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Composition and Anti-ulcer Activity of the Essential Oil from *Citri* Reticulatae *Pericarpium* in Rodents

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HIGHLIGHTS

- The major components of the essential oil of *Citri* Reticulatae *Pericarpium* (EOC) are d-limonene (92.3%) and β-Myrcene (1.3%).
- EOC protects against gastric mucosal lesions induced by HCl/EtOH and indomethacin.
- EOC heals acetic acid-induced gastric ulcer.
- EOC preserves antioxidant enzyme and reduces oxidative stress in gastric mucosa.

Abstract: The essential oil from *Citri* Reticulatae *Pericarpium* (EOC) was analyzed by GC/MS. The major component of EOC was found to be d-limonene (92.3% of the total essential oil). Anti-ulcer and antioxidant activities of EOC were evaluated in several gastric ulcer models. The ulcer index or area and biochemical markers of superoxide dismutase (SOD) and malondialdehyde (MDA) were determined. EOC significantly inhibited gastric mucosal lesions induced by ulcerogenic agents with an inhibitory rate of nearly 50% at an oral dose of 85 mg/kg, and exerted a healing effect on acetic acid-induced ulcers with a curative rate of 63%. HCl/EtOH administration caused severe oxidative stress and lipid peroxidation, which was confirmed by significantly decreased SOD activity and increased MDA levels in mice gastric tissues. EOC pretreatment significantly increased SOD activity and decreased MDA levels, indicating that EOC protects the gastric mucosa against development of numerous lesions by inhibiting oxidative stress. These results demonstrate that EOC has anti-ulcerogenic activity and the mechanism underlying its gastric protective effect is associated with its antioxidant properties.

Keywords: *Citri* Reticulatae *Pericarpium*; essential oil composition; d-limonene; gastric ulcer; antioxidant.

INTRODUCTION

Peptic ulcer disease (PUD) is a chronic condition of the stomach and duodenum affecting approximately 5–10% of the world population [1]. Its etiology has not been fully elucidated, but it is generally accepted that PUD results from an imbalance of gastric mucosal aggressive factors (gastric acid, pepsin) and defensive factors (prostaglandins, mucosal blood flow, mucus and bicarbonates) [2]. PUD is a heterogeneous disease of multifactorial etiology [3]. The hypersecretory acidic environment of the stomach has been believed to be the main cause of PUD, but advances in clinical practice over the years have shown that *Helicobacter pylori* infection, the use of nonsteroidal anti-inflammatory drugs (NSAIDs), heavy drinking, cigarette smoking, and a stressful lifestyle can frequently lead to PUD [4]. Currently, mainstream therapy for the treatment of PUD is drugs that inhibit gastric acid secretion, including H_2 receptor blockers and proton pump inhibitors. Although the use of these medicines, especially the latter, can facilitate the repair of gastric mucosal damage and improve the clinical outcome of PUD through the potent suppression of gastric acid secretion and acid-protease activity, long term repeated use of these drugs can cause serious adverse reactions due to the low acidic environment in the stomach [5]. Consequently, PUD continues to be a challenge owing to the high incidence of relapse, undesirable side effects from treatment, and growing *H. pylori* resistance to antibiotics [6]. Therefore, there is a need for the development of novel anti-ulcer agents that are safe and therapeutically effective.

Citri Reticulatae *Pericarpium* (CRP), referred to as Chenpi in Chinese, is the dried ripe peel of *Citrus* reticulate Blanco and its cultivated varieties [7]. Owing to its effect in strengthening stomach function and aiding digestion, CRP has been popularly used as a flavoring agent and a source of food in China [8]. In traditional Chinese medicine, CRP has mostly been used for treating gastritis and other stomach disorders. The use of tangerine peels in dried powder form for the treatment of stomach ache, a folk remedy in some parts of China, has been shown to be efficacious with good ulcer healing properties and few recurrences [9]. Modern medicine has demonstrated that CRP possesses various pharmacological effects, including antibacterial, anti-inflammatory, and antioxidant effects [10-12]. The aqueous extract and hesperidin from orange (*Citrus sinensis* L.) peels exerted protective effects against oxidative stress and ulcers induced by alcohol in rats [13]. These data suggest that the essential fraction in CRP might possess anti-ulcer activity. Previous reports from Brazil have demonstrated the gastroprotective and healing effects of d-limonene and the essential oils from other *Citrus* species including *aurantium* and *lemon* on experimentally induced gastric ulcers [14,15]. The gastroprotective mechanisms of the essential oils were associated with increasing gastric mucus production by modulating prostaglandin E_2 (PGE_2) levels. The doses of the essential oils used in these rat models were as high as 250 mg/kg, and limonene, the major component of the oils, was responsible for the reduction in PGE_2 levels [16,17].

