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Effect of Butanol Fraction of *Panax ginseng* Head on Gastric Lesion and Ulcer

Choon Sik Jeong
College of Pharmacy, Duksung Women's University, Seoul 132-714, Korea

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From our previous result that *Panax ginseng* head extract had inhibition of gastric damages, the extract was fractionated. Among the hexane, chloroform, butanol and water fractions, butanol fraction showed the most potent inhibition of HCl·ethanol-induced gastric lesion, aspirin-induced gastric ulcer, acetic acid-induced ulcer and Shay ulcer. Butanol fraction showed significant increase in mucin secretion, and inhibited malondialdehyde (MDA) and H^+/K^+ATPase activity in the stomach. This results indicate that the effectiveness of the fraction on gastric damages might be related to inhibition of acid secretion, increment of mucin secretion and antioxidant property.

Key words: Head of *Panax ginseng*, Butanol Fraction, Gastric lesion, Ulcer

INTRODUCTION

The Panax ginseng Radix has been used in Oriental medicine for the treatment of diabetes, hepatitis and used as a tonic to elevate mood and reduce fatigue (Jiansu New College Medicine Eds, 1977; Chung *et al.*, 2001) and the head of *Panax ginseng* C. A. Meyer has been widely used for supplying energy to weaklings (Hur, 1989). We previously reported that the methanol extract of *Panax ginseng* head showed anti-ulcerative action in rats (Chung, 1996). However, the underlying mechanisms of the head of *Panax ginseng* with the active components are not sufficiently clarified. As a precedent stage for isolation of active components, the active fraction was obtained and some of the action mechanisms were studied.

This study deals with the effect of butanol fraction of *Panax ginseng* head on gastric lesion, ulcer, mucin secretion, DPPH activity, MDA level and H^+/K^+ATPase activity, and some histological changes were observed.

MATERIALS AND METHODS

Materials and chemicals

The head of *Panax ginseng* (10kg) collected from the herbal market (Seoul, Korea) was used after well-washed and dried. The methanol extract was made by reflux with 95% methanol for 4 h three times at 70°C in a water bath. Methanol extract (2.6 kg) was fractionated in succession with hexane (120 g), chloroform (220 g), butanol (921 g) and the residues water fraction (1.3 kg). The butanol fraction was used as a test substance.

Bovine serum albumin, 1-diphemyl-2-picrylhdrazyl (DPPH), thiobarbituric acid, 1,1,3,3-testraethoxypropane, L-ascorbic acid and dioctyl sodium sulfosuccinate were purchased from Sigma Chem. Co., and alcian blue (Janssen Chimica), sucrose (Shinyo Pure Chem. Co., Ltd.), carboxymethyl cellulose (Junsei Chem. Co.), cimetidine (Choongwea Pharm. Co., Ltd.) were used. Other reagents and solvents for extraction were pharmaceutical or reagent grade for analysis.

Animals

Male Sprague-Dawley rats weighing 180-200 g were purchased from Samyook Animal Laboratories, Kyunggi-do. Solid food and water were supplied *ad libitum.* All animals were housed in a temperature-controlled (22-25 °C) room and 12 h on and off lighting. Fresh hog stomach was purchased from the Majang market, Seoul, Korea. The test substance suspended in 0.5% CMC solution was administered in a volume of 0.5 ml/100 g (b.w.). Control group received only 0.5% CMC solution.

Correspondence to: Choon Sik Jeong, 419 Ssangmoon-dong, Dobong-gu, Seoul
E-mail: choonsik@center.duksung.ac.kr

HCl· ethanol-induced gastric lesion

According to the method of Mizui and Dodeuchi (1983), male rats fasted for 24 h with free access to water before the experiment were orally administered 0.5 mL/100 g of HCl · ethanol (60% ethanol with 150 mM HCl) solution. One hour later, the animals were anesthetized with ether, and each stomach was excised. Then, 12 mL of 2% formalin was infused into the stomach and the stomach was immersed 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 length was expressed as a lesion index. The test substance was given orally 30min prior to administration of HCl·ethanol solution.

