

Protective Effect of Orally Administrated Dandelion Against Carrageenan-induced Ankle Arthritis in Rats

Bong-Jun Sur¹, Yoon-Jeong Koh², Hee-Don Choi²,
Insop Shim³, Hyejung Lee¹, Dae-Hyun Hahm^{1*}

¹Acupuncture & Meridian Science Research Center, Kyunghee University,
Seoul 130-701, ²Korea Food Research Institute, Seongnam 463-746,

³Department of Integrative Medicine, The Catholic University of Korea
College of Medicine, Seoul 137-701, Korea

ABSTRACT

Dandelion (DA) possesses the therapeutic ability to eliminate heat and alleviating swelling, choleresis, diuresis, and inflammation. In order to investigate the anti-arthritis effect of DA, several behavioral parameters such as paw volume, squeaking score, and weight distribution ratio were investigated in a carrageenan-induced arthritis rat model. At the maximum severity of arthritis, the daily administration of DA was initiated and lasted for 9 days. The therapeutic effects of DA were observed on 9th day after the arthritis induction, as compared to saline-treated control group. Oral administration of DA significantly alleviated apparent symptoms of paw volume, squeaking score, and weight distribution ratio in rats. In conclusion, DA was found to be effective in alleviating the inflammatory response and thus arthritic symptoms in carrageenan-induced arthritic rats.

Key words: dandelion, arthritis, carrageenan, paw volume, squeaking score, weight distribution ratio

INTRODUCTION

Arthritis is a common response of joint tissues which have undergone multiform injuries. Its causes include joint deformation, genetic inheritance, physical force, inner-cellular changes and biochemical factors (Braunwald et al., 2002). Its pathological symptoms usually result from the failure of the cartilage repairing function to keep up with cartilage

breakdown followed by accompanying inflammation. Common symptoms of arthritis are pain, stiffness, limited motion of joints, swelling, and creaking of joints, and the ankle joint is the most frequently affected by osteoarthritis (Bennell and Hinman, 2005). Osteoarthritis is found in approximately 10% of the population over the age of 65 (Krohn, 2005). Surgical approaches, several pharmacologic and non-pharmacologic methods are available, and interestingly, non-pharmacologic methods including exercise, weight loss, rest, use of canes and crutches, physical therapy, osteopathic manipulative treatment, and braces received attention (Rubin, 2005).

Some plants of the genus *Taraxacum*, known as

*To whom correspondence should be addressed.

TEL: 02-961-0366, FAX: 02-966-2175

e-mail: dhhahm@khu.ac.kr

Received November 17, 2008

Accepted for publication December 8, 2008

dandelion, have long been used in folk medicine to treat hepatic disorders and some women's diseases, such as breast and uterus cancers, and as lactating, choleric, diuretic, and anti-inflammatory remedies (Ahmad et al., 2000; Kisiel and Barczak, 2000). Pharmacological activities of *Taraxacum* plants, especially *Taraxacum officinale* F. Weber ex Wiggers (Asteraceae), have been in part evaluated so far (Schutz et al., 2006). *Taraxacum officinale* was assessed to contain acute anti-inflammatory activity by showing its protective effect against cholecystokinin-induced acute pancreatitis in rats (Seo et al., 2005).

Additionally, other species of the genus *Taraxacum* were elucidated to contain various other pharmacological activities. The aqueous extract of *Taraxacum mongolicum* was elucidated to interact with a fluoroquinolone-type antibiotic, ciprofloxacin, which modifies its bioavailability and disposition (Zhu et al., 1999). *Taraxacum japonicum* exhibited strong anti-tumor-promoting activities on the two-stage carcinogenesis of mouse skin tumor induced by an initiator and a promoter, suggesting its chemopreventive activity (Takasaki et al., 1999). In the recent analysis, two new guaianolide glucosides, deacetylmatricarin 8-O- β -glucopyranoside and 11 β -hydroxyleukodin 11-O- β -glucopyranoside, were isolated from *Taraxacum obovatum* (Michalska and Kisiel, 2003), and taraxafolide and (+)-taraxafolin-B were newly identified from *Taraxacum formosanum* (Leu et al., 2005). Differences in constituents and pharmacological activities of various species in the genus *Taraxacum* remain obscure. In this study, the anti-arthritis effects of dandelion were investigated in a carrageenan-induced arthritis rat. To assess the arthritic symptoms of pain and inflammation, several preclinical and behavioral tests, such as paw volume, squeaking score, and weight distribution ratio, were measured and their accuracy and effectiveness as an arthritic indicator were compared.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats, weighting 170 g, were used for the experiments. All experimental animals were obtained from Samtaco Animal Co. (Kyungki-do, Korea). They were kept under controlled envi-

