The Inhibitory Effects of *Portulaca oleracea* L. on HCl-ethanol Induced Gastritis in Rats

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**ABSTRACT**

**Objectives** : The objective of this study was to examine the effects of *P. oleracea* into the HCl-ethanol induced gastritis in rats, and to isolate and determine the chemical compounds from *P. oleracea*.

**Methods** : The rats were orally administered with crude extract or fractions or isolated compounds of *P. oleracea* 30 mins before the induction of gastric lesion by oral administration of HCl-ethanol. The gastric lesional area was measured using pixel counting software. Then the chemical compounds from *P. oleracea* was isolated and determined by LC-MS and NMR.

**Results** : The inhibition effect of oral administration of crude extract of *P. oleracea* at a dose of 500 mg/kg in HCl-ethanol induced gastritis was similar to cimetidine. Then, aqueous fraction at a dose of 240 mg/kg exhibited the effects similar to cimetidine. Then, the aqueous fraction was further separated by MPLC and yielded four sub fractions. Among those sub fractions, agent II at a dose of 40 mg/kg possessed the strongest effect in the HCl-ethanol induced gastritis. The water fraction yielded-Uridine, Adenosine, Guanosine, which were characterized by Mass, 1H-NMR, 13C-NMR.

**Conclusions** : This study suggest that a *P. oleracea* and its compounds showed potent efficacy on the development of HCl-ethanol induced gastritis. Thus, *P. oleracea* can be a potential natural resource for the management of gastritis although the mechanism of action involved in the treatment remains to be explored.

**Key words** : Portulaca oleracea, gastritis, HPLC

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Introduction

The use of herbal products in the purpose of prevention or treatment of several chronic diseases has been practiced traditionally in various ethnic societies worldwide\textsuperscript{1}. However, the restraining effect and involved mechanisms to the gastritis of herbal products have not been cleared yet. \textit{P. oleracea} (Portulacaceae) which has been ranked the eight most common plants in the world\textsuperscript{2} is listed in the World Health Organization as one of the most used medicinal plants and it has been given the term 'Global Panacea'\textsuperscript{3}.

\textit{Portulacae Herba} is sour in flavor, cold in nature, nonpoisonous and attributable to large intestine and liver meridians. Medicinal properties of \textit{P. oleracea} include away heat, relieving toxin, cooling the blood, treating dysentery, stopping bleeding, reliving swelling and treating stranguria. It is used for dysentery, eczema, erysipelas, snake-bite poisoning, and hemorrhoids with blood\textsuperscript{4-7}.

The reported pharmacological effects of this plant include antibacterial\textsuperscript{8,9}, anti-inflammatory and analgesic effects\textsuperscript{10}, skeletal muscle-relaxant\textsuperscript{11}, potassium channel opening effect\textsuperscript{12}, relaxant effect on smooth muscle\textsuperscript{13}, wound-healing activities\textsuperscript{14} and it is important in preventing heart attacks and strengthening the immune system\textsuperscript{15} and reducing the occurrence of cancer\textsuperscript{16}.

Acute gastritis is an acute mucosal inflammatory process, usually of a transient nature. The inflammation may accompanied by hemorrhage into the mucosa and, in more severe circumstances, by sloughing of the superficial mucosal epithelium (erosion). The pathogenesis is poorly understood, in part because normal mechanisms for gastric mucosal protection are not totally clear. Acute gastritis is frequently associated with heavy use of nonsteroidal anti-inflammatory drugs (NSAIDs), excessive alcohol consumption, heavy smoking, treatment with cancer chemotherapeutic drugs, uremia, systemic infections, severe stress, ischemia and shock, suicide attempts with acids and alkali, mechanical trauma, reflux of bilious material after distal gastrectomy\textsuperscript{17,18}.

Ethnopharmacological studies have reported that a variety of botanical products possess anti-ulcer activity. A few published studies showed the \textit{P. oleracea} has protective effects on the gastric mucosal lesion\textsuperscript{19,20}. This study was designed to examine the effects of \textit{P. oleracea} into the HCl-induced gastritis in rats, and determine the chemical compounds from \textit{P. oleracea} responsible for those effects.

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 Dongshin University. A voucher specimen was deposited at the herbarium of College of Oriental Medicine Dongshin University.

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°C) 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. Extraction and fractionation

The dried \textit{P. oleracea} (5 kg) were extracted three times with 90% ethanol (5 L) by maceration at room temperature for 24 h. The extracts were filtered and dried at 40°C under vacuum. The yield (w/w) of the ethanolic extract was 8.8% (440 g).
The EtOH extract (44 g) was suspended in H2O, and partitioned with n-hexane, EtOAc and n-BuOH, successively to give fractions of n-hexane (7.6 g), EtOAc (2.8 g) and n-BuOH (4.2 g) and water (29.4 g), respectively (Fig. 1). Thereafter, the water fraction was analyzed. Waters 1525 LC systems were used for the HPLC analysis. The water fraction was separated into five peaks. And then the water fraction was refractionated by MPLC for compounds determination. Five peaks were shown by four agents. 1st and 2nd peak labelled as 1st, 3rd peak as 2nd, 5th peak as 3rd and 4th peak as 4th.

4. HCl-ethanol induced gastric mucosal membrane lesion

1) Administration of the crude extract of *P. oleracea*

The method is based on the modification of Mizui and Doteuchi\(^\text{21}\). 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, while animals in group 2 were treated with cimetidine (20 mg/kg) and served as treated control. Groups 3, 4, and 5 were treated with 100, 300 and 500 mg/kg of *P. oleracea* extract, respectively. All treatments were done via a stainless steel intubation needle. Thirty minutes later, 1ml 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. After opening along the greater curvature and spreading out on a board, the area (mm\(^2\)) of mucosal erosive lesions was measured using a pixel-counter which is a program made by Computer Engineering, Department in Chon-nam national university.

