大韓本草學會誌 제24권 제1호(2009년 3월)
Kor. J. Herbology 2009 ; 24(1) : 35-40

염산-에탄올에 의해 유발된 흰쥐 위염에 대한 마치현의 항산화 작용

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Anti-oxidant Effects of *Portulaca oleracea* L. on HCl-ethanol Induced Gastritis in Rats

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ABSTRACT

Objectives : The purpose of this study is to evaluate whether or not a pretreatment with *Portulaca oleracea* has an antioxidant effect in HCl-ethanol induced gastric mucosal damage.

Methods : We elucidated the level of reactive oxygen species (ROS), lipid peroxidation, and two important constituents of antioxidant defense such as superoxide dismutase (SOD), glutathione (GSH) in these effects.

Results : The oral administration of crude extract from *P. oleracea* attenuated the gastritic lesion area, submucosal edema and hemorrhage, and mucosal necrosis induced by HCl-ethanol. The MDA levels of control group were higher than those in the rats given the *P. oleracea* pretreatment. While the GSH levels of control were decreased, the GSH activity on the gastric mucosal layer maintain normal level in rats given the Portulaca oleracea pretreatment before HCl-ethanol induced gastritis significantly increased. However, the SOD activites were not altered by *P. oleracea*.

Conclusions : The administration of *Portulaca oleracea* have a protective antioxidant effect against the gastric lesion induced by HCl-ethanol and may therefore be a promising drug for gastritis and gastric ulcer.

Key words : *Portulaca oleracea*, reactive oxygen species, glutathione, superoxide dismutase

Introduction

P. oleracea (Portulacaceae) is listed in the World Health Organization as one of the most widely used medicinal plants and it has been given the term ' Global Panacea'[1], and it is widespread as a weed and has been ranked the eight most common plants in the world[2]. *P. oleracea* L. (Chinese name Ma-Chi-

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· Acceptance : 2009. 2. 6 · Adjustment : 2009. 3. 12 · Adoption : 2009. 3. 20

Xian) is widely used in China not only as an edible plant, but also as a traditional Chineseherbal medicine for alleviating pain and swelling[3]. Also in Korea, *P. oleracea* can be found growing wild, but rarely cultivated[4].

Ulcerative lesions of gastrointestinal tract are one of the major side effects associated with alcohol consumption[5]. At concentrations greater than 40%, it causes marked mucosal hyperemia, necrosis, edema and mucosal or submucosal hemorrage[6,7]. The formation of lesions may be mediated by oxygen-derived free radicals[8-10]. Experimental and clinical evidence suggests that gastric mucosal damage by ethanol, nonsteroidal anti-inflammatory drugs, and by *Helicobacter pylori* is mediated through the ROS[11].

Normally, the level of ROS is regulated by antioxidants including vitamin C, cellular reduced glutathione etc, but levels of these antioxidants are decreased during an infection. The increased levels of pro-oxidative factors and decreased levels of antioxidants result in the accumulation of ROS, which can modulate many processes in the gastric epithelium.

P. oleracea is well-known as a strong free radical scanvenger. and there has been reported that the administration of *P. oleracea* has numerous beneficial effects against many diseases. The ameliorative effects of a *P. oleracea* against oxidative stress may have been proposed as a key mechanism for its beneficial effects[12,13].

Only a few published studies showed the P. oleracea has protective effects on the gastric mucosal lesion[14,15]. Thus, the present study was intended to evaluate the possible mechanisms of protective effect of *P. oleracea* on the HCl-ethanol induced gastric ulcer in rats and to investigate the antioxidant effects of *P. oleracea* on HCl-ethanol induced gastritis. Measurement of gastric mucosal malondialdehyde (MDA) concentration, which is the end-product of lipid peroxidation, was used to assess oxidative damage to membranes. Mucosal glutathione (GSH) concentrations were also measured in order to assess whether reactive oxygen species generation affects levels of the antioxidant peptide. The influence of *P. oleracea* on morphological gastritis and the multi-targeted activity of the enzyme defense system such as superoxide dismutase (SOD) is a marker of oxidative stress.

