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Anti-inflammatory Activities of Hederagenin and Crude Saponin isolated from *Sapindus mukorossi* GAERTN

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The anti-inflammatory activities of hederagenin and crude saponin isolated from *Sapindus mukorossi* GAERTN were investigated utilizing carrageenin-induced edema, granuloma pouch and adjuvant arthritis in rats. The effects of these agents on vascular permeability and acetic acid-induced writhing in mice were also examined. In some experiments, the results were compared with those obtained with saikogenin A, crude platycodin, platycodigenin and oleanolic acid. Anti-inflammatory activity on carrageenin edema was observed with *i.p.* and *p.o.* administered crude saponin, while hederagenin and the other agents used showed activity only when administered *i.p.* Hederagenin, 100 and 200 mg/kg *p.o.* per day for 7 days, showed no significant inhibitory effect on granuloma and exudate formations in rats, while crude saponin, 100 and 200 mg/kg *p.o.*, showed significant effects. Crude saponin, 200 mg/kg *p.o.* per day for 21 days, significantly inhibited the development of hind paw edema associated with adjuvant arthritis in rats, but hederagenin, 50—200 mg/kg *p.o.*, did not. Crude saponin, 400 mg/kg *p.o.*, inhibited the increase in vascular permeability and the number of writhings induced by acetic acid in mice. The results suggest that hederagenin and crude saponin, as well as the other agents used, show some degree of anti-inflammatory activity, especially in the case of saponin.

Keywords—hederagenin; *Sapindus mukorossi*, crude saponin of; saikogenin A; platycodigenin; crude platycodin; oleanolic acid; phenylbutazone; prednisolone; anti-inflammatory activity

The fruit husk of *Sapindus mukorossi* GAERTN has been used as a soap. Hederagenin isolated from *S. mukorossi* was demonstrated to be a naturally occurring triterpene having a

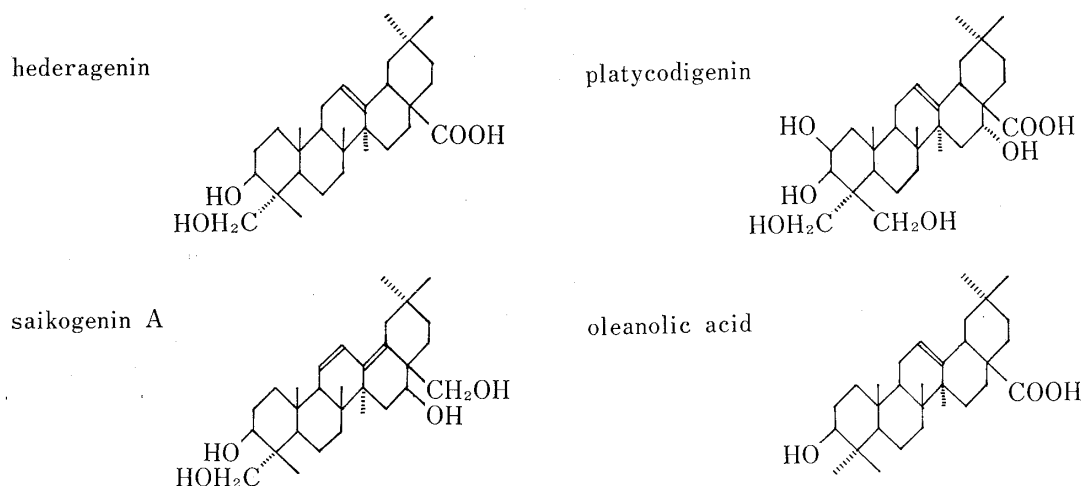


Fig. 1. Chemical Structures of the Triterpenoids used

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hydroxymethyl group at C-4 in ring A.²⁾ The anti-inflammatory activities of crude platycodin and saikogenin A, which are also triterpenoids, have been reported.³⁾

The present study was undertaken to investigate the anti-inflammatory activities of hederagenin and crude saponin isolated from *S. mukorossi*, utilizing carrageenin-induced edema in the rat hind paw, rat granuloma pouch and adjuvant arthritis in rats. The effects of these compounds on vascular permeability and acetic acid-induced writhing in mice were also investigated. In some experiments, the results were compared with those obtained with saikogenin A, crude platycodin, platycodigenin, oleanolic acid, phenylbutazone and prednisolone. Fig. 1 shows the chemical structures of the triterpenoids used.