However, the dose-response relationship and mechanism of action of the essential oil from CRP (EOC) against gastric mucosal lesions have not been elucidated. Therefore, the objective of this study was to evaluate the anti-ulcer activities of EOC in several acute and chronic experimental gastric ulcer models and elucidate its possible mechanism of action in repairing gastric damage.

MATERIAL AND METHODS

Plant materials and essential oil preparation

Peels of *C. reticulatae* fruits were collected in November 2019, in Nanfeng, Middle East Jiangxi province, China. *Citrus* species were identified by Professor Z. C, Liang, Department of pharmacy, Jinggangshan University Medical School, and voucher specimens were deposited at the School Herbarium (JU 191018). Dried *C. reticulatae* peels were powdered using a high-speed grinder (Feida medical equipment factory, Ruian, China) and the resultant powder had a particle size of less than 0.6 mm. The essential oil was extracted by employing hydro-distillation using a Clevenger type device (Jigao experimental equipment Co. Ltd, Tianchang, China). The powered peels (500 g) were used for the preparation of essential oil extraction and dried over anhydrous sodium sulfate. The total quantity of 9.4 mL essential oil was obtained. The sample was stored in hermetically sealed glass containers and kept at a low temperature (4ºC) until analysis. The yield of the sample extract was 1.87%. The quantities used in the treatment of animals and GC-MS analysis were 6.0 mL and 1.0 mL, respectively.

Identification of compounds

The EOC sample was analyzed using a GC/MS (Agilent 7890B-5977B, Agilent Technologies, Wilmington, Delaware, USA) equipped with a capillary column of fused silica (DB-wax, 30 m × 0.25 mm × 0.25 µm). A

diluted sample (1/100) of oil in hexane at a 5:1 split ratio, was injected at a volume of 1.00 µL. The injector and detector were set at 260°C in split injection mode and helium (99.999%) was used as the carrier gas at a flow rate of 1.0 mL/min. The oven temperature was set at 50°C for 3 min, and then increased to 250°C at 5°C /min for 10 min. The ionization mode was EI+ 70ev, and the mass ranges were from m/z 33 to 450. Chemical identification of the unknown compounds was performed by comparison of the obtained mass spectra to the GC/MS system database (NIST2014lib.), the literature, and the Kovats retention indices.

Animals

150 Male Chinese Kun Ming (KM) mice aged 4 weeks weighing between 20–25 g and 24 male Sprague Dawley (SD) rats aged 8 weeks weighing between 200–250 g was obtained from Hunan SJA Laboratory Animal Co., Ltd (Changsha, China). The animals were left to acclimatize to laboratory conditions for one week before subjecting them to the study. They were provided with a standard pellet diet and had access to water ad libitum. The animals were housed at a temperature of 23 ± 2°C and exposed to a 12 h light/dark cycle, as well as moderate humidity (50% ± 5%) throughout the experimental period. All animal experiments were carried out according to international standards and guidelines for the care and use of laboratory animals. Ethical clearance for performing the experiments on animals was obtained from the Institutional Animal Ethics Committee of Jinggangshan University (20190406).

