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Effects of Crude Platycodin on Gastric Secretion and Experimental Ulcerations in Rats

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Effects of crude platycodin on gastric secretion and experimental ulcerations were studied in the rat. Crude platycodin markedly inhibited gastric secretion and prevented peptic ulcers in the pylorus ligated rat but had a rather slight effect on stress induced ulceration. Curative effect of crude platycodin (25 mg/kg/day) on acetic acid induced ulceration was also observed.

Platycodi radix (*Platycodon grandiflorum* A. DC.) has been prescribed frequently for cough and bronchitis as an expectorant in oriental medicine. Recently, pharmacological studies of crude platycodin, saponin fraction of platycodi radix extract, have been carried out in our laboratory.[2] Crude platycodin has been found to have remarkable anti-inflammatory, analgesic and anti-pyretic activities as well as anti-tussive activity.

Many anti-inflammatory drugs, whether they are steroidal or nonsteroidal, have been reported to cause various gastrointestinal disturbances.[3] It is suggested that crude platycodin may have not only anti-inflammatory, but also anti-peptic-ulcer activities, because platycodi radix has also been prescribed in combination with other crude drugs for gastrointestinal disorders such as peptic ulcer and gastritis in oriental medicine.

In the present study, effects of crude platycodin on gastric secretion and experimental ulcerations were investigated in the rat.

Experimental

Crude platycodin, a dry brownish powder, mainly contains saponins. The preparation of crude platycodin was reported by Akiyama, *et al.*[4]

FM 100 is a fraction from licorice root (*Glycyrrhiza glabra*) and is a dry brownish powder. General pharmacological activities of FM 100 was reported by Takagi and Ishii.[5]

Atropine sulfate was supplied by Merck.

1) Gastric secretion in pylorus ligated rats: Male Donryu rats weighing 160 to 200 g were fasted overnight but allowed free access to water before surgery. The operative procedure of this technique was identical to the method described by Shay, *et al.*[6] Crude platycodin dissolved in tap water was administered intraduodenally, and atropine sulfate dissolved in saline was administered subcutaneously immediately after the pyloric ligation. Control animals were given tap water intraduodenally. After 4 hours, animals were sacrificed and the stomach was removed. The gastric contents were emptied into calibrated centrifuge tubes and centrifuged at 3000 rpm for 15 minutes and the volume was measured. The free and total acidity were determined by the titration with 0.1 N NaOH using Töpfer reagent and phenolphthalein as indicators. The pH was determined by using glass electrode pH meter. The pepsin was determined by the method of Bock[7] using bovine serum albumin as a substrate.

1) Location: *Bunkyo-ku, Tokyo.*
2) K. Takagi and E.B. Lee, *Yakugaku Zasshi*, in preparation.
3) K.W. Anderson, "Non-steroidal anti-inflammatory drugs," Excerpta Medica Foundation, Amsterdam, 1964, p. 245.
4) T. Akiyama, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), in preparation.
5) K. Takagi and Y. Ishii, *Arzneim. -Forsch.*, **17**, 1544 (1967).
6) H. Shay, S.A. Komarov, S.S. Fels, D. Merance, M. Gruenstein, and H. Siplet, *Gastroenterology*, **5**, 43 (1945).
7) J. Bock, *Scand. J. Clin. Lab. Invest.*, **6**, 237 (1954).

2) Shay Ulceration: Male Donryu rats weighing 160 to 200 g were fasted for 48 hours before use, but were allowed free access to water. Surgical procedure were carried out according to the routine method mentioned above. Drugs were administered intraduodenally or subcutaneously at the pyloric ligation. After 16 hours, animals were sacrificed and the stomachs were removed. The volume, acidity, pH, and pepsin were determined as mentioned above. The stomachs were opened along the greater curvature and were macroscopically examined. The severity of the gastric ulcer was scored according to the total area of lesions and expressed as an "Ulcer Index." And the perforated stomach was scored 50 in total.[5]

3) Stress induced Ulceration: Male Donryu rats weighing 230 to 250 g were placed in a stress cage and immersed into a water bath (23°) for 20 hours to the level of xiphoid process according to the method reported by Takagi and Okabe.[8] Drugs were administered orally or subcutaneously 30 minutes before the stress. Control animals received tap water orally. At the end of the stress, the animal was killed by a blow on the head, the stomach was removed, inflated with 1% formalin solution and placed into the same solution for 5 minutes. Then, the stomach was cut open along the greater curvature and examined grossly for lesions in the glandular portion. The "Ulcer Index" was calculated as the sum of the area of each lesion in the stomach. Preventive ratio is calculated as follows;

$$\text{preventive ratio (\%)} = \frac{\text{ulcer index (control)} - \text{ulcer index (drug)}}{\text{ulcer index (control)}} \times 100$$

