Arch. Pharm. Res. Vol.19, No.5, pp. 337-341, 1996

Anti-inflammatory Activity of Propolis

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(Received June 13, 1996)

Propolis (bee-glue), known as a folk medicine, is a lipophilic material found in honeybee hives. In the present study on the anti-inflammatory effect of Korean propolis, it was extracted with ethanol, and used as a test material. The LD_{50} value with the oral administration of ethanolic extract of Korean propolis (EEKP) was higher than 2 g/kg in mice. The oral administration of the propolis extract (100 mg/kg) significantly inhibited the development of hind paw edema induced by carrageenin in rats. The oral pretreatment of the propolis extract markedly inhibited the increase in vascular permeability and the number of writhing induced by acetic acid in mice. Propolis extract, 50 and 100 mg/kg p.o. per day for 7 days, produced a significant inhibitory effect on granuloma and exudate formation in rats. This inhibitory effect was enhanced with the concomitant use of prednisolone (2.5 mg/kg). These results suggest that Korean propolis apparently has a strong anti-inflammatory activity.

Key words : Korean propolis, Animal model, Acute toxicity, Anti-inflammatory activity, Analgesic effect

INTRODUCTION

Propolis (bee-glue) is a natural resinous substance produced by honey bees and used by them to strengthen their nests. Its chemical composition was found to be a very complex mixture of compounds including benzoic acid and its esters, substituted phenolic acid and its esters, terpenoids, and flavonoid aglycones (Nagy *et al.*, 1985). Propolis has been widely used as 'folk medicine' for centries and was shown to have beneficial effects in many pathological processes (Kravchuk, 1982). In recent years, propolis extract is thought to exhibit a broad spectrum of activities including antibacterial, antifungal, cytostatic, and antioxidative properties (Hladon *et al.* 1980; Koshihara *et al.* 1984; Krol *et al.* 1990; Grange and Davey, 1990; Amoros *et al.*, 1992). Recent studies by Scheller *et al.* (1989) indicated that ethanol extract of propolis possessed higher survival of mice with Ehrlich ascites carcinoma.

Ethanol extract of propolis has been shown to possess immunological properties in animals and in patients (Scheller *et al.* 1988; Frankiewicz and Scheller, 1984). However, the experimental studies on the anti-inflammatory activity of propolis have not been performed extensively. In this paper, we describe an investigation of Korean propolis on the anti-inflammatory activity, utilizing carrageenin-induced edema in the rat hind paw, rat granuloma pouch, and adjuvant arthritis in rats. The effects on vascular permeability and acetic acid-induced writhing in mice were also examined.

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MATERIALS AND METHODS

Materials

Propolis was obtained from a farm in the area of Chungju city, and was extracted with 10-fold volume of absolute ethanol for 1 week. The ethanolic extract of Korean propolis (EEKP) was then filtered through Whatman #4 paper, and evaporated to dryness under vacuum. Its yield was 40%, which was calculated from the weight of propolis used. The dried residual powder was kept in -20°C to minimize bacterial contamination, and was dispersed in 10% Tween 80 solution immediately prior to use.

Prednisolone, phenylbutazone, sesami oil and light minimal oil were obtained from Sigma Chemical Co. *Mycobacterium butyricum* was obtained from Difco Co. Carrageenin, croton oil and acetic acid were purchased from Yakuri Pure Chemicals Co. Evans-blue was obtained from Tokyo Kasei Chemical Co.

Animals

Male ICR mice and female Sprague-Dawley rats were housed in an animal room under conditions of

24±1°C and 12-h light-dark cycle with humidity (55±5%), fed a commercial diet and allowed tap water *ad libitum*.

Acute toxicity in mice

Male ICR mice, weighing 25±2 g, were used and the oral LD_{50} were determined. Mortality was recorded 72 hours after administration.

Carrageenin-induced edema in the rat hind paw

Edema on the right hind paw of the animals were induced by subcutaneous injection of carrageenin (0.1 ml/rat) prepared as 1% suspension in saline(Winter *et al.*, 1962 ; Han *et al.*, 1994). After the treatment with carrageenin, the volume of the foot was determined every 1 hr for 5 hours, and the increase in the foot volume was taken as the volume of edema. The volume of the hind paw was measured by immersing the limb in a Plethysmometer (UGO Basile). The agents tested were orally administered 60 minutes before the carrageenin treatment. The percent inhibition of edema induced by each agent was calculated for each animal group with respect to its vehicle-treated control group.

Granuloma pouch in rats

The granuloma pouch was induced in rats weighing 110 to 150 g according to the method described by Takagi *et al.* (1980). The injection of 20 ml of air under the dorsal skin of the rat, followed by the injection of 0.5 ml of 1% solution of croton oil in sesami oil, was carried out under ether anesthesia. The agents tested were orally administered once a day for 7 days after the treatment. The animals were killed on the 8th day and the pouch was dissected. The exudate was collected in a graduated test tube and its volume was measured. The granulomatous tissues were then isolated and weighed.