ronmental conditions ($20\pm2^\circ\text{C}$ and a light-dark cycle of 12/12 h) for at least 1 week prior to the start of the experiment. Food and water were available ad libitum. All subjects were habituated to the behavioral test chambers and handled with special care to minimize stress. All methods were approved by the Animal Care and Use Committee of Kyung Hee University. All procedures were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals", published by the Korean National Institute of Health.

Preparation of dandelion extract

Powdered dandelion were obtained from Uiryung Goon, Kyung Nam. Leaf of dandelion were steeped in boiling distilled water for 3 h and filtered through a Whatman No.1 paper. After the supernatant of the dandelion was freeze dried. And it was dissolved in 0.9% saline solution for the subsequent experiments.

Experimental groups

Rats were randomly divided into five treatment groups: saline-treated arthritic group (CON, n=5), carrageenan-induced and 20 mg/kg DA-treated group (DA-20, n=5), carrageenan-induced and 100 mg/kg DA-treated group (DA-100, n=5), carrageenan-induced and 200 mg/kg DA-treated group (DA-200, n=5), and carrageenan-induced and 10 mg/kg prednisolone-treated group (PRE, n=4). DA dissolved in saline solution with the concentrations of 20, 100, and 200 mg/kg was intramuscularly injected immediately after the induction of carrageenan-induced arthritic pain in rats.

Induction of arthritis

In this study, arthritic inflammation was induced by injection of 3% carrageenan (Sigma-Aldrich, St. Louis, MO, USA) in 100 μl of pyrogen-free saline into the left tibiotarsal ankle (Hansra et al., 2000) (Fig. 1).

Behavioral & pre-clinical parameters

1. Evaluation of paw volume: The procedure was performed as previously described by Kwon et al. Paw volumes of both hind paws were measured using a water displacement plethysmometer (Ugo-Basile Biological Research Apparatus Co., Comerio-

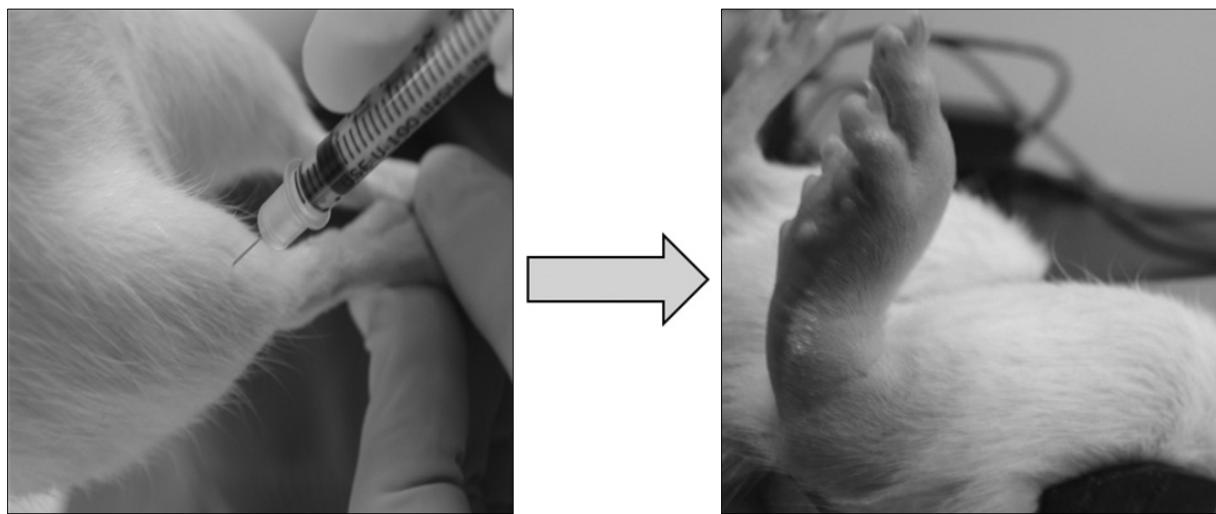


Fig. 1. Photographs of induction of carrageenan-induced ankle arthritis rat.



