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Inhibitory Effects of Plant Extracts on Adjuvant-induced Arthritis

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Twenty seven plant extracts were selected on the basis of ancient literature search for rheumatoid arthritis or similar syndrome. Methanol extract of each plant was prepared and administered orally to rats everyday at a dose of 200 mg/kg/day. Experimental arthritis was induced by subplantar injection of heat-killed *Mycobacterium butyricum* to right hind paw of rats. This treatment provoked swelling of the treated paw in two phases, acute primary swelling and secondary arthritic swelling. An inhibition of secondary swelling was considered to be antiarthritic activity. Several plant methanol extracts such as *Akebia quinata* (caulis), *Ephedra sinica* (herba) and *Sophorae subprostrata* (radix) were found to show significant inhibitory activity against secondary swelling at the dose tested. Our results strongly suggested an antiarthritic potential of these plant extracts.

Keywords : Inflammation, Plant extract, Adjuvant-induced arthritis, Rheumatoid arthritis, Antiarthritic activity, *Akebia quinata, Ephedra sinica, Sophorae subprostrata*

INTRODUCTION

Clinically used anti-inflammatory agents are mainly classified as nonsteroidal anti-inflammatory drugs (NSAID) and steroidal anti-inflammatory drugs (SAID). Using these drugs, most acute inflammatory disorders are well treated. However, it is still problematic to cure chronic inflammatory diseases such as rheumatoid arthritis and these drugs limit their long-term use because of serious adverse effects (Kim, 1990). Thus, there is an urgent need for new drugs having a novel action mechanism. Several selective cyclooxygenase-II (COX-II) inhibitors, phospholipase A_2 inhibitors or interleukin-6 antagonist were revealed to have a potential to treat rheumatoid arthritis (Sugita *et al.*, 1993; Bomalaski and Clark, 1993; Meade *et al.*, 1993). But it is far from clear that they could become a magic bullet to chronic inflammatory disorders. Therefore, numerous researchers are investigating natural products having anti-inflammatory activity with less side-effects (Duwiejua *et al.*, 1993; Perez *et al.*, 1995; Lee *et al.*, 1997).

In this study, for our continuing effort to find new agents acting on chronic inflammatory conditions, antiarthritic activity of 27 plant extracts were investigated.

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MATERIALS AND METHODS

Materials

Tripterygium regelii and *Viscum album* var. *coloratum* were collected in Andong area (Korea) and identified. The other 25 plants were obtained from Kyungdong crude drug market in Seoul (Table I). These plants were selected from ancient literature (Huh, 1613). Each dried plant (100 g) was refluxed in 500 ml methanol for 3 hrs and this procedure was repeated three times. The combined methanol extract was evaporated *in vacuo* to dryness.

Animals

Female Sprague-Dawley rats were kindly provided from Yuhan Pharmaceutical Co. and acclimatized at least for 1 week prior to use. Animals were fed with mouse pellet lab. chow (Sam Yang Co.) and water *ad libitum* under the condition of $22\pm1^{\circ}C$.

Rat adjuvant-induced arthritis

Experimental arthritis was induced by subplantar injection of *Mycobacterium butyricum* (Difco Co.) suspended in mineral oil according to the procedure of Kubo *et al.* (1984). *Mycobacterium butyricum* (0.6 mg/rat) was injected to right hind paw of rats (100 ± 10 g). Swelling of paw volume was measured in every two

Table I. Twenty seven plants evaluated in this study

Plant name	Part used
Aconitum carmichaeli	tuber
Aconitum loczyanum	radix
Akebia quinata	caulis
Anemarrhena asphodeloides	rhizoma
Angelica gigas	radix
Aralia cordata	radix
Asarum sieboldii	radix
Belamcamda chinensis	rhizoma
Bupleurum falcatum	radix
Carthamus tinctorius	flower
Cimicifuga haracleifolia	rhizoma
Citrus unshiu	pericarp
Cnidium officinale	rhizoma
Ephedra sinica	herba
Epimedium koreanum	herba
Ligustrum japonicum	fructus
Liriope platyphylla	tuber
Ostericum koreanum	radix
Paeonia japonica	radix
Plantago asiatica	semen
Platycodon grandiflorum	radix
Schizonepeta tenuifolia	herba
Sophora subprostrata	radix
Scutellaria baicalensis	radix
Taraxacum platycarpum	herba
Tripterygium regelii	caulis and folia
Viscum album var. *coloratum*	herba