Adjuvant-induced arthritis

Using the method described by Person *et al.* (1961), adjuvant arthritis was induced in rats weighing 160 to 200 g at the start of the experiment. The subplantar injection of 1% suspension (0.05 ml) of heat-killed *Mycobacterium butyricum* in liquid paraffin into the right hind paw of the rats was carried out. The volume of the injected hind paw was measured as mentioned above, prior to the injection and 1, 3, 5, 7, 14 and 21 days after the injection. The contralateral paw volume and nodules appeared on tails were also checked. The agents tested were orally administered to the rats once a day for 21 days.

Vascular permeability and acetic acid-induced writhing

Using the method described by Whittle (1964), the effect on capillary permeability was determined by injecting 0.1 ml per 10 g body weight i.v. of 4% pontamine sky blue solution into mice which had been administered with the agent 20 minutes previously. For the measurement of analgesic activity, each animal was placed in an individual cage and the number of writhings of each animal was recorded for 20 min.. The animals were then killed by dislocation of the neck. The viscera were exposed and washed with distilled water. The washing fluid was filtered through glass wool, made up to 10 ml in a graduated test tube and the light absorption was read at 630 nm with a spectrophotometer. Then amount of the dye in the fluid was determined from the calibration curve.

Statistical analysis

Student t-test was used throughout the experiments for evaluating the statistical analysis and considered as statistically significant when P values were less than 0.05.

RESULTS AND DISCUSSION

In this work, the anti-inflammatory effect of ethanolic extract made from Korean propolis were examined using various experimental animal models.

Acute toxicity in mice

LD_{50} value was determined by the oral administration of the extract into mice. The measured LD_{50} value was estimated to be higher than 2 g/kg. Weak tremor and reduced spontaneous movement were observed at the higer doses. The Korean propolis extract was not toxic to mice in the range of dosages tested. This is similar to the result obtained for propolis from other area (Arvouet *et al.*, 1993).

Carrageenin-induced edema in the rat hind paw

Studies on rat paw edema in response to carrageenin suggest that acute vascular responses, vasodilation and increased vascular permeability result from the sequential release of low molecular weight mediators-histamine, serotonin, bradykinin and prostaglandins (Vane and Botting, 1987).

In carrageenin-induced rat paw edema, an acute inflammatory model, EEKP 50, 100 and 200 mg/kg showed a significant inhibition of rat paw edema when orally administered (Table I). Phenylbutazone 100 mg/kg, also produced significant inhibition of edema. The inhibitory effect of phenylbutazone could be enhanced by concomitant administration of EEKP

Table I. Effects of orally administered ethanol extract of Korean propolis (EEKP) on carrageenin-induced hind paw edema in rats

Group	Dose (mg/kg, p.o.)	Edema index[a]	Inhibition (%)
Contorl	-	60.8±5.9	-
EEKP	50	46.3±7.4	28.8
	100	34.5±7.7*	43.2
	200	21.4±8.8*	64.8
Phenylbutazone	100	25.1±4.9*	58.8
Phenylbutazone/EEKP	100/50	14.1±8.1**	76.3

[a]The volume of edema was measured at 4 hours after the treatment of carrageenin. Edema index indicates the percentage of the increase of swelling. Each value represents mean±S.E. The number of animals in each group was 6. *P<0.05, **P<0.01

(Table I). The inhibitory effect of EEKP were stronger at the higher dose, indicating the dose-response relationship. Considering that EEKP is a mixture of various unknown compouds, the inhibitory effect of its active principle in the carrageenin-induced edema seems to be potent.

Granuloma pouch in rats

The inhibitory effects of EEKP, 50 and 100 mg/kg, on the granuloma and exudate formations in rats were examined. The results are shown in Table II. In the subacute inflammatory model used, EEKP were orally administered for 7 days. EEKP 100 mg/kg produced siginificant inhibitory effects of 28.8 and 31.8% on the weight of granuloma and the volume of exudate, respectively. Prednisolone (2.5 mg/kg *p.o.*) also significantly inhibited the granuloma and exudate formations. Simultaneous administration of EEKP (50 mg/kg) and prednisolone (2.5 mg/kg) inhibited the granuloma and exudate formation by 56% and 91%, respectively. These results show that EEKP contains anti-inflammatory effect in the subacute inflammation model.