Fig. 2. Schematic diagram of a novel type of analgesia instrument.

Varese, Italy) everyday for 9 days after carrageenan injection and the mean values were recorded. Paw volume measured prior to carrageenan injection was used as control volume. Paw volumes were expressed as relative values to that of day 1 which is maximum.

2. Squeaking test: Arthritis-induced hyperalgesia was assessed by quantifying total number of vocalization evoked by ankle flexion or extension. The ankle joints of hind limbs were gently bended and extended. The numbers of vocalizations emitted during both flexion and extension periods were then quantified. The cyclic procedure consisting of a flexion and an extension was repeated every 5 s until

a total of five procedures had been delivered to each hind limb. A vocalization rating of 0 (no vocalization) or 1 (vocalization) was given in response to each flexion or extension stimulus. The vocalization rating was thus between 0 and 10 for each hind limb. The squeaking test was performed every day in each animal.

3. Evaluation of weight distribution ration: To estimate the pain levels due to the ankle arthritis, the weight-bearing forces of both hind limbs were measured with an incapacitance meter. The animals were placed in a test box of incapacitance meter in which a slanted plank is located. The bearing force of each hind limb was quantified by two mechanotransducers (Cass Co., Seoul, Korea) (Fig. 2), separately placed below two hind limbs: one is normal and the other is arthritic limb. The bearing force of each hind limb was estimated as a 5-s average, and the mean bearing force was calculated from four separate estimations. The WDR percentage was calculated as % WDR=100× (weight borne by ipsilateral limb/total weight borne by both limbs).

Data analysis

The data were presented as the mean±S.E.M. The significance of the differences between groups was assessed by a one-way analysis of variance (ANOVA), followed by the Tukey HSD post-hoc analysis. Difference was considered significant at $p<0.05$.

RESULTS

Using a carrageenan-induced arthritis rat, the therapeutic effect of dandelion for arthritis was investigated in this study. Behavioral tests such as paw volume, squeaking score were measured, and those results were compared with weight distribution ratio.

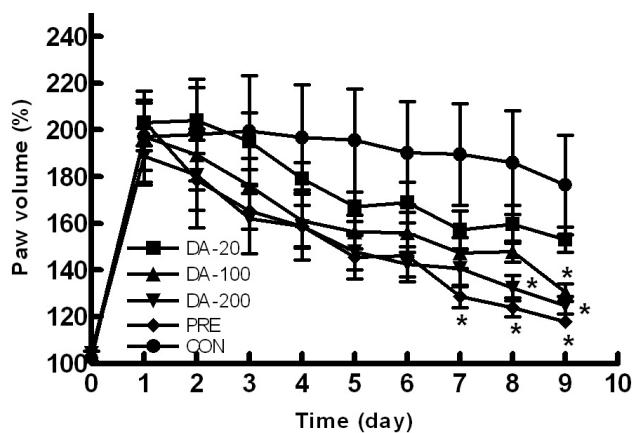


Fig. 3. Effect of the dandelion therapy on paw volume of carrageenan-induced arthritic rat. The CON (N=5), DA-20 (N=5), DA-100 (N=5), DA-200 (N=5), and PRE (N=4) mean the experimental groups. The Y-axis was indicated by gain of paw volume after day 0. The mean numbers of paw volume date were calculated and analyzed by repeated ANOVA followed by the Tukey HSD post hoc test for further confirmation. Significance was indicated as * $p<0.05$ vs. CON group.

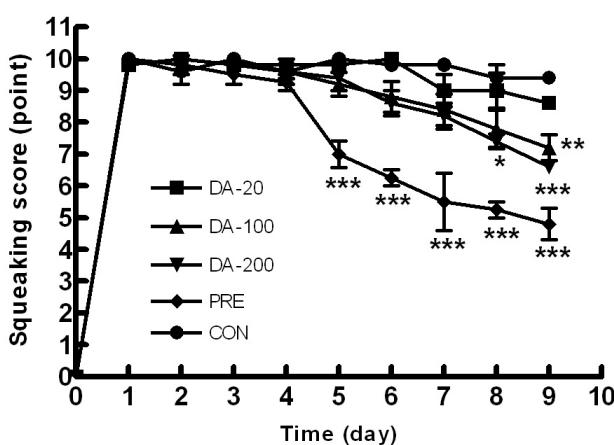


Fig. 4. Effect of the dandelion therapy on squeaking behavior of carrageenan-induced arthritic rat. The CON (N=5), DA-20 (N=5), DA-100 (N=5), DA-200 (N=5), and PRE (N=4) mean the experimental groups. The mean numbers of squeaking score date were calculated and analyzed by repeated ANOVA followed by the Tukey HSD post hoc test for further confirmation. Significance was indicated as * $p<0.05$ vs. CON group, ** $p<0.01$ vs. CON group, and *** $p<0.001$ vs. CON group.

