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Gastroprotective Activity of the Unripe Fruit Extract of *Juglans mandshurica* in Rats

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Abstract – The unripe fruit extract of *Juglans mandshurica* showed significant inhibition on aspirin and indomethacin induced gastric lesion at an oral dose of 1000 mg/kg. The extract did not show inhibition of aspirin induced ulcer and Shay ulcer at the doses of 500 and 1000 mg/kg. The results indicate that the extract had a gastroprotective activity.
Key words – *Juglans mandshurica*, Juglandaceae, unripe fruit, gastroprotection, rats

Introduction

The unripe fruit of *Juglans mandshurica* Maximowicz (Juglandaceae) has been used as a folk medicine for treatment of convulsive stomach pain, gastritis, gastric ulcer and duodenal ulcer in Asian countries (Jiansu New College Medicine, Eds, 1977), and used for tonic and anti-inflammatory astringent agent (Yook, 1981). Epidermis of unripe fruit of *J. mandshurica* contains hydrojuglone and leaf contains kaempferol (Yook, 1981). Three new glycosides (Lee *et al.*, 2000) and other three new glycosides that have cytotoxic property (Kim *et al.*, 1998) in the roots were reported. It was reported that the constituents of *J. mandshurica* stem bark inhibited human immunodeficiency virus type 1 reverse transcriptase and ribonuclease H activities (Min *et al.*, 2000). This study deals with the effectiveness of unripe fruit of *J. mandshurica* on gastric lesion and ulcer models in rats.

Materials and Methods

Plant material and chemicals – The unripe fruits of *Juglans mandshurica* were collected from the mountain around Seoul, Korea and directly chopped up. The extract (JM-f) was made by reflux in 95% MeOH three times for 4 hr at 70°C in a water bath. Ranitidine (Union Quimico Farmaceutica S.A., Spain) and indomethacin (Sigma Chemical Co.) were used. Other drugs used were of either pharmaceutical or reagent grade.

Animals – Male Sprague-Dawley rats were supplied from breeding facilities of Natural Products Research Institute, Seoul National University. Solid food (Samyang Yuji Co. Ltd.) and tap water were provided *ad libitum*. All animals were housed for 1 week in a controlled 12 hr light-dark environment at 22±1°C. The extract was suspended in 0.5% CMC in distilled water and a volume of 0.5 ml/100g (b.w.) was administered. Control group received only 0.5% CMC solution.

HCl·aspirin induced gastric lesion – According to the method of Guth *et al.* (1979), rats (180-200 g) were fasted for 24 hr with free access to water before experiment. The animals were given orally 150mM HCl with aspirin 200 mg/kg suspended in 5% gum acacia in a volume of 0.5 ml/100 g. One hr later, the animals were sacrificed with ether, and each stomach was excised. Then, 12 ml of 2% formalin was infused into the stomach and the stomach was soaked in 2% formalin for 10 min. The stomach was incised along the greater curvature and examined the presence of hemorrhage in the glandular portion. The length (mm) of each lesion was measured under the dissecting microscope (10×), and total value was expressed as a lesion index. JM-f was given orally 30 min prior to administration of HCl·aspirin solution.

Indomethacin induced gastric lesion – According to the method of Suzuki *et al.* (2000), rats (180-200

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g) were fasted for 24 hr with free access to water before experiment. The extract was orally given and 30 min later indomethacin 35 mg/kg suspended in 0.5% CMC was injected subcutaneously. The animals were sacrificed 7 hr after indomethacin injection and the excised stomach was treated as described above and the area (mm^2) of hemorrhagic lesion developed in the corpus mucosa was measured.

Aspirin induced gastric ulcer – According to the method of Okabe *et al.* (1974), rats (200-220 g) were fasted for 24 hr with free access to water before experiment. The pylorus of each animal was ligated under ether anesthesia. The extract was given intragastrically after pylorus-ligation. Ten min later, aspirin 150 mg/kg suspended in 0.5% CMC was administered orally in a volume of 0.5 ml/100 g. Seven hr after aspirin treatment, the animals were sacrificed and each excised stomach was treated as described above and glandular portion was examined to evaluate the ulcers.

Shay ulcer – Rats (210-230 g) were fasted for 36 hr with free access to water prior to the experiment. The pylorus of each animal was ligated under ether anesthesia as described by Shay *et al.* (1945). The extract was given intraduodenally immediately after pylorus-ligation. Fifteen hr later, the animals were sacrificed and the excised stomach was treated as described above and examined for gastric ulcers in the forestomach. The area of each ulcer was measured and summed (Takagi *et al.*, 1974). The ulcer index was graded from 1 to 5 according to the size of the area; i. e., 1-10 mm^2 as 1, 11-20 mm^2 as 2, 21-30 mm^2 as 3, 31-40 mm^2 as 4, 41-50 mm^2 as 5 or perforated cases as 5.

Statistical analysis – All data represent means±S.E. Statistical analyses of the data were performed using analysis of variance followed by Student's *t*-test. All data were evaluated at the $p<0.05$ level of significance.

Results

HCl·aspirin induced gastric lesion – As shown in Fig. 1, JM-f at a dose of 1000 mg/kg p.o. showed significant inhibition by 44.3%. Ranitidine at 150 mg/kg showed 69.1% inhibition.