Experimental

Carrageenin-induced Edema in the Rat Hind Paw—Female Wistar rats weighing 150 to 170 g were used. The volume of the rat hind paw was measured according to the method reported by Van Arman *et al.*⁴⁾ The foot above the topmost callus pad was immersed in a pool of mercury. The pressure increase caused by the slight rise in mercury level was transmitted to a pressure transducer (Nihon Kohden, LPU-0.1). The recordings were made on an ink-writing oscillograph (Nihon Kohden WI-260) through a carrier amplifier (Nihon Kohden, MP-3). The subplantar injection of 0.1 ml of 1% carrageenin in physiological saline solution was carried out. After the treatment with carrageenin, the volume of the foot was determined every 1 hr for 5 hr, and the increase in the volume was taken as the volume of edema. The agents tested were administered *i.p.* or *p.o.* 30 or 60 min before the carrageenin treatment, respectively. 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 female Wistar rats weighing 110 to 150 g according to the method described by Robert and Nejamis.⁵⁾ The injection of 20 ml of air under the dorsal skin of the rat, followed by the injection of 0.5 ml of a 1% solution of croton oil in sesame oil, was carried out under ether anesthesia. The agents tested were administered *p.o.* once a day for 7 days after this 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 Arthritis in Rats—Using the method described by Pearson *et al.*,⁶⁾ adjuvant arthritis was induced in female Wistar rats weighing 160 to 200 g at the start of the experiment. The subplantar injection of 0.05 ml of a 1% suspension of heat-killed *Mycobacterium tuberculosis* 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 increase in volume was taken as the volume of edema. The agents tested were administered *p.o.* to the rats once a day for 21 days. The percent inhibition of edema produced by each agent was calculated for each animal group with respect to its vehicle-treated control group.

Vascular Permeability and Acetic Acid-induced Writhing—Male ddy mice weighing 18 to 20 g were used. Using the method described by Whittle,⁷⁾ 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 min previously. After 10 min, 0.1 ml per 10 g body weight of 0.6% acetic acid solution was injected *i.p.* to the animals. 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 adsorption was read at 590 m μ with a spectrophotometer (Hitachi, model 124).

Agent used—Hederagenin, crude saponin of *Sapindus mukorossi* GAERTN, crude platycodin, platycodigenin, saikogenin A and oleanolic acid, which were kindly supplied by Drs. Shibata and Akiyama, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Tokyo, were suspended in a 1% CMC saline solution. Phenylbutazone (Fujisawa Pharmaceutical Co.) and prednisolone acetate (Tokyo Kasei Co.) were also suspended in a 1% CMC saline solution.

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Results

Carrageenin-induced Edema in the Rat Hind Paw

The anti-inflammatory effects of hederagenin, 200 and 400 mg/kg *p.o.* and 100 and 200 mg/kg *i.p.*, and crude saponin of *S. mukorossi* (crude saponin), 200 and 400 mg/kg *p.o.*, and 25 and 50 mg/kg *i.p.*, on carrageenin-induced edema in the rat hind paw were investigated and the results were compared with those of oleanolic acid, saikogenin A, crude platycodin, platycodigenin and phenylbutazone. The results are shown in Figs. 2 and 3.