HCl/EtOH-induced gastric ulcers in mice

This experimental procedure was carried out according to the method described by Mizui and coauthors [18] with slight modifications. The mice were randomly divided into five groups (n = 10). The vehicle-treated group received 0.1 mL/10g of 2% Tween® 80 p.o. The other four groups were pre-treated with various doses of EOC (85, 170 and 255 mg/kg, p.o.) and omeprazole (OME, 8 mg/kg, p.o.), respectively. The period of pretreatment lasted for 5 days. The mice were left in a fasted state for 24 h prior to administering 0.3 M HCl in 60% EtOH (0.2 mL/animal, p.o.) to induce gastric lesions, 1 h after the last pretreatment dose. After 1 h, the mice were sacrificed by cervical dislocation，followed by immediate ligation of the cardia and pylorus prior to injecting 1 ml of a 2% formaldehyde solution into the stomach of each animal. The stomach was then removed from each animal and fixed in 2% formaldehyde solution for 30 min. The stomachs were cut along the greater curvature and the gastric mucosal lesions were characterized by hemorrhage or erosion. Bleeding points, plaques, and strips were measured, and the total sum of lengths was expressed as an ulcer index (UI).

$$\text{Ulcer inhibitory (\%)} = (\text{UI vehicle} - \text{UI test}) / \text{UI vehicle} \times 100 \quad (1)$$

Indomethacin-induced gastric ulcers in mice

This procedure was carried out according to the method described by Nwafor and coauthors [19]. The mice were randomly divided into five groups (n = 10) and the treatment of each group was the same as described above. The pretreatment period was 5 days and the mice were left in a fasted state for 24 h prior to administering indomethacin (30 mg/kg) intraperitoneally (i.p.) to induce gastric lesions, 1 h after the last pretreatment dose. The mice were sacrificed by cervical dislocation after 7 h. The degree of gastric mucosal lesions in the glandular stomach was evaluated using the ulcer index and the percentage of inhibition as described above.

Acetic acid-induced gastric ulcers in rats

This experiment was carried out according to the method described by Takagi and coauthors [20] with slight modifications. The SD 24 rats were randomly divided into 4 groups, containing sham operation group, vehicle-treated group, EOC group and pantoprazole group. After 12 h of fasting, the rats were anesthetized using 1% pentobarbital sodium (0.5 mL/100 g, i.p.). A laparotomy was performed through a midline epigastric incision approximately 2 cm away from the xiphoid process. The stomach was then removed from the abdominal cavity, following which 0.05 mL (v/v) of a 10% acetic acid solution was injected into the subserosa layer in the glandular portion of the anterior wall of the stomach until a translucent white spot with a diameter of 3 mm appeared. The stomach was then bathed with saline (20°C) to avoid adherence to the external surface of the ulcerated region before being inserted back into the abdominal cavity, covered with omentum and peritoneum, and the muscular layer and skin sutured to close the abdomen.

On the third day after the operation, the sham operation group and the vehicle-treated group received 2% Tween® 80 (10 mL/kg) via intragastric administration, while the other two groups were respectively treated

with EOC (170mg/kg, p.o., a medium effective dose for acute ulcer models in mice) and pantoprazole (PAN, 4mg/kg, p.o.), once a day for 12 consecutive days. After the last treatment, the rats were fasted for 24 hours before they were sacrificed. Their stomachs were removed and opened at the greater curvature. The ulcers were easily identified on the inner gastric surface of the anterior wall injected with acetic acid, and the maximum lengths and widths of the ulcers were measured using vernier calipers. The ulcer area (UA) was calculated as the length times the width of the ulcer, and ulcer healing (%) was determined as (UA vehicle – UA test) / UA vehicle × 100.