Aspirin-induced gastric ulcer

According to the method of Okabe *et al.* (1974), male rats were fasted for 24 h, and the pylorus of each animal was ligated under ether anesthesia. The test substance was given intraduodenally after pylorus-ligation. Ten minutes later, aspirin 150 mg/kg suspended in 0.5% CMC was administered orally in a volume of 0.5 mL/100 g. Seven hours after aspirin treatment, the animals were sacrificed and each excised stomach was treated with formalin solution as described above and the ulcer index was measured.

Shay ulcer

According to the method of Shay *et al.* (1945), male rats were fasted for 36 h, and the pylorus of each animal was ligated under ether anesthesia. The test substance was given intraduodenally immediately after pylorus-ligation. Fifteen hours 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. The ulcer index was expressed according to the severity of ulcer: 1, no lesion; 2, bleeding or light; 3, moderate; 4, severe; 5, perforation.

Malondialdehyde assay

Malondialdehyde (MDA) reactive substance in the stomach was measured as a maker of lipid peroxidation by the method Ohkawa *et al.* (1979). Briefly, 0.2 mL of forestomach tissue homogenates which were obtained from the above experiment was added to 0.8 ml of 20% acetic acid, adjusted to pH 3.5, 0.4 mL of 8% sodium dodecyl sulfate, and 0.4 mL of 0.8% thiobabituturic acid. The mixture was vortexed and then boiled for 60 min at 95°C. The colored product was cooled and centrifuged at 12,000 g for 15min. The supernatant was collected and the absorbance at 520 nm (exitation) and 535 nm (emission) was measured for the determination of TBA reactive substances.

Acetic acid-induced ulcer

According to the method of Takagi and Okabe (1968), male rats were fasted for 24 h before the experiment, the stomach was exposed under ether anesthesia, and 20 μl of 20% acetic acid was injected in subserosal layer of forestomach and sutured. Ampicillin 50 mg/kg was orally administered for preventing infection. The test substance was orally administered once a day from the 4th day to 11th day after surgery. On the 15th day after the surgery, animals were sacrificed and the excised stomach was treated as described above and the area (mm^2) of each ulcer was measured and summed.

Mucin secretion

Male rats were fasted for 24 h before the experiment. Absolute ethanol was orally administered to rats by 1 mL/100 g 30 min after oral administration of the test substance according to the method of Robert (1979), and 1h later the animals were sacrificed. The mucin content was measured by the method of Kitagawa *et al.* (1986). Briefly, the stomach was removed immediately and the gastric lumen was rinsed with 10 ml of ice-cold 0.25 M sucrose. 0.1% Alcian blue dissolved in 0.16M sucrose buffered with 0.05M CH_3COONa adjusted to pH 5.8 with HCl, in a volume of 10 mL was infused into the stomach to stain for 2 h the intragastric mucus alone, and the esophagus and pylorus were clamped. The lesser curvature of the stomach was cut and was everted. Dye-mucus complex was extracted for 2 h with 10 mL of 30% dioctyl sodium sulfosuccinate dissolved in 70% ethanol. The supernatant of extract obtained by centrifugation for 10 min at 3000 rpm was measured at 620 nm.

H^+/K^+ATPase activity in hog gastric microsome

Gastric microsomal vesicles were prepared from hog stomach by a modification of the method of Saccomani *et al.* (1977). Briefly, tissue was homogenized in isotonic medium and the microsomal fractions were obtained by differential centrifugation. This material was separated on a discontinuous density gradient and the fraction at the interface between 0.25M sucrose and 0.25M sucrose with 7% Ficoll layers was taken. The interface fraction was diluted in two volumes of homogenization buffer, spun down and freeze-dried overnight. The lypophilized vesicle were resuspended in homogenation buffer and stored at -70°C. Protein concentration was determined by the Lowry method (Lowry *et al.*, 1951).

ATPase was preincubated in 5 mM imidazole buffer (pH 7.4) and the test substance for 30 min at 37°C. The enzyme activity was determined at 37°C in the presence of 20 mM $MgCl_2$, 20 mM Na_2ATP and 200 mM imidazole buffer (pH 7.4) with or without 100 mM KCl and incubated for 15 min at 37°C. The reaction was teminated by adding 1.0 mL of ice-cold 22% trichloroacetic acid. After centrifugation at 3000 rpm for 15 min, supernatant was obtained. The inorganic phosphate hydrolyzed from ATP was measured by the method of Fiske and Subbarow (1924).