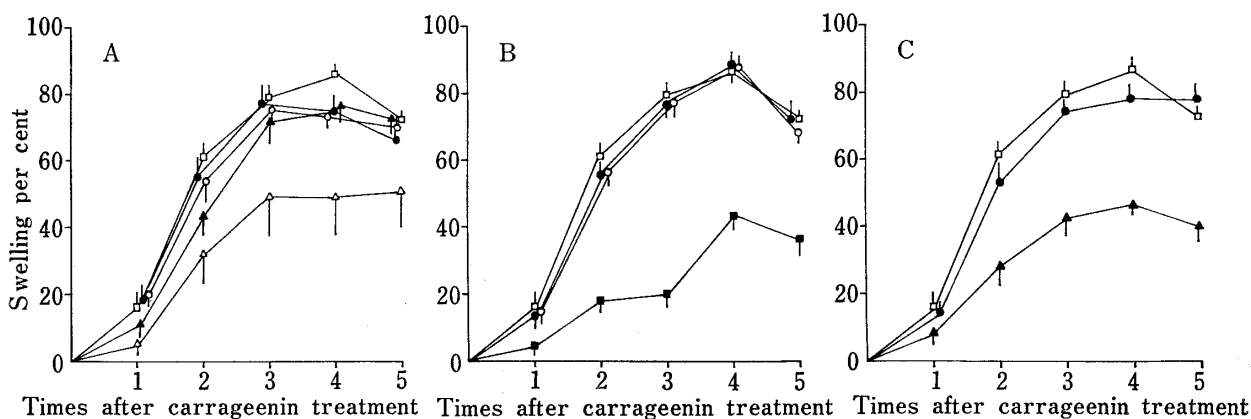


Fig. 2. Effects of Orally Administered Hederagenin, Crude Saponin, Oleanolic Acid, Saikogenin A, Platycodigenin, Crude Platycodin and Phenylbutazone on the Carrageenin-induced Edema in the Rat Hind Paw

Each point represents the mean value obtained from seven rats. Vertical bars represent standard errors of mean values.

- A) □ control (1% CMC), ● hederagenin 200 mg/kg *p.o.*, ○ hederagenin 400 mg/kg *p.o.*, ▲ crude saponin 200 mg/kg *p.o.*, △ crude saponin 400 mg/kg *p.o.*
- B) □ control (1% CMC), ● oleanolic acid 200 mg/kg *p.o.*, ○ saikogenin A 200 mg/kg *p.o.*, ■ phenylbutazone 200 mg/kg *p.o.*
- C) □ control (1% CMC), ● platycodigenin 200 mg/kg *p.o.*, ▲ crude platycodin 200 mg/kg *p.o.*

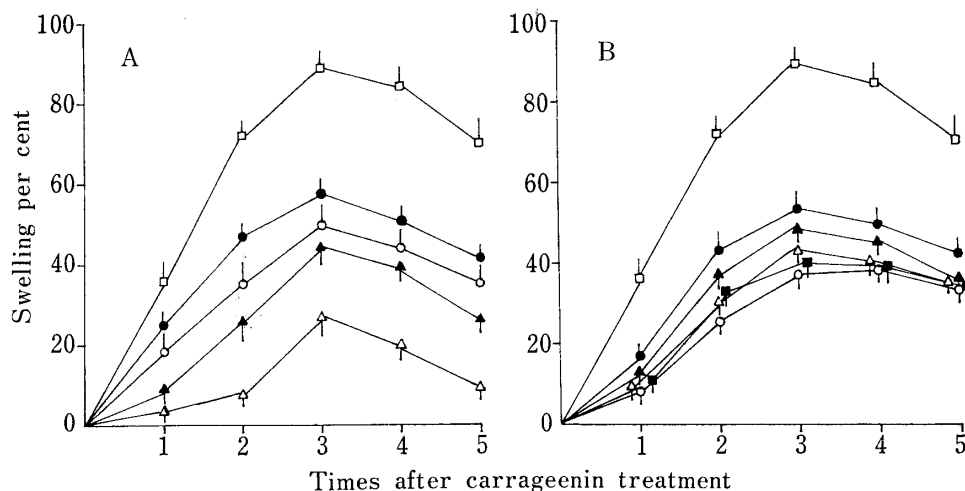


Fig. 3. Effects of Intraperitoneally Administered Hederagenin, Crude Saponin, Oleanolic Acid, Saikogenin A and Phenylbutazone on the Carrageenin-induced Edema in the Rat Hind Paw

Details are as in Fig. 2.