Adjuvant arthritis in rats

To investigate the chronic anti-inflammatory activity of EEKP, adjuvant induced arthritis in rats was carried out. The inhibitory effects of 21-day administrations of EEKP 50 mg *p.o.* on the edema formation in rat hind paw were examined. EEKP 50 mg/kg *p.o.* inhibited the development of edema (Fig. 1). The inhibitory effect was also observed with phenylbutazone 50 mg/kg *p.o.* Simultaneous administration of EEKP 50 mg/kg and phenylbutazone 50 mg/kg inhibited the edema more strongly than phenylbutazone 50 mg/kg alone. These results indicate that EEKP is also efffective in the animal model of chronic inflammation.

Table II. Effects of ethanol extract of Korean propolis (EEKP) on granuloma and exudate formation induced by croton oil in rats[a]

Group	Dose (mg/kg, p.o.)	Wet weight of granuloma (g/100 g)	Volume of exudate (ml/100 g)
Control	-	2.4±0.3	4.4±0.1
EEKP	50	2.1±0.2 (12.5)	3.5±0.1* (20.5)
	100	1.8±0.1* (25.0)	3.0±0.2* (30.7)
Prednisolone	2.5	1.2±0.2* (50.0)	1.2±0.5** (72.7)
Prednisolone/EEKP	2.5/50	1.1±0.2** (54.2)	0.4±0.1** (90.9)

[a]Wet weght of granuloma and volume of exudate were measured after 7-day administration of tested agents. Each value represents mean±S.E. (n=6). Figures in parentheses indicate percent inhibition from control value. *P<0.05, **P<0.01

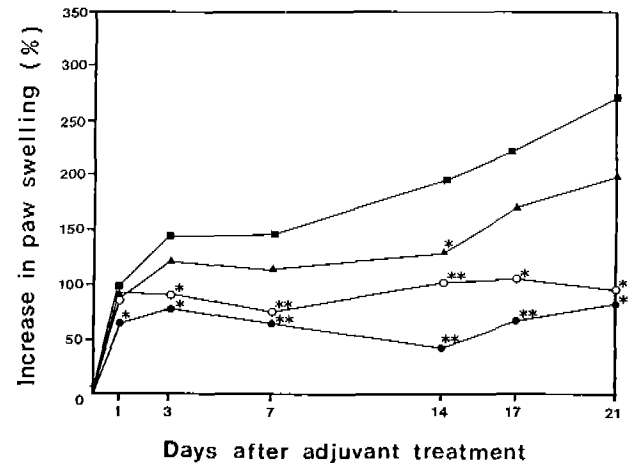

Fig. 1. Effects of 21-day administrations of ethanolic extract of Korean propolis (EEKP) on the development of hind paw edema associated with adjuvant arthritis in rats. *P<0.05, **P<0.01, ■: Control, ▲: EEP 50 mg/kg, PB 50 mg/kg, EEP 50+PB 50 mg/kg

Vascular permeability and acetic acid-induced writhing in mice

The effects of EEKP on capillary permeability and writhing induced by acetic acid in mice were investigated. Oral administration of EEKP, 50 and 100 mg/kg, caused siginificant inhibitions (24.8 and 47.5%) of the dye leakage into the abdominal cavity, respectively (Table III). Phenylbutazone, a positive control compound, also showed a strong effect. The inhibitory effect of EEKP was found to be comparable to that of phenylbutazone. EEKP 50, 100 and 200 mg/kg caused siginificant inhibitory effects (25.6, 34.3, and 44.1%) in the number of writhings induced by acetic acid in mice, respectively (Table IV). This effect

Table III. Effects of ethanol extract of Korean propolis (EEKP) on leakage of dye into the peritoneal cavity induced by acetic acid a in mice

Group	Dose (mg/kg, p.o.)	Leakage of dye (μg/10 g)[b]	Inhibition (%)
Control	-	73.1± 5.0	-
EEKP	50	58.5±19.1	24.8
	100	49.6± 7.6*	47.5
Phenylbutazone	100	43.3± 3.1**	68.6

[a]Acetic acid was intraperitoneally injected with a volume of 0.1 ml per 10 g body weight.
[b]Each value represents the mean±S.E. (n=7).
*P<0.05, **P<0.01

Table IV. Effects of ethanol extract of Korean propolis (EEKP) on writhing induced by acetic acid in mice[a]

Group	Dose (mg/kg, p.o.)	Number of writhing (times/15 min)	Inhibition (%)
Control	-	42.4±6.9	-
EEKP	50	31.6±4.8	25.6
	100	27.9±5.4	34.3
	200	23.7±3.3*	44.1
Pheylbutazone	100	16.3±3.4**	61.6

[a]Acetic acid was intraperitoneally injected with a volume of 0.1 ml per 10 g body weight. Each value represents the mean±S.E. (n=7).
*P<0.05, **P<0.01

was based on the relationship of dose and response.