Materials and method

1. Preparation of *Portulaca oleracea*

Portulaca oleracea was purchased from Omni-herb (Dae-gu, ROK) and identified by Department of prescription, College of Oriental Medicine Dong-shin University. A voucher specimen was deposited at the herbarium of College of Oriental Medicine Dongshin University. The dried portulaca oleracea (5 kg) were extracted with 90% ethanol (5 L)by maceration at room temperature for 24h three times. The extracts were filtered and dried at 40℃ under vacuum. The yield (w/w) of the ethanolic extracts were 8.8% (440 g).

2. Experimental animal

Male Spraque Dawley rats weighing approximately 250-280 grams were housed under standard laboratory conditions. The animals were maintained in a temperature-controlled room (25℃) and kept on 12:12 light dark cycle (lights on at 08:00 and off at 20:00). Food and water were provided ad libitum. Food was restricted but free access to water was allowed for 24 h before the experiment to ensure an empty stomach. And, all animals were kept in raised mesh-bottom cages to prevent coprophagy. The experiments were carried out from 10:00 to 12:00. There were 5 rats in each group.

3. Administration of the crude extract of P. oleracea

The method is based on the modification of Mizui and Doteuchi[16]. The rats were randomly allocated into 5 groups of 5 animals each. Prior to the experiment, animals were fasted for 24 hours

but water was provided ad libitum. Animals in group 1 were treated with normal saline at 4 mL/kg and served as untreated control. Groups 2 were treated and 500 mg/kg of P. oleracea extract. All treatments were done via a stainless steel intubation needle. Thirty minutes later, 1 ml of 60% ethanol in 150 mM HCl was administered orally. Each animal was anesthetized with overdose of urethane 1 h after the administration of necrotizing agents and the stomach was excised and gently rinsed under running tap water.

4. Histological procedures

For the histological examination, segmental biopsies of the stomach were fixed in 10% formaldehyde, embedded in paraffin, cut into 4 µm sections, de-waxed, and stained with hematoxylin- eosin.

5. TBARS assay

Lipid peroxidation was measured using the kits purchased from Oxis Research (USA) according to the manufacturer's instructions. Briefly, 10 µl of probucol was added to each assay tube. 200 µl of sample or standard and 640 µl of diluted R1 reagent (one volume of 100% methanol to three volumes N-methyl-2-phenylindole, in acetonitrile) was added to each tube and mixed each tube by vortexing gently. 150 µl of concentrated hydrochloric acid was added. Each tube was stoppered, mixed, and incubated at 45℃ for 60 mins. Samples were centrifuged (10,000×g for 10 mins). The clear supernatant was transferred to a cuvette, and then absorbance was measured at 586 nm.

6. Glutathione activity assay

The GSH activity was measured using the kits purchased from OxisResearch(USA) according to the manufacturer's instructions. Briefly, 200 µl of sample supernatant or calibrator was added to a test tube or spectrophotometric cuvette. 200 µl buffer (potassium phosphate, Diethylenetriaminepentaac etic acid, Lubrol) was added to the reaction mixture. 200 µl reducing agent (Tris(2-carboxyethyl) phosphine in HCl) was added to the reaction mixture, and mixed well. 200 µl Chromogen (1-Methyl-4-chloro- 7-trifluoromethylquinolinium methylsulfate in HCl) and 200 µl Color Developer (NaOH in water) was added to the reaction mixture. Test tube or spectrophotometric cuvette was incubated at room temperature in the dark for 30 mins and then measured the absorbance at 420 nm.

7. Superoxide dismutase activity (SOD) assay

The SOD activity was measured using the kits purchased from Dojindo (Japan) according to the manufacturer's instructions. Briefly, 20 µl of sample solution was added to each sample and blank 2 well. 20 µl of double distilled water was added to each blank 1 and blank 3 well. 200 µl of WST working solution was added to each well, and mixed. 20 µl of dilution buffer was added to each blank 2 and blank 3 well. 20 µl of enzyme working solution was added to each sample and blank 1 well, and then mixed thoroughly. The plate was incubated at 37℃ for 20 min. and then measured the absorbance at 450 nm using a microplate reader. The SOD activity (inhibition rate %) was calculated using the following equation.

$$\text{SOD activity(inhibition rate \%)} = [\,[(A_{blank1} - A_{blank3}) - (A_{sample} - A_{blank2})]\,/(A_{blank1} - A_{blank3})] \times 100$$

8. Data expression and statistical analysis

Data are expressed as means±SEM (standard error of means). Statistical differences between means were determined by the Student's t-test. Differences between multiple groups were tested using analysis of variance (ANOVA) for repeated measures. P value below than 0.05 was considered as significant data.