- A) □ control (1% CMC), ● hederagenin 100 mg/kg *i.p.*, ○ hederagenin 200 mg/kg *i.p.*, ▲ crude saponin 25 mg/kg *i.p.*, △ crude saponin 50 mg/kg *i.p.*
- B) □ control (1% CMC), ● oleanolic acid 100 mg/kg *i.p.*, ○ oleanolic acid 200 mg/kg *i.p.*, ▲ saikogenin A 100 mg/kg *i.p.*, △ saikogenin A 200 mg/kg *i.p.*, ■ phenylbutazone 100 mg/kg *i.p.*

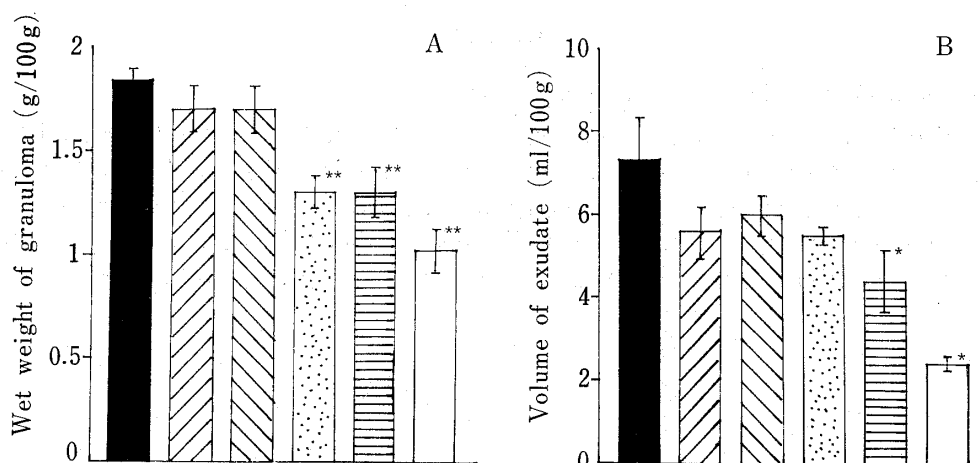


Fig. 4. Effects of 7-day Administrations of Hederagenin, Crude Saponin and Prednisolone on Granuloma Weight (A) and Exudate Formation (B) induced by Croton Oil in Rats

Each column represents the mean value obtained from seven rats. Vertical bars represent standard errors.

■ control (1% CMC),
 ▨ hederagenin 100 mg/kg/day p.o.,
 ▩ hederagenin 200 mg/kg/day p.o.,
 ▤ crude saponin 100 mg/kg/day p.o.,
 ▥ crude saponin 200 mg/kg/day p.o.,
 □ prednisolone 2.5 mg/kg/day p.o.,
 * $p < 0.05$
 ** $p < 0.01$

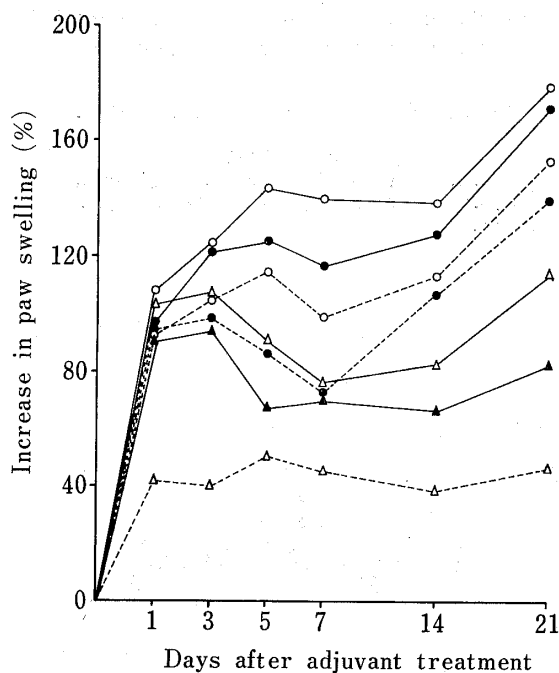


Fig. 5. Effects of 21-day Administrations of Hederagenin, Crude Saponin and Phenylbutazone on the Development of Hind Paw Edema associated with Adjuvant Arthritis in Rats

Each point represents the mean value obtained from seven rats.