DPPH anti-oxidative assay

According to the partly modified method of Uchiyama *et al.* (1968), radical scavenging activity was determined. The test substance at various concentrations dissolved in MeOH was added to 1.5×10^{-4}M 1,1-diphenyl-2-picryl-hydrazyl (DPPH) methanol solution. Concentrations of the substance were 40, 80, 160, 240 μg/mL. Then the solution with the substance was shaken vigorously and kept in the dark for 30 min at 25°C. The absorbance of the substance was measured on a spectrophotometer at 520 nm. L-ascorbic acid was used as a positive control.

Histological examination

The stomach fixed for 48 h with 10% formalin was dehydrated by passing successively in different mixture of ethyl alcohol-water (50, 80, 95% and finally in absolute alcohol), cleared in xylene and embedded in paraffin. Section (4-5 μm thick) were prepared and then stained with hematoxylin-eosin dye for microscopic observation (40 ×).

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

Effect of the butanol fraction on HCl·ethanol-induced gastric lesion

Intragastric administration of HCl plus ethanol to rats produced large hemorrhagic erosion in the glandular stomach, of which lesion index was 48.2. The test substance at an oral dose of 500 mg/kg remarkably pre-vented the gastric lesion by 56.2%. Cimetidine at an oral dose of 150 mg/kg also prevented the lesion by 87.3% (Fig. 1).

Effect of the butanol fraction on aspirin-induced gastric ulcer

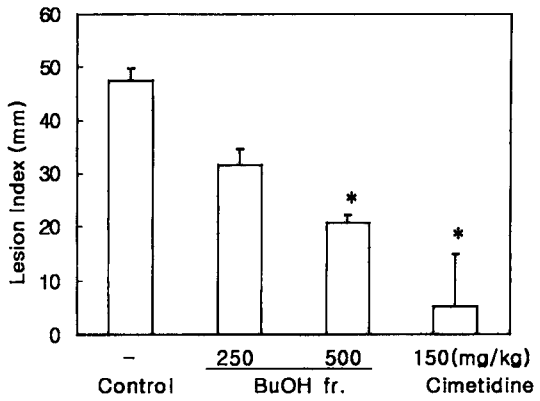

Fig. 1. Effect of the butanol fraction on HCl · ethanol-induced gastric lesion. *p<0.01; Significantly different from control group. (n=6)

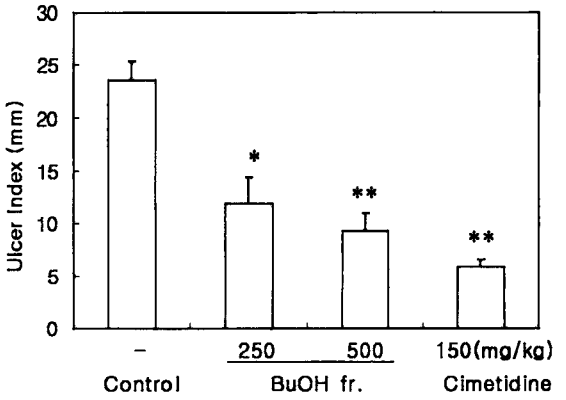

Fig. 2. Effect of the butanol fraction on aspirin-induced gastric ulcer. *p<0.05, **p<0.01; Significantly different from control group. (n=8)

The intragastric administration of aspirin in pylorus-ligated rats produced gastric ulcer in the glandular stomach of which ulcer index is 23.6. The substance at the intraduodenal doses of 250 and 500 mg/kg significantly inhibited the gastric ulcer in a dose-dependent manner. Cimetidine at an oral dose of 150 mg/kg also significantly inhibited the ulcer (Fig. 2).

Effect of the butanol fraction on Shay ulcer and MDA content

The Shay ulcer was remarkably produced in the forestomach of the control rats of which ulcer index was 3.9. As shown in Fig. 3, the substance at an intraduodenal dose of 500 mg/kg showed significant inhibition of the ulcer by 56.4%. Cimetidine at an oral dose of 150 mg/kg also significantly inhibited the ulcer. In this Shay ulcer test, the substance at an intraduodenal dose of 500 mg/kg significantly lowered MDA level in the forestomach by 87 %. Cimetidine at an oral dose of 150 mg/kg also lowered MDA level by 68% (Table I).