days during experiments using water displacement method. Each plant extract dissolved in DMSO (200 ul) was administered orally at dose of 200 mg/kg/day for 18-20 days starting from 0 day at 1 hr prior to administration of adjuvants. At least 5-6 animals were used per a group. Inhibitory activity of arthritis was measured on the basis of inhibition of secondary inflammation of right hind paw (treated side) and inhibition of swelling of untreated left hind paw during 12-18 days after administration of adjuvant. Only data for treated right hind paw were represented in this study. Percent inhibition was calculated compared to control group having adjuvant as follows:

$$\text{Percent inhibition of arthritic inflammation} = \left(1 - \frac{\text{Tested group-Control w/o adjuvant}}{\text{Control w/ adjuvant-Control w/o adjuvant}}\right) \times 100$$

Statistical analysis was evaluated using one way ANOVA and P values less than 0.05 were regarded to be significantly different.

RESULTS AND DISCUSSION

Rat adjuvant-induced arthritis is an experimental animal model of human rheumatoid arthritis. Although this model does not reflect human disease completely, it has been widely used for screening of antiarthritic agents by many researchers. Actually, most clinically used drugs on rheumatoid arthritis show inhibitory activity in this model (Sedgwick and Willoughby, 1989).

Fig. 1. clearly showed typical pattern of experimental arthritis evoked by subplantar injection of *M. butyricum* to rats. There were two phases of inflammation (swelling), primary swelling (2~6 days) and late secondary inflammation starting about 10 days after administration of adjuvant. The secondary inflammation (swelling) is considered to be an arthritic inflammation accompanied by deformation of bones and formation of nodules on a tail (Newbould, 1963). It was found that prednisolone used as a reference compound strongly inhibited secondary inflammation as well as primary swelling at 20 mg/kg/day. This result was well matched with the previous results of ours (Lee *et al.*, 1995) and other investigator (Waltz *et al.*, 1971).

The results of evaluation of 27 plant extracts were shown in Fig. 1-10. Although there was an ancient literatural background used in rheumatoid arthritis or similar conditions for these plants, plant extracts except *Angelica gigas, Akebia quinata, Ephedra sinica, Liriope platyphylla, Plantago asiatica, Schizonepeta tenuifolia* and *Sophora subprostrata* did not show significant inhibitory activity either against primary inflammation or secondary inflammation at oral dose of 200 mg/kg/day. There may be several explanations for these plants showing no inhibition. Either the plants may not possess antiarthritic activity in spite of literatural background, or the dose/treatment regimen selected may not be suitable to show inhibitory activity. Another possible explanation may be due to the nature of adjuvant induced arthritis model, in which most immunosuppressive agents and cyclooxygenase inhibitors are sensitive in contrast to immunoenchancing agents.

As represented in Fig. 3., methanol extract of *Plantago asiatica* showed potent inhibition against primary inflammation, but it also showed toxicity at the dose tested. It was not possible to continue experiment over 12 days. *Angelica gigas, Akebia quinata, Ephedra sinica, Liriope platyphylla* and *Schizonepeta tenuifolia* showed significant inhibition against primary inflammation. These results indicated that they may possess anti-inflammatory activity against acute inflammation. Among these extracts, only *Akebia quinata, Ephedra sinica* and *Sophora subprostrata* showed significant antiarthritic activity by inhibition of secondary swelling. The average inhibition at 16 days were found to be 22%, 36% and 13% for *Akebia quinata, Ephedra sinica* and *Sophora subprostrata,* respectively. *Anemarrhena asphodeloides, Angelica gigas* and *Schizonepeta tenuifolia* showed weak inhibitory activity but not statistically significant. Methanol extract of *Akebia quinata* showed sig-