Biochemical determination of SOD and MDA in serum and gastric tissue

SOD and MDA were detected using the HCl/EtOH-induced gastric ulcer models. The experimental method is described above. However, during this study, the pretreatments included the oral administration of EOC (85, 170 mg/kg) or PAN (8 mg/kg). Blood samples were drawn from the retro-orbit plexus of the mice and placed at 25°C room temperature. The samples were then centrifuged at 3000 g for 10 min in order to separate the serum. After collection of the blood samples, the mice were sacrificed, their stomachs immediately removed, opened along the greater curvature, washed in ice-cold normal saline, and weighed. The samples of serum and gastric tissue were stored in a refrigerator set at -80°C for subsequent biochemical analyses. Prior to analysis, the stomach samples were thawed to 25°C room temperature, diluted in 0.9% normal saline (1:9, w/v), and homogenized using a high-speed homogenizer at 15000 rpm during 10 s for 3 times. The resultant homogenates were centrifuged at 3000 g during 10 min to obtain the supernatants. Quantification of SOD and MDA was then performed using the SOD and MDA kit according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute). The hydroxylamine method was used to measure SOD at an absorbance of 550 nm, and the results were expressed as U/mL serum or U/g tissue, while the thiobarbituric acid method was used to measure MDA at an absorbance of 532 nm, and the results were expressed as nmol/mL serum or nmol/g tissue.

Statistical analysis

All experimental results were recorded as the mean ± standard error of the mean (S.E.M.) The significance of difference was assessed using one-way analysis of variance (ANOVA), followed by the Dunnett's post hoc test or the student's *t*-test for comparison between two groups. The level of significance was set at $P < 0.05$.

RESULTS

Chemical composition of EOC

The compounds identified in EOC are shown in Table 1. Analysis of the oil using GC/MS led to the identification of 21 different compounds (97.6% of the total essential oil). The major constituent of EOC was d-limonene (92.3%), followed by β-myrcene (1.3%), and γ-terpinene (1.2%). In addition, monoterpene hydrocarbons with the molecular formula $C_{10}H_{16}$, were characterized as the most abundant chemical structure (96.1%). The total ion chromatograms (TIC) of EOC are depicted in Figure 1.