2) Administration of the fractions of extract

The method was same as above. The rats were orally administered with different fractions (240 mg/kg) of n-hexane, EtOAc and n-BuOH and water respectively.

3) Administration of the compounds separated from water fraction

The method was same as above. Five peaks were shown by four agents. 1st and 2nd peak labelled as 1st, 3rd peak as 2nd, 5th peak as 3rd and 4th peak as 4th. The rats were orally administered with those agents (40 mg/kg) respectively.

5. Isolation and Structure Determination

*P. oleracea* extract was fractionated with organic solvents, and the water fraction showed the most
strong protective effect in the gastric mucosal injury. The water fraction was analyzed. Waters 1525 LC systems were used for the HPLC analysis. The separation was performed on a Develosil ODS UG-5 column (4.6 x 150 mm, 5 \( \mu m \); Nomura chemical, Japan). A mobile phase consists of 95% water (containing 1% acetic acid) and 5% acetonitrile (containing 1% acetic acid). The flow rate was kept at 1 ml/min. The sample injection volume was 10 \( \mu l \). Absorbance was measured at 260 nm. The water fraction was separated into five peaks (Fig. 2). And then the water fraction was refractionated by MPLC for compounds determination. The water fraction were characterized by Mass, 1H–NMR, 13C–NMR.

6. 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) parallel to the long axis of the stomach in rats. The results are shown in Fig. 3, 4 and 5. In the crude extract of \( P. \) oleracea, the extract exhibited significant antiulcer activity in rats at doses of 300, and 500 mg/kg, similar to the reference drug cimetidine. The lesion was 51.32 mm\(^2\) in control group and 2.66 mm\(^2\) in \( P. \) oleracea pretreatment (500 mg/kg) group. \( P. \) oleracea treated groups were decreased the development of HCl–ethanol induced gastritis significantly and dose-dependently. These data suggested that \( P. \) oleracea and cimetidine have similar pharmacological activities. In administration of the fractions of extract, The
Table 1. $^{13}$C NMR (100 MHz) Data of Isolated Compounds from *P. oleracea* Water Fraction and Standard Compounds

<table>
<thead>
<tr>
<th>C</th>
<th>Fr-II</th>
<th>Uridine</th>
<th>Fr-III</th>
<th>Adenosine*</th>
<th>Fr-IV</th>
<th>Guanosine*</th>
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<tr>
<td>2</td>
<td>151.10</td>
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<td>149.30</td>
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<td>62.15</td>
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</table>

* : $^{13}$C NMR data were found in reference (Breitaimer E and Voelter W [1990] In Carbon-13 NMR spectroscopy [3rd ed.] VCH. New York).

2. Isolation and Structure Determination using LC–MS and NMR

Water fraction was further separated by MPLC and yielded four sub-fraction, Fr–I–Fr–IV. HPLC chromatograms of each sub-fraction were shown in Fig. 2. Fr-I contained several peaks and pure compound was not isolated from this fraction.

Compound isolated from Fr–II was analyzed using LC–MS and NMR. In the ESI–MS spectrum, the peak at m/z 243 was determined as the quasi-molecular ion $[M-H]^-$ and the molecular weight of the compound was 244. In the $^{13}$C NMR spectrum, five ribosyl carbons and four pyrimidin ring carbons were detected. HMQC and HMBC spectra also proved the presence of ribose and uracil. From the above results the structure of isolated compound from Fr–II was determined as uridine.

The ESI–MS spectrum of compound isolated from Fr–III showed the peak at m/z 268 corresponding to $[M+H]^+$ in the positive mode and m/z 266 corresponding to $[M-H]^-$ in the negative mode. Thus, the molecular weight of the compound was determined to be 267. In the $^{13}$C NMR spectrum, five ribosyl carbons and four purine ring carbons were detected. From the above results the structure of isolated compound from Fr–III was determined as adenosine.

The ESI–MS spectrum of compound isolated from Fr–IV showed the peak at m/z 284 corresponding to $[M+H]^+$ in the positive mode and m/z 282 corresponding to $[M-H]^-$ in the negative mode. Thus, the molecular weight of the compound was determined to be 283. In the $^{13}$C NMR spectrum...
spectrum, five ribosyl carbons and four purine ring carbons were detected. From the above results the structure of isolated compound from Fr-IV was determined as guanosine.

The water fraction yielded- Uridine, Adenosine, Guanosine which are nucleosides which possessed anti-oxidant and anti-ulcerogenic activities although the effects were not restricted to only these components.

Conclusions

1. The oral administration of *P. oleracea* inhibited the HCl-ethanol induced gastritis by dose-dependantly.
2. The aqueous fraction exhibited the effects similar to cimetidine. Among those sub fractions, agent II possessed the strongest effect in the HCl-induced gastritis.
3. The water fraction yielded- Uridine, Adenosine, Guanosine.
4. *P. oleracea* could be an option in the treatment of gastritis.

This study suggest that a *P. oleracea* and its compounds showed potent efficacy on the development of HCl-induced gastritis. Thus, *P. oleracea* can be a potential natural resource for the management of gastritis although the mechanism of action involved in the treatment remains to be explored.

References

2. Isin Y, Ismail T, Askim HS, Tijien D. Salinity tolerance of purslane (*Portulaca oleracea* L.) is achieved by enhanced antioxidative system, lower level of lipid peroxidation and proline accumulation. Environmental and Experimental Botany. 2007 ; 61 : 49-57.
14. Singleton VL, Rossi JA. Colorimetry of total