nificant inhibitory activity in this animal model (Fig. 5). The major constituents in *Akebia quinata* were reported to be hederagenin glycosides (Ikuta and Itokawa, 1989; Higuchi and Kawasaki, 1976a and b). It has been reported by us that hederagenin and its glycoside, loniceroside A, possessed antiarthritic activity (Son *et al.*, 1994; Lee *et al.*, 1995). Therefore, it is reasonable to suggest that hederagenin and its glycosides in methanol extract of *Akebia quinata* may participate in antiarthritic activity, at least in part. *Scutellaria baicalesis* did not show inhibition (Fig. 8), in contrast to the previous reports of Kubo *et al.* (1984) showing potent inhibition by methanol fraction and flavonoid constituents. *Aconitum carmichaeli* failed to show positive response in this model (Fig. 8), as reported previously by Hikino *et al.* (1980). It is worth to note that *Trypterigium regelii* did not show inhibition (Fig. 10). *Trypterigium wilfordii*, a closely related species, was known to possess potent antiarthritic activity (Gu *et al.*, 1992) and it is clinically used currently in China. *Sophora subprostrata* showed antiarthritic activity. It has been primarily used for antidote, diuretics, anti-inflammatory agent, etc., and previously reported to show antiarrhythmic activity, antiulcer activity, antiathmatic activity and antineoplastic activity by total extract or several constituents