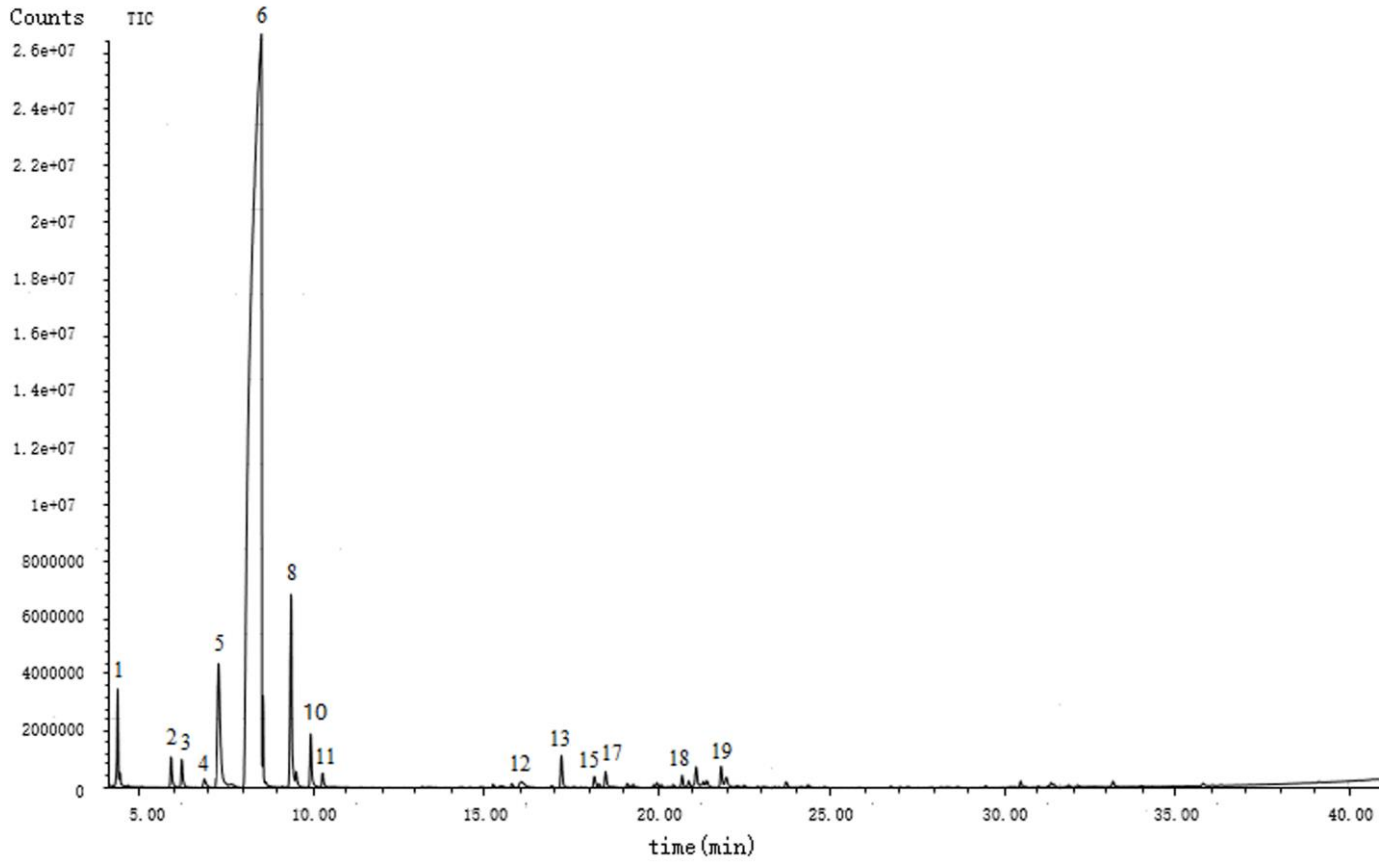


Figure 1. GC/MS chromatogram following the analysis of the essential oil from *Citri* Reticulatae *Pericarpium*.

Table 1. Chemical composition of the essential oil from *Citri* Reticulatae *Pericarpium*

NO.	Compound	RI[a]	RI[b]	Composition (%)	Identification
1	d-α-Pinene	1044	1043	0.4	GC/MS, RI
2	(-)-β-Pinene	1106	1106	0.2	GC/MS, RI
3	Sabinene	1119	1119	0.2	GC/MS, RI
4	3-Carene	1145	1145	0.1	GC/MS, RI
5	β-Myrcene	1162	1162	1.3	GC/MS, RI
6	d-Limonene	1211	1211	92.3	GC/MS, RI
7	β-Phellandrene	1213	1213	0.2	GC/MS, RI
8	γ-Terpinene	1244	1244	1.2	GC/MS, RI
9	β-Ocimene	1249	1248	0.1	GC/MS, RI
10	p-Cymene	1265	1265	0.3	GC/MS, RI
11	Terpinolene	1278	1278	0.1	GC/MS, RI
12	Decanal	1497	1497	0.1	GC/MS, RI
13	Linalool	1545	1545	0.2	GC/MS, RI
14	Diethylene glycol monoethyl ether	1546	1577	0.2	GC/MS, RI
15	β-Elemene	1583	1583	0.1	GC/MS, RI
16	Bicyclosesquiphellandrene	1584	1530	0.1	GC/MS, RI
17	(-)-4-Terpineol	1597	1597	0.1	GC/MS, RI
18	α-Terpineol	1692	1692	0.1	GC/MS, RI
19	α-Farnesene	1742	1742	0.1	GC/MS, RI
20	(-)-trans-Isopiperitenol	1747	1745	0.1	GC/MS, RI
21	Geranyl acetate	1750	1750	0.1	GC/MS, RI
	TOTAL			97.6	

RI[a]: Retention indices (DB-wax column); RI[b]: Retention indices searched in the National Institute of Standards and Technology (NIST) WebBook Database (https://webbook.nist.gov) and citations therein.

Effect of EOC on mouse models of acute gastric ulcers

The anti-ulcer activity of EOC in HCl/EtOH and indomethacin-induced gastric ulcer models is shown in Table 2.

In studies involving the use of HCl/EtOH to induce gastric ulcers, the ulcer index significantly increased in the EOC and OME-treated groups compared to the vehicle-treated group ($P < 0.05$ and $P < 0.01$, respectively). These results indicated that gastric mucosal injury induced by HCl/EtOH was significantly inhibited by pretreatment with 85, 170 and 255 mg/kg oral doses of EOC. Their percentage inhibition scores were 45.0%, 53.2%, and 66.5%, respectively, while that of 8 mg/kg OME was 61.6%.