The present study demonstrated the strong anti-inflammatory effects of ethanol extract of Korean propolis on carrageenin-induced edema, granuloma pouch and adjuvant arthritis. This agent also showed inhibitory activity on vascular permeability and analgesic effect. However, further investigation should be done to explain the mechanism and active principles of Korean propolis, which is responsible for its anti-inflammatory and analgesic effect. Finally, it may be concluded that 1) EEKP is not toxic, 2) The ethanol extract of Korean propolis (EEKP; 100 and 200 mg/kg, *p.o.*) shows significant inhibition on carrageenin edema, 3) EEKP (100 mg/kg, *p.o.* per day for 7 days) also shows significant inhibitory effect on granuloma formation and exudate formation induced by croton oil in rats, 4) EEKP (100 mg/kg, *p.o.*) markedly inhibit vascular permeability increase, and 5) EEKP (200 mg/kg, *p.o.*) shows analgesic effect on acetic acid-induced writhing test.

ACKNOWLEGEMENT

The authors thank to Miss Ja-Hoon Kahng for helping the preparation of this manuscript.

This work was partly supported by research grant to Eun-Hee Park from Sookmyung Women's University.

REFERENCES CITED

Amoros, M., Simoes, C, M. O. and Girre, L., Synergistic effect of flavones and flavonols against herpes simplex virus type 1 in cell culture. Comparison with the antiviral activity of propolis. *J. Nat. Prod.*, 55, 1732 (1992).

Arvouet, G. A., Lejeune, B., Bastide, P., Pourrat, A., Privat, A. M. and Legret, P., Propolis extract. I. Acute toxicity and determination of acute primary cutaneous irritation index. *J. Pharm. Belg.*, 48, 165 (1993).

Frankiewicz, L. and Scheller, S., Bienen-Kittharz Stimuliert das Immunsystem. Arzneimittel Praxis, 2869 (1984).

Grange, J. M. and Davey, R. W., Antibacterial properties of bee glue. *J. Royal Soc. Med.*, 83, 159 (1990).

Han, B. H., Suh, D. Y., Yang, H. O., Lee, S. J. and Kim, H. P., Reduced anti-inflammatory activity of Acetylsalicylic Acid maltol ester, Aspalatone. *Arch. Pharm. Res.*, 17(3), pp 166-169 (1994).

Hladon, B., Bylka, W., Wojtaszek, M. E., Skrzypczale, L., Szafarele, P., Chodera, A. and Kowalewski, Z., *In vitro* studies on the cytostatic activity of propolis extracts. Arzneim-Forsch. *Drug Res.*, 30, 1847 (1980).

Koshihara, Y., Neichi, T., Murota, S. L., Lau, A., Fujimoto, Y. and Tatsuno, T., Caffeic acid is a selective inhibitor for leukotriene biosynthesis. *Biochim. Biophys. Acta*, 792, 92 (1984).

Kravchuk, P. A., "Propolis", Zdarovje, Kiev (1982).

Krol, W., Czuba, Z., Scheller, S., Gabrys, J., Grabies, S. and Shani, J., Anti-oxidant property of ethanol extract of propolis as evaluated by inhibiting the chemiluminescence of luminal. Biochem. Intern., 21, 593 (1990).

Nagy, E., Papay, V., Litkei, G. and Dinya, Z., in "Flavonoids and Bioflavonoids." ed. Farkas, L., G bor, M. and Kallay, F. Elsevier, Amsterdam, pp. 223-232 (1985).

Person, C. M., Waksman, B. H. and Sharp, J. T., Studies of arthritis and other lesions induced in rats by injection of mycobacterial adjuvant. J. Exp. Med., 113, 485 (1961).

Scheller, S., Gazda, G., Gabrys, J., Szumulas, J., Eckert, L. and Shani, J., The ability of EEP to stimulate plaque formation in immuized mouse spleen cells. *Pharmacol. Res. Comm.*, 20, 323 (1988).

Scheller, S., Krol, W., Swiacik, S., Owczarek, S., Gabrys, J. and Shani, J., Antitumoral property of ethanolic extract of propolis in mice-bearing Ehrlich carcinoma, as compared to Bleomycin. Z. Naturforsch, 44C, 1063 (1989).

Takagi, K., Park, E. H. and Kato, H., Anti-inflammatory activities of hederagenin and crude sa-

ponin isolated from Sapidus mukorossi GAERTIN. *Chem. Pharm. Bull.*, 28, 1183 (1980).

Vane, J. and Botling, R., Inflammation and the mechanism of action of anti-inflammatory drugs. FASEB J. 1, 89 (1987).

Whittle, B. A., The use of changes in permeability in mice to distinguish between narcotic and non-narcotic analgesics. Brit. J. Pharmacol., 22, 246 (1964).

Winter, C. A., Risley, E. A. and Nuss, G. W., Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Pro. Soc. Exp. Med.*, 111, 544 (1967).

Zumla, A. and Lulat, A., Honey-a remedy rediscovered. *J. Royal Soc. Med.*, 82, 384 (1989).