Indomethacin induced gastric lesion – The effect of JM-f on indomethacin induced gastric lesion was represented in Fig. 2. It showed significant inhibition at a dose of 1000 mg/kg p.o. by 28.1%. Ranitidine at 150 mg/kg showed 88.4% inhibition.

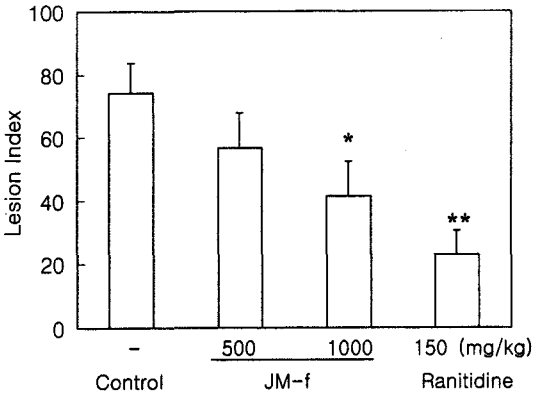

Fig. 1. Effect of JM-f on HCl·aspirin induced gastric lesion. *p<0.01, **p<0.001; Significantly different from the control group (n=8).

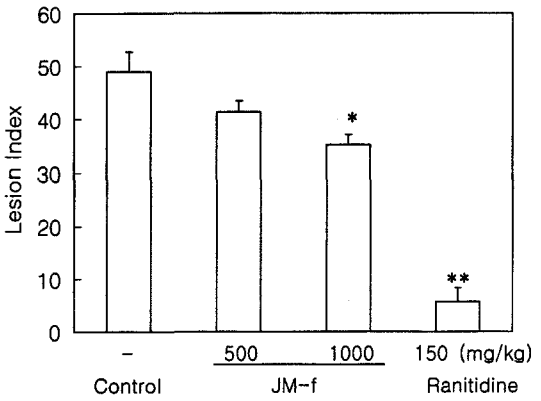

Fig. 2. Effect of JM-f on indomethacin induced gastric lesion. *p<0.01, **p<0.001; Significantly different from the control group (n=8).

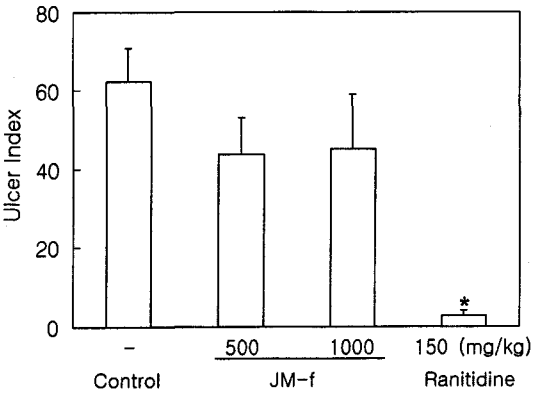

Fig. 3. Effect of JM-f on aspirin induced gastric ulcer. *p<0.01; Significantly different from the control group (n=8).

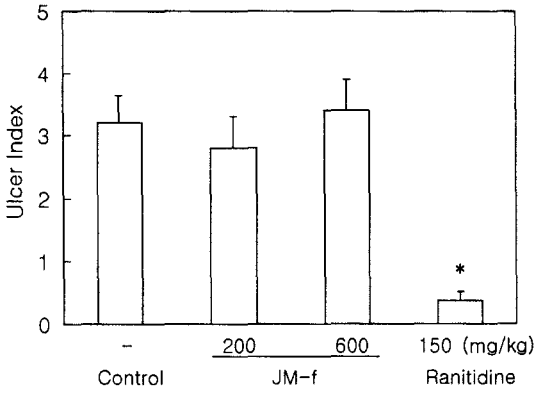

Fig. 4. Effect of JM-f on Shay ulcer. *p<0.01; Significantly different from the control group (n=8).

Aspirin induced gastric ulcer – As shown in Fig. 3, JM-f at the intragastric doses of 500 and 1000 mg/kg did not show inhibition of the ulcer. Ranitidine at 150 mg/kg showed 95.5% inhibition of the ulcer.

Shay ulcer – As shown in Fig. 4, JM-f at the intraduodenal doses of 500 and 1000 mg/kg did not affect the ulcer. Ranitidine at 150 mg/kg showed 87.3% inhibition of the ulcer.

Discussion

Effects of JM-f on two models of gastric lesion and two models of gastric ulcer in rats were investigated. JM-f showed significant inhibition on HCl·aspirin and indomethacin induced gastric lesion. The effectiveness of JM-f on these gastric lesions might be related to direct protection of mucosal damages by chemical inducers, indicating cytoprotective activity. On the pathogenesis of lesion induced by indomethacin, Takeuchi *et al.* (1986) reported that an increase of gastric motility in rats may play a role. However, the cause of effectiveness is unknown at present except cytoprotective effect.

JM-f did not affect aspirin induced and Shay ulcer. It is indicated that the negative anti-ulcer activity of JM-f might be related to no effects on gastric acid secretion. In conclusion, our results indicate that JM-f possesses pronounced inhibitory action on gastric lesion, but does not show anti-ulcer activity in rats.

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