In the mouse models where indomethacin was used to induce gastric ulceration, the ulcer index significantly decreased in EOC and OME-treated groups compared to the vehicle-treated group ($P < 0.05$ and $P < 0.01$, respectively), indicating that pretreatment with EOC at the investigated doses (85, 170 and 255 mg/kg) inhibited gastric mucosal damage induced by indomethacin. The observed percentage inhibition values were 48.6%, 55.0% and 61.9%, respectively, while that of 8 mg/kg OME was 58.8%.

Table 2. Effect of EOC on gastric ulcers induced by HCl/EtOH and indomethacin in mice

Pretreatment	Dose (mg/kg)	Ulcer index of HCl/EtOH	Inhibition (%)	Ulcer index of indomethacin	Inhibition (%)
Vehicle	-	37.29±12.47	0	6.75±1.08	0
EOC	85	23.14±9.46*	45.0	3.47±1.78*	48.6
	170	17.46±8.85*	53.2	3.04±1.33*	55.0
	255	12.50±8.53**	66.5	2.57±1.47**	61.9
OME	8	14.33±7.98 **	61.6	2.78±1.72*	58.8

Note: Vehicle (2% Tween® 80, 10 mL/kg, p.o.); EOC (The essential oil from *Citri* Reticulatae *Pericarpium*, p.o.); OME (Omeprazole, p.o.). Results are expressed as mean ± S.E.M., $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the vehicle-treated group.

Effect of EOC on acetic acid-induced gastric ulcers in rats

The macroscopic typical ulcers were observed to be round or oval with deep central fovea. In the vehicle-treated group, the ulcers were larger and deeper with white moss on the bottom, and the surrounding mucosa was edematous and uplifted compared to the EOC or PAN-treated groups. Table 3 shows that the mean ulcer area in the vehicle-treated group was 38.75 mm^2, while the mean areas in the EOC-treated (170 mg/kg) group and PAN-treated (4 mg/kg) group after 12 days of treatment were 14.33 and 9.46 mm^2, respectively. There were significant differences between the vehicle-treated group and the EOC and PAN-treated groups ($P < 0.01$), indicating that oral treatment with EOC could promote healing of the damaged gastric mucosa. Although the percentage of healing of ulcer in the PAN group (75.59%) was higher than that of the EOC group (63.02%), the difference between these two groups was not statistically significant ($P > 0.05$).

Table 3. Effect of EOC on gastric ulcers induced by acetic acid in rats

Treatment	Dose(mg/kg)	Ulcer area (mm^2)	Healing (%)
Sham	-	0	-
Vehicle	-	38.75±18.21	0
EOC	170	14.33±4.63**	63.0
PAN	4	9.46±7.22**	75.6

Note: Sham (no treatment); Vehicle (2% Tween®80, 10 mL/kg, p.o.); EOC (The essential oil from *Citri* Reticulatae *Pericarpium*, p.o.); PAN (Pantoprazole, p.o.). Results are expressed as mean ± S.E.M., $^{**}P < 0.01$ compared to the vehicle-treaded group.

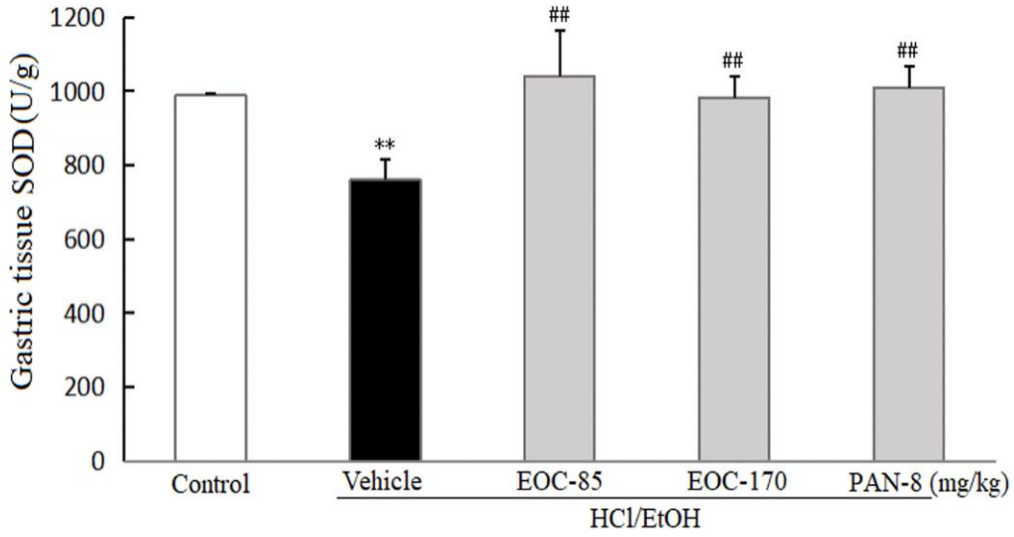
Effect of EOC on SOD and MDA in gastric ulcers induced by HCl/EtOH