○—○ control,
 ●—● hederagenin 50 mg/kg/day p.o.,
 ○—○ hederagenin 100 mg/kg/day p.o.,
 ●—● hederagenin 200 mg/kg/day p.o.,
 △—△ crude saponin 100 mg/kg/day p.o.,
 ▲—▲ crude saponin 200 mg/kg/day p.o.,
 △—△ phenylbutazone 30 mg/kg/day p.o.

After the *p.o.* administration, hederagenin, 200 and 400 mg/kg, and crude saponin, 200 mg/kg, as well as oleanolic acid, saikogenin A and platycodigenin, 200 mg/kg, caused no significant changes in the induced edema. Crude saponin, 400 mg/kg, crude platycodin, 200 mg/kg, and phenylbutazone, 200 mg/kg, significantly inhibited the development of the edema ($p < 0.05$).

In contrast, when administered *i.p.*, hederagenin, 100 and 200 mg/kg, and crude saponin 25 and 50 mg/kg, caused significant inhibitions of 36, 44, 51, and 71% on average 3 hr after the treatment with carrageenin, respectively. Oleanolic acid and saikogenin A, 100 and 200 mg/kg, and phenylbutazone, 200 mg/kg, also produced significant inhibitions of the edema ($p < 0.05$).

Granuloma Pouch in Rats

The inhibitory effects of hederagenin and crude saponin, 100 and 200 mg/kg, and prednisolone, 2.5 mg/kg *p.o.* per day for 7 days, on the granuloma and exudate formations in rats were investigated. The results are shown in Fig. 4.

Hederagenin, 100 and 200 mg/kg *p.o.*, produced a slight inhibition. Significant inhibitory effects of 28 and 40% with crude saponin, 200 mg/kg *p.o.*, on the weight of granuloma and the volume of exudate were

observed, respectively ($p < 0.05$). Prednisolone, 2.5 mg/kg *p.o.*, also significantly inhibited the granuloma and exudate formations by 44 and 67% on average, respectively ($p < 0.05$).

Adjuvant Arthritis in Rats

The inhibitory effects of 21-day administrations of hederagenin, 50, 100, and 200 mg/kg, crude saponin, 100 and 200 mg/kg, and phenylbutazone, 30 mg/kg *p.o.*, on the edema formation in the rat hind paw were determined. The results are illustrated in Fig. 5.

Hederagenin, 50–200 mg/kg *p.o.*, tended to inhibit the development of edema dose-dependently. Crude saponin, 200 mg/kg *p.o.*, significantly inhibited the edema by 51% on average on the 21st day ($p < 0.01$). The inhibitory effect was also observed with phenylbutazone, 30 mg/kg *p.o.*

