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Antiinflammatory Action of *Phytolacca* Saponin

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Abstract—The antiinflammatory activity of *Phytolacca* saponin and phytolaccagenin isolated from the roots of *Phytolacca americana* has been investigated. When *Phytolacca* saponin and phytolaccagenin were given parenterally exhibited potent inhibitory effects on acute edema in rats and in mice. While oral administration required 6 times more dosage in comparison with that of intraperitoneal injection. The anti-exudative and anti-granulomatous activities of the saponin showed as much as the dose of 8 times higher than that of hydrocortisone. The saponin appeared to be of no effects on adrenal, however, showed a severe thymolysis at high dose.

Phytolaccae radix has been used as an indigenous medicine against edema and rheumatism from ancient times. In the previous paper, separation and antiinflammatory effect of the saponin mixture obtained from the roots of *Phytolacca americana* were briefly described[1].

The present communication deals with some detailed works regarding antiinflammatory activity of the saponin. Phytolaccagenin which is a major component as aglycon, was also tested for its antiedemic property in order to understand a possible relationship in antiinflammatory activity between saponin and sapogenin.

EXPERIMENTAL

Materials—The saponin was isolated from methanol extract of the roots of *Phytolacca americana* and its procedure in detail was described in the previous paper[1].

The saponin consisted of 10 phytolaccosides, component E of which is largest in quantity. Phytolaccagenin was obtained by acid hydrolysis of the saponin. Glycyrrehetic acid and oleanolic acid were obtained from *Glycyrrhiza uralensis* and *Aralia elata*, respectively. Cortisone acetate (Tokyo Kasei Co.,) and hydrocortisone acetate (Pfizer Inc., N.Y., U.S.A.) were obtained commercially.

Acute toxicity—Albino mice of either sex weighing 17~23 g and male albino rats weighing 110~150 g were used. The saponin and phytolaccagenin were injected either intraperitoneally or orally, and the animals were observed for 4~5 hr for any apparent changes in the behavior due to the compounds and again 72 hr later to assess mortality. LD_{50} were computed by the method of Litchfield-Wilcoxon[2] and up and down method.

Carrageenin-induced edema test in rats—Edema in rats was induced by the method described by Winter and others[3].

A volume of 0.1 ml of 1% carrageenin in 0.9% NaCl was injected into the subplantar side of right hind paws of the rats. The test compounds suppended in 0.9% NaCl were injected intraperitoneally or subcutaneously 30 min or orally 1 hr before the injection of the irritant. The volume of the paw was measured plethysmometrically[4] immediately and 3 hr after the carrageenin treatment. Degree of edema was expressed as the rate of volume increased after carrageenin injection to the uninjected paw volume.

Carrageenin-induced edema test in mice—Edema in mice was induced by injecting 0.025 ml of 1% carrageenin solution into the subplantar region of the right hind paw, the other paw using as control[5]. The animals were killed with ether 4 hr later and the difference between the weights of the paws of treated and untreated animals was a measure of the suppresive action of the compounds tested.

Granuloma pouch method—Granuloma pouch was induced by the method of Selye[6] by using croton oil as a phlogistic agent. Twenty ml of air was injected sc into the dorsum of each rat under ether anesthesia.

Immediately after air injection, 1 ml of 1% croton oil in olive oil was injected into the pouch and administrations of test compounds were followed.

The test compounds, suspended in saline solution, were administered im into the gluteal muscles or directly into pouch daily for 7 days.

Control group received saline solution only. Net body weight was checked at the end of the experiments. On the 8 th day, the animals were killed by cutting carotid artery. The exudate in the pouch was collected by making a cut in the skin into a beaker and the volume was estimated.

The capsule of the granulomatous tissue was removed, and then its wet weight was measured. Both adrenals and thymus were dissected and weighed. Hydrocortisone acetate was used as a reference compound.

RESULTS

Acute toxicity—LD_{50} values determined by intraperitoneal administrations of the saponin were shown in Table I, and they were about 181 mg/kg for mice and 208 mg/kg for rats. At the lethal doses, the animals showed a stimulant action such as sudden runs and jumps during first several minutes, and a depression and subsequent death followed.

Some animals showed extensive uresis and catharsis. In contrast to saponin, there was no

mortality of mice in the group treated ip with phytolaccagenin upto a dose of 2 g/kg, indicating it to be physiologically innocuous. No mortality was also found when crude saponin was administered orally upto a dose of 1.5 g/kg.

Table I—Acute toxicities of the saponin and phytolaccagenin

Compounds	Species	Route	LD_{50}(mg/kg)
Saponin	Mouse	ip	181(175.7~186.4)[a]
	Mouse	po	1500<
	Rat	ip	208[b]
Phytolaccagenin	Mouse	ip	2000<

a) Litchfield-Wilcoxon method. Figures in parenthesis indicate the 95% confidential limits.
b) Up and down method. The animals were observed for 72 hr after dosing.