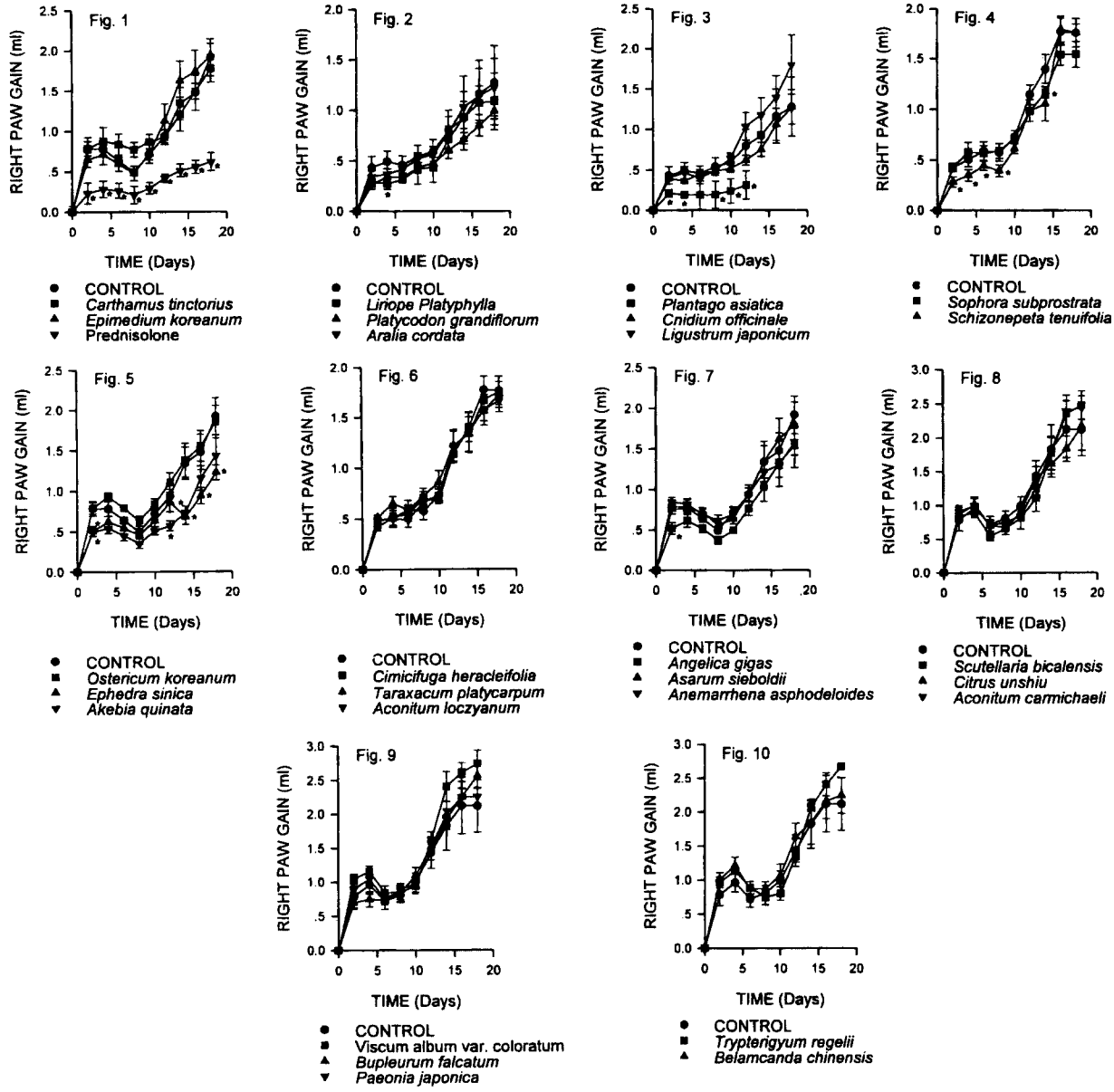

Fig. 1-10. Antiarthritic activity of 27 plant extracts. All plant extracts were orally administered to SD rats at 200 mg/kg/day. Prednisolone (Fig. 1) was administered orally at 20 mg/kg/day. All data were represented as mean±SE. *: Significantly different from control group, $P< 0.05$ by one way ANOVA.

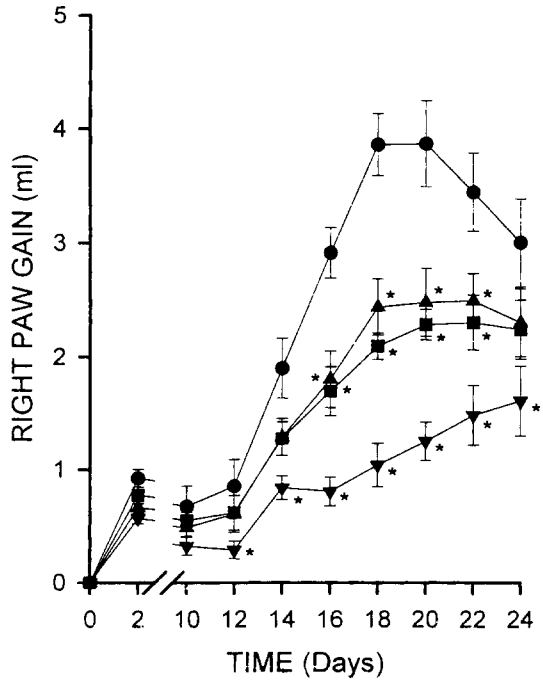

Fig. 11. Antiarthritic activity of fractions of *Sophora subprostrata* All fractions were orally administered to SD rats at 50 mg/kg/day. Prednisolone (▼) was administered orally at 10 mg/kg/day. All data were represented as mean±SE. *: Significantly different from control group, $P< 0.05$ by one way ANOVA. Control with adjuvant (●), Ethyl acetate fraction (■), n-butanol fraction (▲).

such as alkaloids and flavonoids (Tang and Eisenbrand, 1992). In order to localize its antiarthritic activity, chloroform, ethyl acetate, n-butanol and water fractions were prepared. Among these fractions, ethyl acetate and n-butanol fractions also showed antiarthritic activity at 50 mg/kg/day in the same animal model (Fig. 11). Further study for isolating active principle(s) is currently under investigation.

The present investigation represented basic screening results of 27 plant extracts on antiarthritic activity. Although rat adjuvant-induced arthritis is not a simple and fast animal model, it is thought to be adequate to evaluate antiarthritic activity of plant extracts. Using this model, *Akebia quinata, Ephedra sinica* and *Sophora subprostrata* were clearly found to possess significant antiarthritic activity orally at dose of 200 mg/kg/day.

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