Paw volume

For measuring the degree of edema in an arthritis-induced ankle joint, the variation of paw volume was analyzed. Fig. 3 displays the effect of dandelion therapy on the paw volume of a carrageenan-induced arthritic rat. A variation in the paw volume of the each groups were observed during the experimental period (9 day). From day 1, paw volume of the DA-100, DA-200 and PRE groups continually decreased, and during days 7 and 9, a significant decrease in paw volume was observed in the DA and PRE groups as compared to that of CON group.

Squeaking score

In the case of the CON group, from 1 day to 9 day, most of the squeaking scores were ten points. However, the score of the DA-100 and DA-200 decreased a little after day 5. Beside, the score of the PRE group continuously decreased a squeaking score. DA-100, DA-200 and PRE groups was significantly less than of the CON group. (Fig. 4)

Weight distribution ratio

Fig. 5 shows the effect of the dandelion therapy on weight distribution between the paws of a carra-

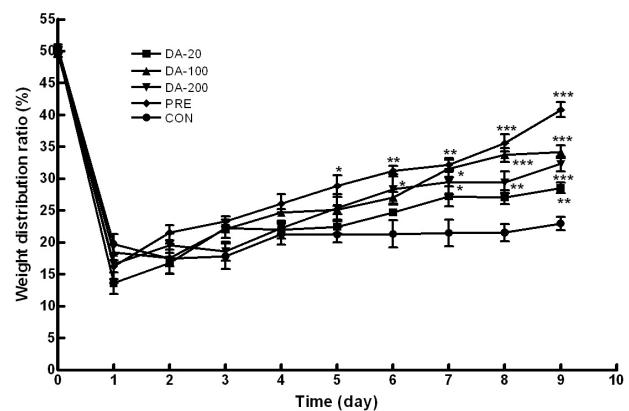


Fig. 5. Effect of the dandelion therapy on the weight distribution ratio of carrageenan-induced arthritic rat. The CON (N=5), DA-20 (N=5), DA-100 (N=5), DA-200 (N=5), and PRE (N=4) mean the experimental groups. The weight distribution ratios of the unilateral paw in normal mice were nearly 50%. The DA, PRE, and CON treatment was started on day 1 and executed every day for 9 days. The mean numbers of weight distribution ratio date were calculated and analyzed by repeated ANOVA followed by the Tukey HSD post hoc test for further confirmation. Significance was indicated as * $p<0.05$ vs. CON group, ** $p<0.01$ vs. CON group, and *** $p<0.001$ vs. CON group.

geenan-induced arthritic rats. The weight distribution ratios of the unilateral paws in the normal mice were nearly 50%. In case of the DA and PRE groups, the weight distribution ratio of the arthritis induced paw (left side) in the early stage was 15%. However, a change in the ratio could be seen from day 2, and the change was significant after day 5. In comparison, the ratio of an arthritis-induced paw in the CON group was maintained at about 20% after the early stage. This means that the saline administration group (CON) did not produce a medicinal effect and the arthritic symptoms worsened as time passed.

DISCUSSION

Arthritis is a chronic, systemic, and inflammatory disease which causes multiple joints in the body to be tender and swollen, and thus the joints become stiff and very sore. Despite of the prevalence and severe symptoms of the disease, no one has identified yet what exactly causes arthritis, and to date, there is no known remedy to completely cure the disease (Yeom et al., 2003).

Carrageenan-induced arthritis in the rat is also a practical model of arthritis. Carrageenan rapidly and reliably induces arthritis and is simple to prepare and administer. The rat is an inexpensive research animal in comparison with larger animals. A more extensive literature on anatomy, immunology and inflammation exists for the rat compared with the rabbit, dog or pig, and immunologic reagents are more readily available for the rat than for the other species. While such reagents are widely available for murine models, the rat is more suitable than the mouse for particular types of studies, where the larger size of musculoskeletal structures and organs provides more tissue and facilitates preparation. For example, immunohistochemical and flow cytometry analysis of bone marrow can be more easily performed with rat bones. The above factors make the rat model of carrageenan arthritis an important addition (Hansra et al., 2000).