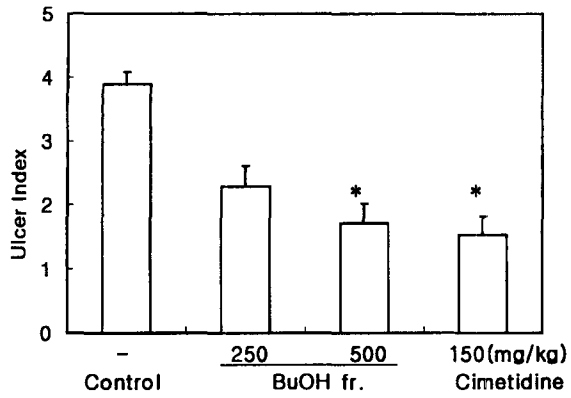

Fig. 3. Effect of the butanol fraction on Shay ulcer. *p<0.01; Significantly different from control group. (n=8)

Table I. Effect of the butanol fraction on malondialdehyde level of Shay ulcer induced rats

Treatment	Dose (mg/kg, i.d.)	No. of animals	Malondialdehyde level (nmol/mg protein)	Inhibition rate (%)
Saline	-	6	0.83 ± 0.01	-
BuOH fr.	500	6	0.10 ± 0.02*	87
Cimetidine	150	6	0.23 ± 0.04*	68

The values are means ± S.E.
*p<0.01; Significantly different from control group

Effect of the butanol fraction on acetic acid-induced ulcer

The ulcer index produced by acetic acid in rats was 5.1. Repeated administration of the substance at the oral doses of 250 and 500 mg/kg for 15 days decreased the ulcer index by 51.6 and 72.3%, respectively. Cimetidine at an oral dose of 150 mg/kg significantly inhibited the ulcer by 74.1% (Fig. 4).

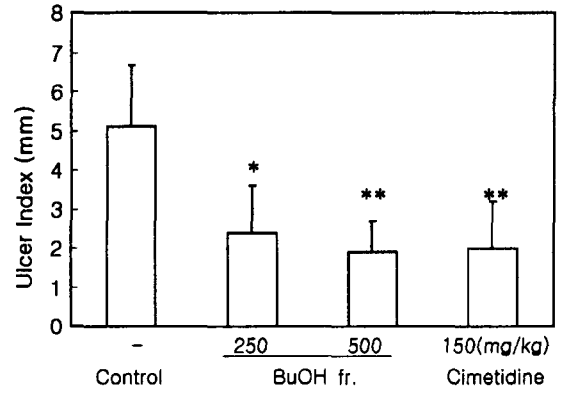

Fig. 4. Effect of the butanol fraction on acetic acid-induced gastric ulcer. *p<0.01, **p<0.001; Significantly different from control group. (n=8)

Table II. Effect of the butanol fraction on mucin secretion in rats

Treatment	Dose (mg/kg, i.d.)	No. of animals	Mucus contents (μg alcian blue)
Saline	-	8	142.7 ± 30.4
BuOH fr.	250	6	187.8 ± 26.0
	500	6	410.7 ± 97.7*
Cimetidine	150	6	152.4 ± 37.8

The values are means ± S.E.
*Significantly different from control group (p<0.05)

Table III. Inhibitory effect of the butanol fraction on H^+/K^+ ATPase activity at pH 6.4 in hog gastric microsomes

Treatment	Stock Concentration (mg/ml)	Inorganic Phosphate (O.D.)			Inhibition (%)	IC_{50} (mg/kg)
		[a]K(+)	[b]K(-)	K(+) - K(-)		
Control	-	0.511	0.162	0.349	-	-
BuOH fr.	15	0.501	0.155	0.346	0.9	
	30	0.506	0.152	0.354	-1.4	77
	60	0.408	0.140	0.269	23.1	
	120	0.164	0.104	0.060	86	

[a]K(+), incubated in the presence of KCl
[b]K(-), incubated in the absence of KCl

Effect of the butanol fraction on mucin secretion

As shown in Table II, the mucus content of control group was 142.7 μg/rat as expressed as alcian blue and the substance at 500 mg/kg significantly increased the mucus content in the stomach to 410.7 μg/rat.

Effect of the butanol fraction on H^+/K^+ATPase assay

Table III shows effects on the H^+/K^+ATPase activity at pH 6.4 in hog gastric microsomes. The substance inhibited H^+/K^+ATPase by 23 and 82% at the doses of 60 and 120mg/kg, and IC_{50} is 77 mg/kg.

