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Studies on the Pharmacological Actions of Cactus: Identification of Its Anti-inflammatory Effect

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The ethanol extracts of *Opuntia ficus-indica* fructus (EEOF) and *Opuntia ficus-indica* stem (EEOS) were prepared and used to evaluate the pharmacological effects of cactus. Both the extracts inhibited the writhing syndrome induced by acetic acid, indicating that they contains analgesic effect. The oral administrations of EEOF and EEOS suppressed carrageenan-induced rat paw edema and also showed potent inhibition in the leukocyte migration of CMC-pouch model in rats. Moreover, the extracts suppressed the release of β-glucuronidase, a lysosomal enzyme in rat neutrophils. It was also noted that the extracts showed the protective effect on gastric mucosal layers. From the results it is suggested that the cactus extracts contain anti-inflammatory action having protective effect against gastric lesions.

Key words : Cactus, Acute toxicity, Analgesic effects, Rat carrageenan edema, Leucocyte migration inhibition, β-glucuronidase activity, Gastric lesions

INTRODUCTION

Cactus (*Opuntia ficus-indica* var. *saboten makino*) is a tropical or subtropical plant which belongs to the a *Cactaceae* family. It has been widely used as 'folk medicine' for burned wound, edema, and indigestion. It contains a complex composition including anhalinin, mescaline, indicaxathin, isobetain, betain, saponin, galactin and alkaloid and so on (Ghansah, *et al.*, 1993).

An extract of the cactus plant has been reported to inhibit virus replication and inactivate extracellular virus (Ahmad *et al.*, 1996). Cactus (*Opuntia* sp.) has been traditionally used by Mexican population for the treatment of diabetes mellius. The cactus extract was found to have hypoglycemic properties when orally administered, in animals with hyperglycemia (Ibanez-Camacho and Roman-Ramos, 1979). The hypoglycemic activity of purified extract from prickly pear cactus (*Opuntia* sp.) was evaluated on streptozocin-induced diabetic rats (Trejo-Gonzalez *et al.*, 1996). Blood glucose levels were reduced to normal values by a combined treatment of insulin and cactus extract. When insulin was withdrawn from the combined treatment, the extract alone maintained normoglycemic state in the diabetic rats. Any other pharmacological actions of cactus have not been reported.

This paper deals with the pharmacological effects of the cactus extracts collected in Korea.

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MATERIALS AND METHODS

Materials

Cacti (*Opuntia ficus-indica*) and their seeds were purchased from a private farm at Hallym area, Chejudo. Heparin, Folin-Ciocalteau's phenol reagent, bovine serum albumin (BSA), triton X-100, glycogen, phenolphthalein glucuronic acid, cytochalasin B, and N-formyl-methyl-leucyl-phenyl alanine were obtained from Sigma Chemical Co. (St.Louis, USA). Carrageenan, croton oil and acetic acid were purchased from Yakuri Pure Chemical Co. (Osaka, Japan). Phenylbutazone was obtained from Heungsung Chemical Co. (Seoul, Korea). Aspirin was from Jansen Pharm. Co.(Japan). Sucralfate was purchased from Jungwye Pharm. Co. (Seoul, Korea).

Animals

Male ICR mice and female Sprague-Dawley rats, which had been commercially purchased, 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*.

Preparation of extract

Cacti or their seeds were boiled with 3-fold volume of absolute ethanol in water bath for 4 hours, which was repeated three times. The extracts of *Opuntia ficus-*

indica fructus (EEOF) and *Opuntia ficus-indica* stem (EEOS) were filtered through Whatman #4 paper and evaporated to dryness under vacuum. Yields of preparation of *Opuntia ficus-indica* fructus (EEOF) and *Opuntia ficus-indica* stem (EEOS) extracts were 12% and 9.5%, respectively, based on the original weights used. The dried residual powder was kept in -20°C to minimize bacterial contamination. They were dispersed in saline just before use.

Acute toxicity test

Male ICR mice, weighing 25±2 g, were used to determine LD_{50} values. Both the extracts were administered to mice orally or intraperitoneally. Mortality was recorded for 1 week after administration.

Analgesic test

Analgesic effects of test materials were evaluated by the ability of mice to inhibit abdominal writhing syndrome induced by 1% acetic acid (0.1 ml/10 g body weight) according to the procedure of Koster *et al.* (1959). Writhing was defined as a characteristic contraction of abdominal muscles accompanied by extension of the hind limbs. The number of writhing were counted immediately after intraperitoneal injection.