Dandelion belongs to the family of compositae, which is a type of traditional herb. Dandelion possesses the therapeutic ability to eliminate heat and toxins, removing swelling, choleresis, diuresis, and anti-inflammation. It has been used in folklore for

the treatment of acute mastitis, lymphadenitis, hepatitis, struma, urinary infections, cold, and fever (Chu et al., 2006). As a therapeutic drug, it has no side effects over prolonged use. Chemical investigations of bioactive ingredients show that dandelion contains mainly flavonoids, phenolic acids, etc (Kisiel and Barszcz, 2000).

In this study, the effect of dandelion therapy was investigated by using a carrageenan-induced arthritis rat. The parameters such as paw volume, squeaking score were measured, and the results were compared to those of the weight distribution ratio. To assess behavioral and pre-clinical arthritic symptoms of a pain animal model, the following experimental methods are generally used - body weight measuring, acetone atomizing and hot plate test, heat sensitivity measuring method of the paw, and the Von Frey hair test. For measuring the degree of edema in the inflamed region of the body, mainly the paw volume, knee circumference, squeaking threshold, and articular index are analyzed. However, just as various analgesia equipment have their merits and demerits, the experimental limits, such as over subjective inclination, measuring the degree of pain and edema, requirement of general anesthesia can also have a similar effect. Therefore more than two methods were generally used at the same time, in order to improve experimental accuracy. For overcoming certain limits, an incapacitance meter, a new type of analgesia meter which is used to measure the weight difference between both soles of the arthritic rat's feet, was used for exclusion of subjective inclination, analyzing pain and edema at once, and the use of anesthesia was not necessary (Schott et al., 1994; Hay et al., 1997).

In the case of paw volume, for investigating the degree of edema caused by local induction of arthritic inflammation, the variation of paw volume was measured. Between the DA and PRE groups, the decrease in the paw volume variation was observed after day 2. After day 7, compared to the CON group, the DA and PRE groups had decreased more, also so much that it was statistically significant. These results indicated that DA was somewhat effective for carrageenan-induced arthritis. In order to obtain a more solid verification, longer-term experiments must be conducted studied.

The squeaking score, using modified methods of Park (2001) and Yu (2002), were used for analyzing rigidity and pain of ankle arthritis. In an ankle joint, arthritis was the cause of sensitized pain receptors, induced rigidity, and restricted extension-reflection. Consequently, squeaking was induced in arthritis-induced animals. In this study, after day 5, squeaking scores in the PRE group were significantly reduced than CON group. Beside, squeaking scores in the DA-200 group were begun to decrease after day 5, and a significantly decrease was observed compared to the CON group on day 8 and 9. Also, in the DA-100 group, a significantly decrease was observed on day 9. These results also indicated that the DA administration was more effective for alleviating arthritic pain.

In the end, using an incapacitance meter, a paw pain measuring unit, the efficacy of the DA therapy for treating arthritis was investigated. This device was designed to detect the difference of weight distribution between the two paws of an arthritis-induced rat, referring to Hay (1997) and Schott (1994). In this animal model, the pain only occurred at the arthritis-induced paw (left side) when body weight was placed onto both hind paws. To avoid or minimize the pain, the rats shifted their body weight onto the healthy paw (right paw). Therefore, the difference of weight distribution between the two paws was attributed to an action for avoiding pain. In the results, the wider the difference in weight distribution ratio means the more painful a paw had become, due to the arthritis.

Before the arthritis induction, the weight distribution between two paws was equal, that is 5 : 5. After arthritis induction, the ratio rapidly changed to 8 : 2 on day 1. In the PRE group, the efficacy of PRE administration was observed on day 2, and the ratio improved to 6 : 4 on day 9. In the case of the DA groups, during the experimental period (9 days), the ratio was continuously cure. The efficacy of the DA-200 group was observed after day 6. And the therapeutic effect of DA-100 was observed after day 7. And 9 day also found the effect of DA-20. For these reasons, the efficacy of the DA administration in terms of WDR recovery was significantly verified in this study.