Result and discussion

1. Effect of *P. oleracea* on HCl-ethanol induced gastritis

HCl-induced gastric damage was observed as elongated black-red lines (1-10 mm long by 0.5-1.5 mm wide) parellel to the long axis of the stomach in rats. In control group, multiple erosions and minor bleeding spots with marked mucosal redness and edema are observed. Animals pretreated with *P. oleracea* showed very mild lesions with interstitial hemorrhage.

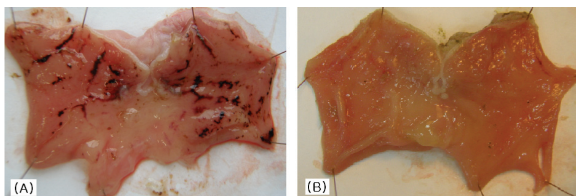


Fig. 1. Gross examination of the stomach in HCl-ethanol alone (A) and in the pretreatment of *P. oleracea* (500 mg/kg) with HCl-ethanol (B)

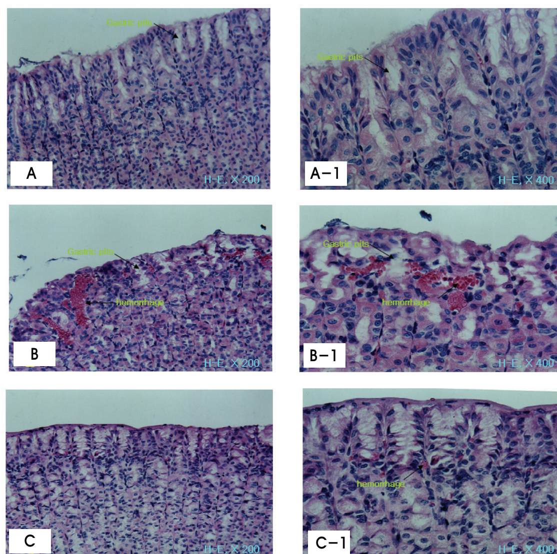


Fig. 2. Microscopic examination of hematoxylin-eosin stained sections

A : Normal histological structure of rat gastric mucosa.

B : Histological appearance of the gastric ulcers 1h after HCl-ethanol treatment. Severs erosion with necrosis of gastric mucosa, detachment of necrotic gastric mucosa.

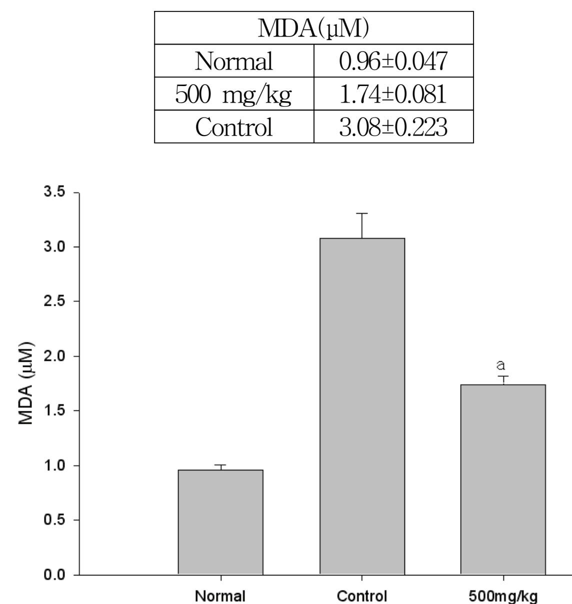
C : Rat pretreated with *P. oleracea* (500 mg/kg), slight erosion of the gastric mucosa is observed.

2. Histological assay

In the section stained with hematoxyline-eosin, there were differences in the cellular architecture between control and *P. oleracea* group (500 mg/kg). In normal group, normal histological structure of rat gastric mucosa was observed. In control group, severs erosion with necrosis of gastric mucosa, detachment of necrotic gastric mucosa was observed. In *P. oleracea* group (500 mg/kg), suppression of diffused mucosal erosion with hemorrhage and edema was observed.