Carrageenan-induced edema in the rat hind paw

Edema on the right hind paw of the animals was induced by subcutaneous injection of carrageenan (0.1 ml/rat) prepared as 1% suspension in saline (Winter *et al.*, 1962). The volume of the foot was determined every hour for 5 hrs, and the increase in 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, Italy). The agents tested were orally administered 60 min before the carrageenan treatment. The inhibition of edema induced by each agent was calculated with respect to its vehicle-treated control group.

Inhibition of leukocyte migration

Inhibition of leucocyte migration was evaluated as described previously (Sedgwick *et al.*, 1983). Ten ml air was injected subcutaneously on the dorsal of rats. After 24 hrs, 5 ml of 2% (w/v) CMC solution dissolved in 0.9% NaCl was injected into the air sac. For the measurement of leucocyte number and protein concentration in the CMC fluid, 1 ml of heparinized saline was injected into the pouch to wash and exudated fluid was taken out at 6 hrs after injection of CMC. After shaking the fluid taken for a few minutes to obtain a homogeneous solution, 0.05 ml of pouch fluid was mixed with 1 ml of Türk solution for staining leucocytes and the number of leucocyte was counted with haemocytometer. Its protein concentration which represented the vascular permeability was measured by Lowry's method (Lowry *et al.*, 1951).

Inhibition of β-glucuronidase release

Preparation of neutrophils: According to the procedure of Cohn and Hirsh (1950), 0.2% glycogen was intraperitoneally injected to rats. After 20 hrs later, rats were sacrificed and the peritoneal cavity was washed with 20 ml saline containing heparin. Each exudate was centrifuged at 1,000 rpm for 10 min and neutrophil pellet was resuspended in phosphate-buffered saline(PBS) containing 1.2 mM of calcium.

Measurement of β-glucuronidase: β-Glucuronidase activity was measured spectrophotometrically, according to Brittinger *et al.* (1968). Aliquots of neutrophil suspension (5×10 cells in 0.5 ml PBS) was preincubated with cytohalasin B (5 μg/ml) for 15 min at 37°C and was centrifuged at 12,000 rpm for 1 min. Supernatant (100 μl) was incubated with 2 mM phenolphthalein glucuronic acid (200 μl) and 0.2 M acetate buffer (200 μl) for 18 hrs at 37°C. Absorbance was read at the wavelength of 550 nm. Triton X-100 was used as standard.

Prevention of gastric necrosis produced by HCl-ethanol

According to the method of Mizui and Doteuchi (1983), rats were fasted except free access to water for 24 hrs before experiments. One ml of 60% ethanol in 150 mM HCl was given orally and animals were sacrificed with ether 1 hr later. Stomachs were dissected and opened through the greater curvature. The length of each lesion was measured and the lesion index was expressed as the sum of lengths of each lesion. Test compounds were orally administered 30 min prior to ethanol treatment.

Statistical analysis

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

RESULTS AND DISCUSSION

In the present work, the pharmacological effects of ethanolic extract prepared from cactus were investigated using various experimental models.

Acute toxicity in mice

LD_{50} value was determined by oral or intraperitoneal adminstration of the extract into mice. The measured

LD_{50} values were estimated to be higher than 2 g/kg (Table I). At the used dosages, no mice were found to die and no other abnormal behavior of mice was observed. These results indicate that the cactus extracts are not toxic to mice in the range of dosages tested.

Analgesic effect

Using the method previously described by Koster *et al.* (1959), the effects of the cactus extracts on the writhing induced by acetic acid in mice were examined (Table II). Aspirin, a positive control compound, showed a strong analgesic effect. The inhibitory effects of the cactus extracts on writhing were found to be comparable to that of aspirin. EEOF 300 mg/kg and 600 mg/kg caused 58.1% and 58.1% inhibition in the number of writhing induced by acetic acid, respectively. EEOS 300 mg/kg and 600 mg/kg also gave significant inhibitions of 37.9% and 56.3% in the number of writhing, respectively. Asprin 200 mg/kg showed about 40% inhibition in the number of writhing (Table II). From the results, EEOF and EEOS were found to show analgesic effect.