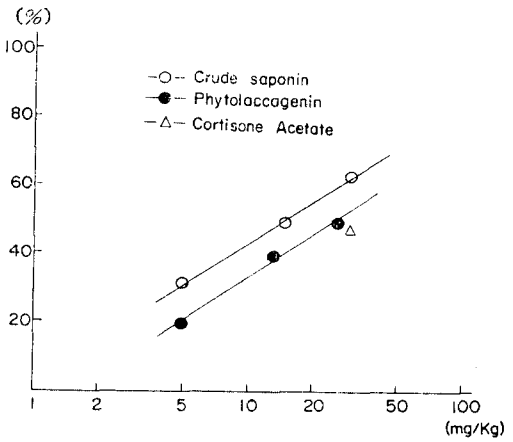

Fig. 1—Dose-response regression lines for inhibition by ip doses in carrageenin edema in rats.

Effects on carrageenin-induced edema—The dose-response curves for the inhibition on carrageenin-induced edema in rats by single ip doses of the saponin and phytolaccagenin were shown in Fig. 1.

The saponin exhibited highly potent inhibitory effects on carrageenin edema, its inhibiting edema by 50% (ED_{50}) being 15.1 mg/kg. The inhibitory potency of the saponin was stronger (15.3%) in comparison with that of cortisone acetate at the same dose level. Phytolaccagenin also showed significant inhibitory effect (ED_{50}, 26 mg/kg), however, was weaker than the saponin. Graded doses of the saponin in the range of 20 mg/kg to 80 mg/kg were injected subcutaneously and were compared with cortisone acetate in its inhibiting effect on the edema in mice (Table II).

The dose-response relationships between the saponin and cortisone acetate were not parallel, but their ED_{50} value calculated were 100.8 mg/kg and 90 mg/kg, respectively. These data indicated that the saponin was almost as active as cortisone.

When the saponin was treated orally, it required about 6 times more dosage than that of intraperitoneal injection to give rise a same inhibitory potency against carrageenin edema in rats (Table III).

Phytolaccagenin was again compared its potency with two other triterpenoids of β-amyrin series which are known to be effective in acute inflammation[7,8].

Table II—Effect of the saponin on carrageenin-induced edema in mice

Compounds	Dose (mg/kg, sc)	No. of mice	Edema (mg)±S.E.	Inhibition (%)	ED_{50} (mg/kg)
Control (saline)	—	10	56.7±4.4	—	
Saponin	80	10	30.3±4.6[a)]	46.6	
	40	10	41.2±5.5[c)]	27.3	100.8
	20	10	45.3±6.5	20.1	
Control (saline)	—	8	57.3±5.5	—	
Cortisone acetate	100	8	29.1±5.0[a)]	51.0	
	50	8	31.4±9.1[a)]	45.2	90.0
	25	8	36.4±5.0[b)]	36.5	

a) $P<0.01$ *vs.* control. b) $P<0.02$ *vs.* control. c) $P<0.05$ *vs.* control.

Table III—Effect of the saponin on carrageenin-induced edema in rats

Compounds	Dose (mg/kg, po)	No. of rats	Degree of edema	Inhibition (%)
Exp. 1				
Control	—	5	0.94±0.13	—
Saponin	400	5	0.31±0.17[a)]	67.0
	200	5	0.35±0.12[a)]	63.1
Cortisone acetate	30	5	0.47±0.13[b)]	50.4
Exp. 2				
Control	—	5	0.81±0.08	—
Saponin	30	5	0.75±0.06	7.4

a) $P<0.01$ *vs.* control. b) $P<0.05$ *vs.* control.
0.05 ml of 1% carrageenin solution in 0.9% NaCl was injected to induce edema.
The compounds were given orally 1hr before the injection of carrageenin solution.
Degrees of edema are shown as means±standard error.

As shown in Table IV, it showed more potent antiedemic activity than that of two other similar compounds.

Effect on the formation of granuloma pouch—Table V shows the results of the effect on the formation of the granuloma pouch by 7 consecutive daily im injections of the saponin. Even at the dose level of 6 times higher than that of hydrocortisone acetate, the saponin exhibited only weak inhibitory effect on both granuloma and exudate formation. At the dose of 10 mg/kg, hydrocortisone acetate showed significant inhibitory effect on both granuloma and exudate formation but accompanied a marked involution of adrenal wet weight. The saponin did not cause any effect on adrenal.

The dosage of the saponin was again increased upto 160 mg/kg to observe its effect on granuloma pouch formation (Table VI). The test compound was administered directly into

Table IV—Comparison of the inhibitory effects of genins in rat carrageenin edema test

Compounds	Dose (mg/kg, ip)	No. of Rats	Degree of Edema	Inhibition (%)
Control (saline)	—	6	1.17±0.10	—
Phytolaccagenin	30	6	0.63±0.10[a]	46.2
Oleanolic acid	30	6	0.76±0.09[b]	35.0
Glycyrrhetic acid	30	6	0.76±0.08[b]	35.0
Cortisone acetate	30	6	0.61±0.08[a]	47.9

a) $P<0.01$ *vs.* control. b) $P<0.02$ *vs.* control.
Degrees of edema are shown as mean±standard error.

