Methods Find Exp. Clin. Pharmacol.* **1991**, *13*, 75-83.
(31) Vane, J. *Drugs.* **1987**, *33*, Suppl 1, 18-27.
(32) Hwang, Y. J.; Song, J.; Kim, H. R.; Hwang, K. A. *BMB Rep*. **2014**, *47*, 524-529.
(33) Lee, W.; Yang, E. J.; Ku, S. K.; Song, K. S.; Bae, J. S. *Inflammation* **2013**, *36*, 94-102.

Received August 8, 2014
Revised September 9, 2014
Accepted October 9, 2014