Effect of the butanol fraction on DPPH anti-oxidative assay

The test substance showed free radical-scavenging

Table IV. Free radical scavenging effect of the butanol fraction

Treatment	Concentration (μg/ml)	Inhibition (%)	IC_{50} (μg/ml)
BuOH fr.	40	29.6	198.0
	80	36.0	
	160	41.7	
	240	62.5	
Ascorbic acid	2.5	3.5	7.5
	5	16.5	
	10	30.4	

effect in a dose dependent manner on DPPH assay (Table IV). The IC_{50} of the substance and ascorbic acid are 198.0 and 7.5 μg/ml, respectively.

Histological examination

In histological examination (Fig. 5), hemorrhages induced by HCl·ethanol solution are markedly reduced, and mucosal layer which is peeled off with lesions is recovered almost to the normal condition in the treatment group.

DISCUSSION

The butanol fraction which is an active fraction obtained from methanol extract of *Panax ginseng* head was investigated on gastric lesion and ulcer models The present study demonstrates that the fraction has inhibitory effect on HCl·ethanol-induced gastric lesion, aspirin-induced gastric ulcer, Shay ulceration and acetic acid-induced chronic ulcer in rats.

HCl·ethanol-induced lesion is known to be produced by direct irritation of gastric mucosal barrier (Seiki *et al.*, 1990). The test substance significantly increased mucin secretion about three-folds high in rats and lowered MDA level in pylorus ligated rats. The substance potentially suppressed gastric acid secretion by inhibition of H^+/K^+ATPase. Thus, the effectiveness of the test substance on HCl·ethanol-induced gastric lesion and the gastric ulcer models might be related to inhibition of acid secretion, increase in mucus secretion and antioxidant property. Only in Shay ulcer, we demonstrated that the effect of the substance is mainly due to inhibition of gastric acid secretion.

The *in vitro* free radical-scavenging effect was shown by treatment of the substance. In general, it is thought that the activity of free radical-scavenging enzymes is elevated to scavenge free radicals generated in injured inflamed tissues. The effect of the substance in the ulcerated region might be shown as a result of the decrease in the generation of free radicals, because lipid peroxidation was markedly increased in the ulcerated region.

Brzozowski *et al.* (2001) reported the natural healing of acetic acid-induced ulcer is originated from increase of PGE_2. However, the effect of the substance on PGE_2 synthesis is unknown at present.

In conclusion, these results indicate that the antiulcer effect of the test substance might result from reduction of acid secretion through inactivation of H^+/K^+ATPase, increases of mucus secretion and antioxidant effect.

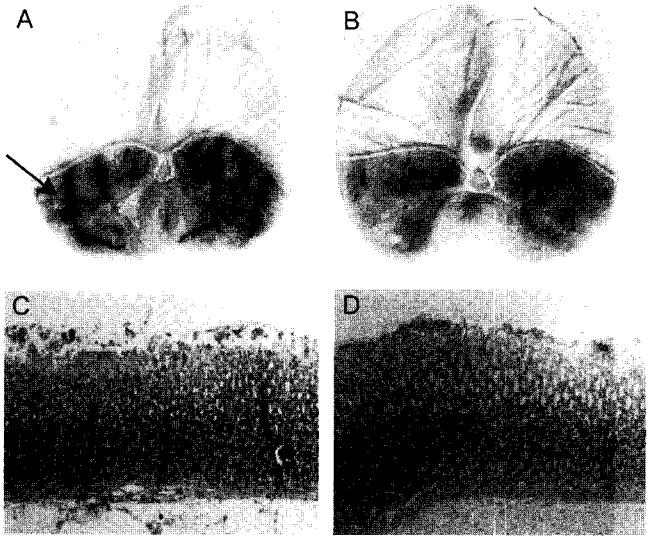

Fig. 5. Histological examination. Photomicrographs of stomach with lesion by HClethanol induction. Submucosal edema and extensive erosion stained with hematoxylin and eosin. Histological sections of stomach compared to the control: A, C (× 40), and lesions are pointed by arrows. The tissue obtained from the rat treated with the butanol fraction (500 mg/kg p.o.) 1hr before HClethanol administration showed no abnormalities : B, D (× 40).

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