Table V—Effects of the saponin on the formation of granuloma-pouch in rats.

Compounds	Dose (mg/kg, im)	No. of rats	Granuloma (g)	Exudate (g)	Adrenal (mg)
Control (saline)	—	5	3.94±0.29	9.4±2.6	49.4±3.8
Saponin	20	5	4.08±0.23	7.0±1.0	43.7±3.3
Saponin	60	5	3.46±0.32	7.8±2.0	50.5±3.2
Hydrocortisone acetate	2	5	3.96±0.19	11.8±4.3	38.2±3.8
Hydrocortisone acetate	10	5	2.26±0.20[b] (43.0)	3.0±0.4[c] (68.1)	19.8±1.4[a] (59.9)

Figures in parenthesis represent % inhibition.
a) $P<0.001$ *vs.* control. b) $P<0.01$ *vs.* control. c) $P<0.05$ *vs.* control.
Data are shown as means±standard error.

Table VI—Effects of the saponin on the formation of granuloma pouch in rats

Compounds	Dose (mg/kg,) intrapouch	No. of rats	Net body wt. (g)	Exudate (g)	Granuloma (g)	Adrenal (mg)	Thymus (mg)
Control (saline)	—	6	136.5±7.9	9.2±3.1	3.9±0.3	44.7±2.1	144.7±24.2
Saponin	160	4	118.0±1.5[c] (13.6)	1.0±0.7[b] (89.1)	0	53.0±2.9	28.0±10.2[a] (80.6)
Saponin	80	6	131.8±5.4	2.6±0.9[c] (71.7)	2.0±0.5[a] (48.7)	53.6±2.6	93.3±17.2

a) $P<0.01$ *vs.* control b) $P<0.05$ *vs.* control. c) $P<0.1$ *vs.* control.
Figures in parenthesis represent % inhibition.
Data are shown as means±standard error.

pouch since its injecting volume was too high. The volume of exudate in the pouch was 10.9% of control by 160 mg/kg and 41.3% by 80 mg/kg. The wet weight of granuloma of the group treated with 160 mg/kg was dramatically involuted and was difficult to assess quantitatively. The group treated with 80 mg/kg showed 48.7% inhibition to control group. Adrenal weight in both groups were rather a little increased compared to control group. But severe thymolysis and the body weight loss occured in the group of 160 mg/kg treatment.

DISCUSSION

It was demonstrated in the present study that a single parenteral administration of *Phytolacca* saponin exhibited strong inhibitory effects on carrageenin edema, which is a typical model for an acute inflammation (Fig. 1, Table II). The saponin mixture isolated from the roots of *Phytolacca americana* consists of ten components, of which phytolaccoside E is the major saponin. The genin of phytolaccoside E is phytolaccagenin[1].

The fact that antiedematous acivity of the saponin (ED_{50}=15.1 mg/kg) is twice than that of phytolaccagenin (ED_{50}=26.0 mg/kg) suggests that sugar moieties of saponin could give rise an enhancement of antiinflammatory action of triterpenoid. In case of oral administration, however, the inhibitory effect of the saponin appeared at relatively high doses in comparison with parenteral administration (Table III). As the data were shown in Table I, there was no mortality upto a dose of 1.5 g/kg when the saponin was treated orally. Such low toxicity and relatively high effective doses of the saponin by oral route, indicates its poor absorptivity in gastro-intestinal tracts. Above phenomenon could also be encountered when treated with escin[9], saicosaponin[10] and other saponins of various sources[11]. Jaligonic acid, free acid form of phytolaccagenin, has been demonstrated to possess a strong inhibitory action comparable to hydrocortisone on carrageenin edema in rats, when given by a single oral administration[12]. This indicates that the triterpenoid can be freely absorbed from the gastrointestinal tracts.

It has been known that the triterpenoids of the oleanane series possess antiinflammatory activity[7,9]. Phytolaccagenin was found to be more potent compared to oleanolic acid and glycyrrhetic acid (Table IV). This supports the hypothesis that the antiinflammatory activity increases with the polarity of the compounds[13].

The potency of the saponin was higher than cortisone in the carrageenin edema test but it was only one eighth as active in the chronic test (Fig. 1, Table VI). This result is similar to other saponin activity[10].

Repeated administrations at moderate doses of the saponin gave no effect on the weights of body, adrenal and thymus. Such results indicate that the antiinflammatory effect of the saponin is specific and not related to its toxicity or peritoneal irritation[13]. But it exhibited severe thymolytic effects at highest dose (160 mg/kg) in the present study.

On the basis of this work, it appears that *Phytolacca* saponin is more effective antiedemic remedy than an agent capable of inhibiting pathological connective tissue behavior. The exact mechanism of antiinflammatory activity of the saponin remains to be elucidated further.

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