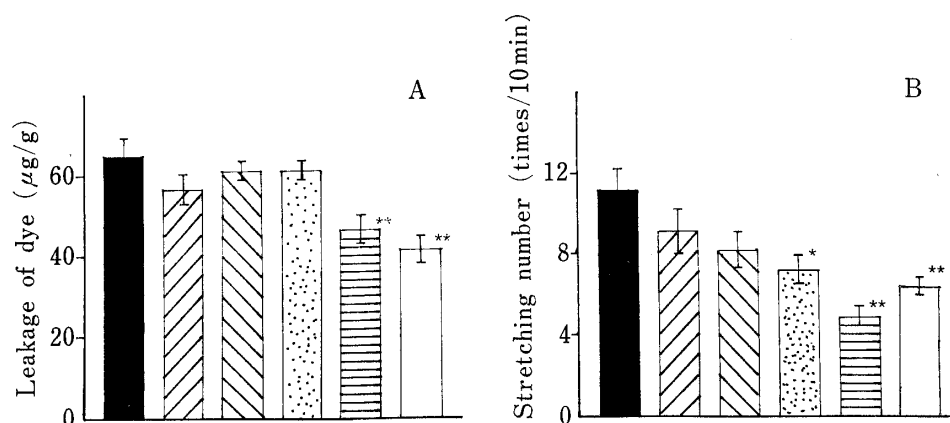


Fig. 6. Effects of Hederagenin, Crude Saponin and Phenylbutazone on Leakage of Dye into the Peritoneal Cavity (A) and Writhing (B) induced by Acetic Acid in Mice

Each column represents the mean value obtained from six mice. Other details are as in Fig. 4.

■ control (1% CMC),	□ phenylbutazone 100 mg/kg <i>p.o.</i> ,
▨ hederagenin 200 mg/kg <i>p.o.</i> ,	▧ hederagenin 400 mg/kg <i>p.o.</i> ,
▩ crude saponin 200 mg/kg <i>p.o.</i> ,	▦ crude saponin 400 mg/kg <i>p.o.</i>
	* $p < 0.05$
	** $p < 0.01$

Vascular Permeability and Acetic Acid-induced Writhing in Mice

The effects of hederagenin and crude saponin, 200 and 400 mg/kg *p.o.*, and phenylbutazone, 100 mg/kg *p.o.*, on increased capillary permeability and writhing induced by acetic acid in mice were investigated. The results are shown in Fig. 6. Crude saponin, 400 mg/kg *p.o.*, and phenylbutazone, 100 mg/kg *p.o.*, caused a significant inhibition of leakage the dye into the abdominal cavity and of the number of writhings induced by acetic acid in mice ($p < 0.01$); the average inhibitions were 27 and 35% for the vascular permeability and 57 and 43% on average for writhings, respectively. Crude saponin, 200 mg/kg *p.o.*, showed a similar effect on writhing, but not on vascular permeability. Hederagenin in the doses used failed to show a significant effect in these experiments.

Discussion

The present study demonstrated the anti-inflammatory effects of crude saponin isolated from *S. mukorossi* on carrageenin-induced edema, granuloma pouch and adjuvant arthritis. This agent also showed inhibitory activity on vascular permeability and analgesic effect. In contrast, orally administered hederagenin failed to show these activities in the doses used. The effects of hederagenin, saikogenin A isolated from *Bupleurum* root and oleanolic acid on

carrageenin-induced edema varied according to its route of administration; a significant effect was observed after *i.p.* administrations, but these agents showed no effect when given *p.o.* Crude saponin of *S. mukorossi* inhibited the development of the edema after not only *i.p.* but also *p.o.* administrations. Similar results were reported in the case of platycodigenin and crude platycodin, which were isolated from *Platycodon* root.⁸⁾ In other words, saponins may show anti-inflammatory effect on the edema after not only *i.p.* but also *p.o.* administrations, while sapogenins, such as platycodigenin, hederagenin and saikogenin A, may show such an effect only after *i.p.* administration.

It is well known that many saponins have a potent local irritant activity even when administered *p.o.* Therefore, it is likely that the activity of a saponin as a counter-irritant, which might be the mechanism of action for the anti-inflammatory effect, may be more potent than that of the sapogenin. It has been shown that the oleanane group of triterpenoids had anti-inflammatory activity.⁸⁾ The saponins and sapogenins used in the present study are oleanane triterpenoids; hederagenin and saikogenin A have a hydroxymethyl group, platycodigenin has two hydroxymethyl groups, and oleanolic acid has two methyl groups at C-4 in ring A. In spite of the variations in the chemical structures, these agents all showed some degree of anti-inflammatory activity, probably involving the same mechanism.

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