4) Acetic Acid induced Ulceration: Male Donryu rats weighing 230 to 250 g at the time of operation were used in this experiment. Surgical procedure was performed according to the method of Takagi, *et al.*[9] Under ether anesthesia laparotomy was performed through a midline epigastric incision. After exposing the stomach, 0.05 ml of 10% acetic acid was injected into subserosal layer in the glandular part of the anterior wall, then abdomen was closed. Animals were fed normally and received drugs once a day orally for 10 days from the second day after the operation. Crude platycodin and FM 100 were dissolved in water and the solutions were adjusted to the neutral pH with diluted NaOH. Animals were sacrificed on the 12th day after the operation. Stomachs were removed and treated as described at the stress induced ulceration. The ulcers produced were oval or round in shape, the length and width were measured and the product was used as the terms of "Ulcer Index." The calculation method of curative ratio is as follows;

$$\text{curative ratio (\%)} = \frac{\text{ulcer index (control)} - \text{ulcer index (drug)}}{\text{ulcer index (control)}} \times 100$$

Result

1) Gastric Secretion in Pylorus Ligated Rats

Effects of atropine sulfate and crude platycodin on gastric secretion are shown in Table I. Atropine sulfate (10 mg/kg *s.c.*) strikingly reduced all the parameters of gastric secretion. Crude platycodin also significantly inhibited gastric secretion. In rats treated with 50 mg/kg of crude platycodin, the volume of gastric juice, acid output and pepsin activity were reduced by about 50% compared with control. Crude platycodin in a dose of 100 mg/kg almost completely inhibited gastric secretion, and gastric samples could be obtained only from 3 and 5 animals out of 10 animals used.

2) Shay Ulceration

Results are summarized in Table IIa and IIb. Atropine sulfate and crude platycodin significantly prevented Shay ulceration. Crude platycodin in a dose of 25 mg/kg *i.d.* was as effective as atropine sulfate (10 mg/kg *s.c.*). On account of perforation, gastric samples from control animals could be obtained only from 2 animals out of 12. No significant differences in the gastric secretion were observed among rats treated with atropine sulfate (10 mg/kg *s.c.*) and crude platycodin (25 mg/kg and 50 mg/kg *i.d.*). In rats treated with 100 mg/kg of crude platycodin, Shay ulceration was almost completely prevented, and all the parameters of gastric secretion were significantly reduced.

8) K. Takagi and S. Okabe, *Japan. J. Pharmac.*, **18**, 90 (1968).
9) K. Takagi, S. Okabe, and R. Saziki. *Japan. J. Pharmac.*, **19**, 418 (1969).

TABLE I. Effect of Crude Platycodin on Gastric Secretin in Pylorus Ligated Rats

Treatment	Dose mg/kg	No. of animals	pH	Volume ml/4 hr	Total acidity mEq/liter	Acid output μEq/4 hr	Pepsin[a] mg tyrosine/4 hr
Control (*i.d.*)	—	12	1.6	4.7±0.6	96.7±6.7	467.6±73.3	83.1±10.4
Atropine sulfate (*s.c.*)	10	12	2.9	0.6±0.1[b]	4.8±0.5[b]	3.0± 1.4[b]	11.5± 5.3[b]
Crude platycodin (*i.d.*)	25	12	1.7	3.5±0.3	83.3±5.1	305.0±49.4	62.7± 9.6
	50	12	1.8	2.1±0.3[b]	89.0±5.6	194.4±36.9[b]	41.9± 6.4[b]
	100	10	3.9	0.4±0.2[b]	6.2±3.0 (3)[c]	5.6± 2.5 (3)[c]	20.7± 7.3 (5)[c]

Values are mean±S.E.
a) Amount of tyrosine, released per 30 min of incubation of bovine serum albumin with the gastric juice collected during 4 hr of pyloric ligation.
b) statistically significant at $P=0.01$
c) Number of samples obtained.

TABLE IIa. Effect of Crude Platycondin on Gastric Ulceration in Pylorus Ligated Rats

Treatment	Dose (mg/kg)	No. of animals	Incidence of perforation(%)	Ulcer index (mean±S.E.)	% inhibition
Control (*i.d.*)	—	12	83.3	47.8±2.3	—
Atropine sulfate (*s.c.*)	10	12	8.3	14.9±5.4[a]	68.9
Crude platycodin (*i.d.*)	25	12	8.3	16.2±5.8[a]	66.4
	50	12	8.3	12.3±4.6[a]	74.3
	100	12	0	0.8±0.8[a]	98.4

a) statistically significant at $P=0.01$

TABLE IIb. Effect of Crude Platycodin on Gastric Secretion in Pylorus Ligated Rats