3. TBARS assay

The concentration of TBARS was evaluated in order to determine the production of malondialdehyde (MDA), the end-product of lipid peroxidation by ROS. The contents of TBARS for the control group were significantly higher than those of the *p. oleracea* treated group (3.08±0.223 μM vs. 1.74±0.081 μM, $p < 0.01$, Fig. 3) suggesting that the administration of *P. oleracea* possessed the protective effect in the HCl-ethanol induced gastritis model against oxidative stress.



MDA(μM)	
Normal	0.96±0.047
500 mg/kg	1.74±0.081
Control	3.08±0.223

Fig. 3. Effect of *P. oleracea* on TBARS levels in gastric mucosa

Data are mean±S.E.M., n=5, $^{a}p < 0.05$ vs control value.

4. GSH assay

P. oleracea group maintained the GSH activity in the gastric mucosa. The level of the GSH activity was 302.98±5.72 μM while that of control was 202.28±2.64 μM after the induction of gastric mucosal damage for 30 mins. The level of GSH in Protulaca pretreated group was approximately fifty percent higher than in the control group

GSH (μM)	
Normal	297.38±3.12
500 mg/kg	302.98±5.72
Control	202.28±2.64

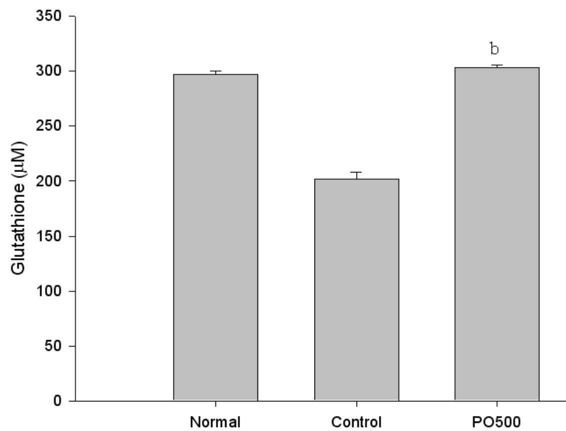


Fig. 4. Effect of *P. oleracea* on GSH levels in gastric mucosa

Data are mean±S.E.M., n=5, $^{b}p < 0.01$ vs. normal.

SOD activity(inhibition rate %)	
Normal	100.82±0.28
500 mg/kg	100.42±0.23
Control	100.05±0.41

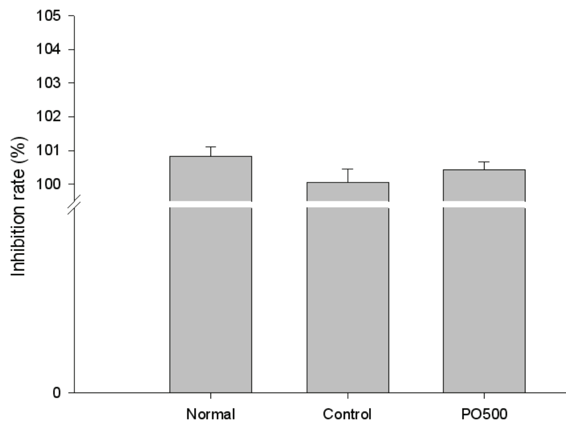


Fig. 5. Effect of *P. oleracea* on SOD activity in gastric mucosa

5. SOD assay

The activity of SOD in *P. oleracea* pretreated group was 100.42±0.23% while that of control was 100.05±0.41% suggesting that pretreatment of *P. oleracea* before HCl-ethanol did not statistically alter the SOD activities compared to control group

Conclusions

In orther to investigate the effects of Portulaca oleracea on the oxygen free radicals, the gastric mucosa antioxidant defense mechanisms (glutathione, superoxide dismutase), the lesion - inducing effects of gastric mucosal malondialdehyde (MDA) concentration, which is the end-product of lipid peroxidation to obtain the following conclusion.

1. Administration of HCl-ethanol caused the marked gastric lesion, submucosal edema, hemorrhage and mucosal necrosis due to oxidative stress.
2. The oral administration of P. oleracea before the administration of induction agent, HCl-ethanol, inhibited all those symptoms.
3. Lipid peroxidation was inhibited by P. oleracea by preserving thelevel of GSH although SOD level remained unchanged.
4. P. oleracea could be an option in the treatment of gastritis.

These results show that the administration of Portulaca oleracea have a protective antioxidant effect against the gastric lesion induced by HCl-ethanol and may therefore be a promising drug for gastritis and gastric ulcer. A continued study on detailed control passway of protective factor will be conducted.

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