Carrageenan-induced edema in the rat hind paw

Previous studies by Vane and Botting (1987) on rat paw edema in response to carrageenan 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. In carrageenan-induced rat paw edema, an acute inflammation model, the oral administration of EEOF 300 mg/kg and 600 mg/kg showed a significant inhibition of rat paw edema (Table III). The oral administration of EEOS also caused the similar degree of inhibition in rat paw edema (Table III). Aspirin, used as a positive control compound, produced a significant inhibition in rat paw edema. However, the effects of EEOF and EEOS were not found in dose-response manner, especially in case of EEOS. According to Di Rosa (1987) and Vineger *et al.* (1976, 1982), histamine and serotonin were mainly released during first 1.5 hr after carrageenan injection. Kinin was released until 2.5 hrs and at last step inflammation was continued till 5 hrs by prostaglandins. From the results obtained in this work, the oral administration of EEOF and EEOS gave stronger inhibition at 3-5 hours after the injection of carrageenan, indicating that their effects might correlate with the release of kinin and prostaglandins. Our results propose that EEOF and EEOS show an anti-inflammatory effect against acute inflammation.

Inhibition of leukocyte migration

Wedmore and Williams (1981) proposed that the character of anti-inflammatory agent might be det-

Table I. Acute toxicity of ethanol extracts of *Opuntia ficus-indica* fructus (EEOF) and *Opuntia ficus-indica* stem (EEOS)

Group	Dose (mg/kg)	Administration route	No. of the treated	No. of the died
EEOF	4,000	p. o.	7	0
	2,000	i. p.	7	0
EEOS	4,000	p. o.	7	0
	2,000	i. p.	7	0

Ethanol extracts of *Opuntia ficus-indica* fructus (EEOF) and *Opuntia ficus-indica* stem (EEOS) were orally administered to animals and the mortality was observed during 1 week.

Table II. Effects of ethanol extracts of *Opuntia ficus-indica* fructus (EEOF) and *Opuntia ficus-indica* stem (EEOS) on the writhing induced by acetic acid in mice[a]

Group	Dose (mg/kg, p.o.)	No. of animals	No. of writhing	Inhibition rate (%)
Control	-	7	24.86±0.38	58.05
EEOF	300	7	10.43±3.80**	58.05
	600	7	10.43±3.80**	37.93
EEOS	300	7	15.43±1.19**	56.32
	600	7	10.86±2.39**	38.33
Aspirin	200	7	16.83±2.39**	

[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.
**$p<0.01$

Table III. Effects of oral administration of ethanol extracts of *Opuntia ficus-indica* fructus (EEOF) and *Opuntia ficus-indica* stem (EEOS) on the carrageenan-induced rat hind paw edema

Group	Dose (mg/Kg, P.o)	No. of animals	Swelling percentage (%)				
			1 hr	2 hr	3 hr	4 hr	5 hr
Control	300	6	33.46±2.24	63.26±2.53	86.88±2.34	91.23±1.96	71.40±2.24
EEOF	600	6	20.61±2.20*	33.73±2.05**	48.49±1.37**	50.75±2.43**	45.02±3.46**
	300	6	25.65±2.70**	35.51±3.87**	43.73±4.19**	45.05±4.95**	41.39±5.59**
EEOS	600	6	19.06±0.59**	31.35±1.10**	40.80±2.53**	45.55±3.17**	36.61±4.36**
	200	6	18.62±2.41*	28.31±2.79**	37.82±3.15**	46.81±3.92**	43.71±4.71**
Aspirin		6	24.25±1.10**	33.74±2.48**	41.65±2.41**	48.37±3.61**	38.86±5.10**

Each value represents the mean value obtained from six rats.
10% Tween 80 was administered to the control group.
*$p<0.05$, **$p<0.01$.

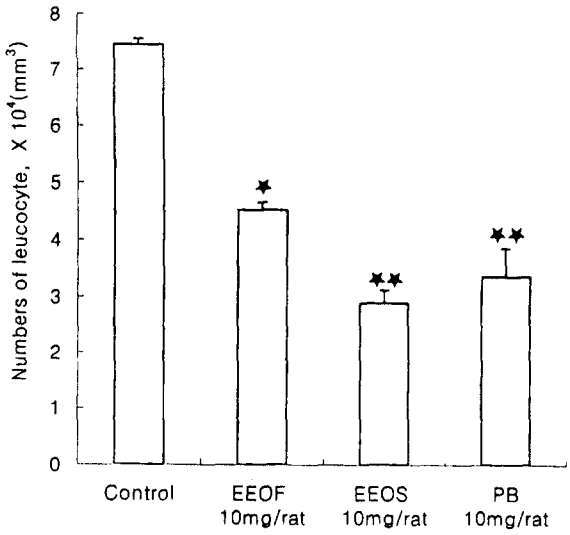

Fig. 1. Effects of EEOF and EEOS on leucocyte migration of CMC-pouch of rats. Each column represents the mean±S.E. for six rats. Control: 0.9% NaCl, EEOF: ethanol extract of *Opuntia ficus-indica* fructus, EEOS: ethanol extract of *Opuntia ficus-indica* stem, PB: phenylbutazone, *p<0.05, **p<0.01.