Treatment	Dose (mg/kg)	No. of animals	pH	Volume ml/16hr	Total acidity Eq/liter	Acid output μEq/16 hr	Pepsin[a] mg Tyrosine/16hr
Condtrol (*i.d.*)	—	2[b]	1.0	11.2	78.5	879.2	265.7
Atropine sulfate (*s.c.*)	10	11	0.9	9.6±0.7	109.1±8.8	1044.1±98.6	201.4±22.0
Crude platycodin (*i.d.*)	25	11	1.1	9.6±0.9	77.9±5.6	743.9±62.0	211.7±18.4
	50	11	1.0	9.4±0.8	94.1±0.8	880.8±91.3	203.1±16.8
	100	12	1.8	4.3±0.7	93.6±4.8	401.8±88.5	92.4±14.1

a) Amount of tyrosine, released per 30 min of incubation of bovine serum albumin with the gastric juice collected during 16 hr of pyloric ligation.
b) Control samples were obtained only from two animals, so standard errors were not calculated.

TABLE III. Effect of Crude Platycodin on the Stress Induced Ulceration

Treatment	Dose (mg/kg)	No. of animals	Incidence of ulceration(%)	Ulcer index (mean±S.E.)	% inhibition
Control (*p.o*)	—	11	100	30.5±3.7	—
Atropine sulfate (*s.c*)	10	12	50	6.7±2.2[a]	78.2
Crude platycodin (*p.o*)	25	5	100	31.0±4.0	−1.6
	50	10	100	28.4±3.6	6.4
	100	11	100	19.2±3.1[a]	37.0

a) statistically significant at $P=0.05$

3) Stress induced Ulceration

The results of effects of atropine sulfate and crude platycodin on stress induced ulceration are presented in Table III. Atropine sulfate significantly reduced the severity of the lesion induced by the stress. Crude platycodin in a dose of 100 mg/kg significantly prevented stress induced ulceration, but in doses of 25 and 50 mg/kg it had no effect. The reduction of Ulcer Index by crude platycodin (100 mg/kg *p.o.*) was as half as that by atropine sulfate (10 mg/kg *s. c.*).

4) Acetic Acid induced Ulceration

Effects of FM 100 and crude platycodin on the acetic acid induced ulcer are presented in Table IV. FM 100 (200 mg/kg) was hightly effective on the healing of acetic acid induced ulceration and its curative ratio was 41%. The curative ratios of crude platycodin in doses of 25 and 50 mg/kg were 46 and 20%, respectively. The higher dose of crude platycodin (50 mg/kg) was less effective than the lower dose (25 mg/kg). The synergistic effect of crude platycodin (25 mg/kg) with FM 100 (200 mg/kg) on the ulcer healing was not observed.

TABLE IV. Effect of Crude Platycondin on Acetic Acid induced Ulceration

Treatment (*p.o.*)	Dose (mg/kg)	No. of animals	Ulcer index (mean±S.E.)	Curative ratio (%)
Control	—	12	19.5±3.1	—
FM 100	200	11	11.4±1.9[a)]	41
Crude platycodin	25	11	10.5±1.9[a)]	46
	50	11	15.7±3.0	20
FM 100 + Crude platycodin	100 / 25	9	14.6±3.4	25

a) statistically significant at $P=0.05$

Discussion

This study demonstrates that crude platycodin has not only inhibitory effect on gastric secretion, but also preventive and curative effects on the experimental gastric ulcerations.

Prevention of Shay ulceration by crude platycodin may be due to the inhibition of gastric secretion and an anti-inflammatory action which was observed in the rat paw edema.[2)] Although crude platycodin was found to cause mydriasis in mice and antagonize against acetylcholine and histamine induced contraction *in vitro* study on the guinea pig ileum,[2)] its anti-cholinergic activity was very weak and less than 1/500 that of atropine. So it is difficult to suppose that crude platycodin inhibited gastric secretion through its atropine-like property at the dose level used in this study.

In the case of stress induced ulceration, crude platycodin was not so effective as in the case of Shay ulceration, and only at high dose (100 mg/kg *p.o.*) significantly reduced the severity of the lesion. There were no direct relationships between the degree of inhibition of gastric secretion and the reduction of severity of the stress induced ulceration by crude platycodin.

Crude platycodin in a dose of 25 mg/kg was significantly effective on the healing of the acetic acid ulcer, but in a dose of 50 mg/kg less effective. The similar phenomenon was observed in the case of FM 100 in which lower dose of FM 100 (400 mg/kg) was more effective than higher dose (800 mg/kg) (unpublished data). The reason of these is not known.

The combined effect of crude platycodin with FM 100 was investigated in this experiment, because in oriental medicine, both platycodi radix and licorice root are most frequently used in combination. But no synergistic effect was observed.