CONCLUSION

In conclusion, for investigating the anti-arthritis effects of dandelion, paw volume, squeaking score, weight distribution ratio of a carrageenan-induced arthritis rat model were measured, as compared to those of that had a saline administrated to a control. In regards to the arthritic model development, the carrageenan injection to the ankle joint produced arthritic symptoms such as edema, pain, stiffness and rigidity. In this study we showed that the effects of dandelion on the carrageenan-induced arthritis rats. The dandelion treatment was more effective for the alleviation of pain and inflammation than the similarly herb. And can be removed the administration of high dose dandelion is essential to achieve herb therapy.

REFERENCES

- Ahmad VU, Yasmeen S, Ali Z, Khan MA, Choudhary MI, Akhtar F, Miana GA and Zahid M (2000) Taraxacin, a new guaianolide from *Taraxacum wallichii*. *J Nat Prod* 63:1010-1011.
- Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL and James JL (2002) Harrison's manual of medicine. McGraw-Hill, New York. p 748.
- Bennell K and Hinman R (2005) Exercise as a treatment for osteoarthritis. *Curr Opin Rheumatol* 17:634-640.
- Chu Q, Lin M and Ye J (2006) Determination of polyphenols in dandelion by capillary zone electrophoresis with amperometric detection. *J Am Assoc Lab Anim Sci* 38:20-24.
- Hansra P, Moran EL, Fornasier VL and Bogoch ER (2000) Carrageenan-induced arthritis in the rat. *J Inflamm* 24:141-155.
- Hay CH, Trevethick MA, Wheeldon A, Bowers JS and de Belleroche JS (1997) The potential role of spinal cord cyclooxygenase-2 in the development of Freund's complete adjuvant-induced changes in hyperalgesia and allodynia. *Neuroscience* 78:843-850.
- Kisiel W and Barszcz B (2000) Further sesquiterpenoids and phenolics from *Taraxacum officinale*. *Fitoterapia* 71:269-273.
- Krohn K (2005) Footwear alterations and bracing as treatments for knee osteoarthritis. *Curr Opin Rheumatol* 17: 653-656.
- Leu YL, Wang YL, Huang SC and Shi LS (2005) Chemical constituents from roots of *Taraxacum formosanum*. *Chem Pharm Bull* 53:853-855.
- Michalska K and Kisiel W (2003) Sesquiterpene lactones from *Taraxacum obovatum*. *Planta Med* 69:181-183.
- Park HJ (2001) The effect of electroacupuncture on the chronic monoarthritis in rats. Thesis for the degree of doctor of philosophy in oriental medicine, graduate school

- of east-west medical science, Kyunghee university, Seoul.
- Rubin BR (2005) Management of osteoarthritic knee pain. *J Am Osteopath Assoc* 105:s23-28.
- Schutz K, Carle R and Schieber A (2006) Taraxacum-a review on its phytochemical and pharmacological profile. *J Ethnopharmacol* 107:313-323.
- Seo SW, Koo HN, An HJ, Kwon KB, Lim BC, Seo EA, Ryu DG, Moon G, Kim HY, Kim HM and Hong SH (2005) *Taraxacum officinale* protects against cholecystokinin-induced acute pancreatitis in rats. *World J Gastroenterol* 11:597-599.
- Schott E, Berge OG, Angeby-Moller K, Hammarstrom G, Dalsgaard CJ and Brodin E (1994) Weight bearing as an objective measure of arthritic pain in the rat. *J Pharmacol Toxicol Methods* 31:79-83.
- Takasaki M, Konoshima T, Tokuda H, Masuda K, Arai Y, Shiojima K and Ageta H (1999) Anti-carcinogenic activity of Taraxacum plant. *Biol Pharm Bull* 22:602-605.
- Yeom MJ, Lee HC, Kim GH, Shim I, LEE HJ and Hahm DH (2003) Therapeutic effects of *Hominis placenta* injection into an acupuncture point on the inflammatory responses in subchondral bone region of adjuvant-induced polyarthritic rat. *Biol Pharm Bull* 26:1472-1477.
- Yu YC, Koo ST, Kim CH, Lyu YS, Grady JJ and Chung JM (2002) Two variables that can be used as pain indices in experimental animal models of arthritis. *J Neurosci Methods* 115:107-113.
- Zhu M, Wong PY and Li RC (1999) Effects of *Taraxacum mongolicum* on the bioavailability and disposition of ciprofloxacin in rats. *J Pharm Sci* 88:632-634.
-