ermined by the inhibition degree in the leucocyte migration. The cactus extracts showed the significant inhibitory effect on leucocyte migration, when compared with phenylbutazone (Fig. 1). EEOF 10 mg/rat and EEOS 10 mg/rat had considerable effect in the inhibition of protein exudation (Data not shown).

Inhibition of β-glucuronidase release

β-Glucuronidase is one of lysosomal enzymes which released from inflammatory cells infiltrated in damaged tissue by phagocytosis. Therefore, the effects of EEOF and EEOS on β-glucuronidase release were examined

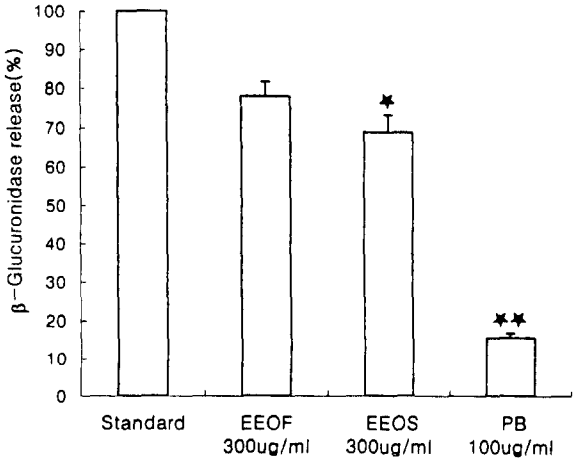

Fig. 2. Effects of EEOF and EEOS on β-glucuronidase release in rat neutrophils. Each column represents the mean±S.E. for six rats. Standard: triton X-100, EEOF: ethanol extract of *Opuntia ficus-indica* fructus, EEOS: ethanol extract of *Opuntia ficus-indica* stem, PB: phenylbutazone, *p<0.05, **p<0.01.

Table IV. Effects of orally administered ethanol extracts of *Opuntia ficus-indica* fructus (EEOF) and *Opuntia ficus-indica* stem (EEOS) on HCl-ethanol gastric lesion

Group	Dose (mg/kg, p.o.)	No.of animals	Lesion length[a] (mm)	Inhibition ratio (%)
Control	-	6	104.22±16.46	
EEOF	300	6	67.88± 7.61	37.87
	600	6	29.55± 5.87**	71.65
EEOS	300	6	53.57± 5.49*	48.59
	600	6	33.42± 8.61*	67.93
Sucralfate	200	6	9.17±11.23**	91.20

[a]Each value represents the mean±S.E.
*p<0.05, **p<0.01

(Fig. 2). EEOF (300 μg/ml) and EEOS (300 μg/ml) caused a decrease in β-glucuronidase release in neutrophils. These indicate that the effects of the cactus extracts may relate with inhibition in the release of inflammatory mediators.

Gastric necrosis

Most non-steroidal anti-inflammatory drugs were found to have the side effect of gastric damage. Therefore, the effects of EEOF and EEOS on the protection of gastric lesions were examined as described by Mizui and Doteuchi(1983). Gastric lesions were produced by the oral administration of 1 ml of 60% ethanol in 150 mM HCl. EEOF and EEOS showed significant reduction in gastric lesions in a dose-response manner (Table IV). These indicate that the cactus extracts show the protective effect in the gastric mucosal layers. However, their protective effects were found to be lower compared with sucralfate. This result proposes that cactus can be used to reduce gastric lesions in long-term patients such as rheumatoid arthritis patients taking non-steroidal anti-inflammatory drug.

The results obtained in present work propose that the cactus extracts (EEOF and EEOS) show anti-inflammatory activity in acute inflammatatory models and a reduction in side effect such as a gastric disorder which non-steroidal anti-inflammatory drugs usually possess.

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