The effect of EOC on SOD in gastric tissue and serum from mice with HCl/EtOH-induced gastric ulcers is shown in Figure 2.

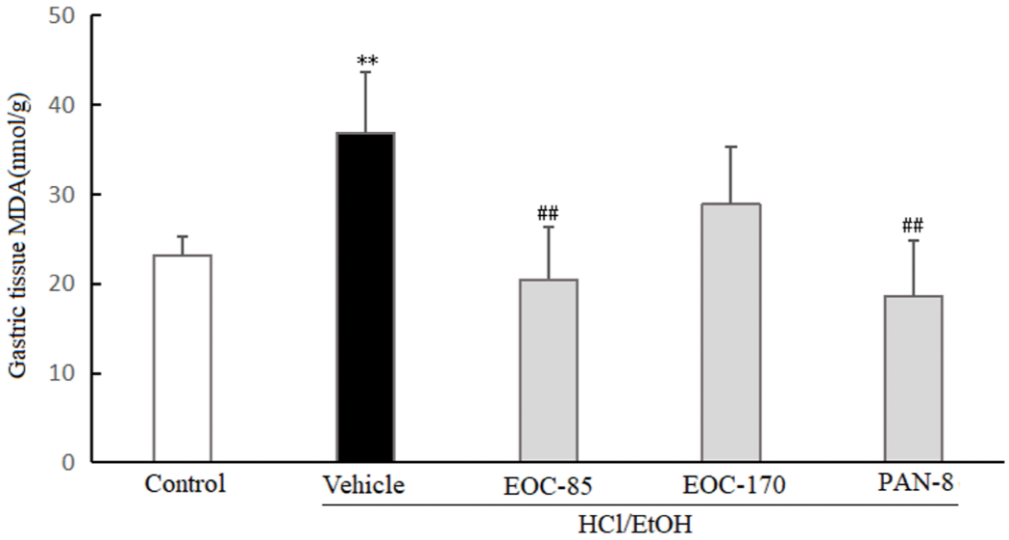
Compared to the control group, the activity of SOD in gastric tissue significantly decreased (989.67 ± 4.21 vs 761.46 ± 124.90 U/g tissue, $P < 0.01$) in the vehicle group, while SOD activity in the gastric tissues increased significantly after pretreatment with EOC (85, 170 mg/kg) or PAN (8 mg/kg). The SOD values of the three groups were 1040.54 ± 54.06, 981.68 ± 57.54 and 1010.89 ± 57.70 U/g tissue, respectively compared to the vehicle group (761.46 ± 124.90 U/g tissue, $P < 0.01$).

Compared to the control group, the activity of SOD in serum significantly decreased (99.30 ± 1.02 vs 79.43 ± 6.66 U/mL serum, $P < 0.01$) in the vehicle group, while the serum SOD activity significantly increased after pretreatment of EOC at 170 mg/kg (not 85mg/kg) or PAN at 8mg/kg. The SOD values of the three groups were 81.07±5.90, 85.92±6.31 and 85.61±3.48 U/mL serum, respectively compared to the vehicle group (79.43 ± 6.66 U/mL serum, $P < 0.01$).

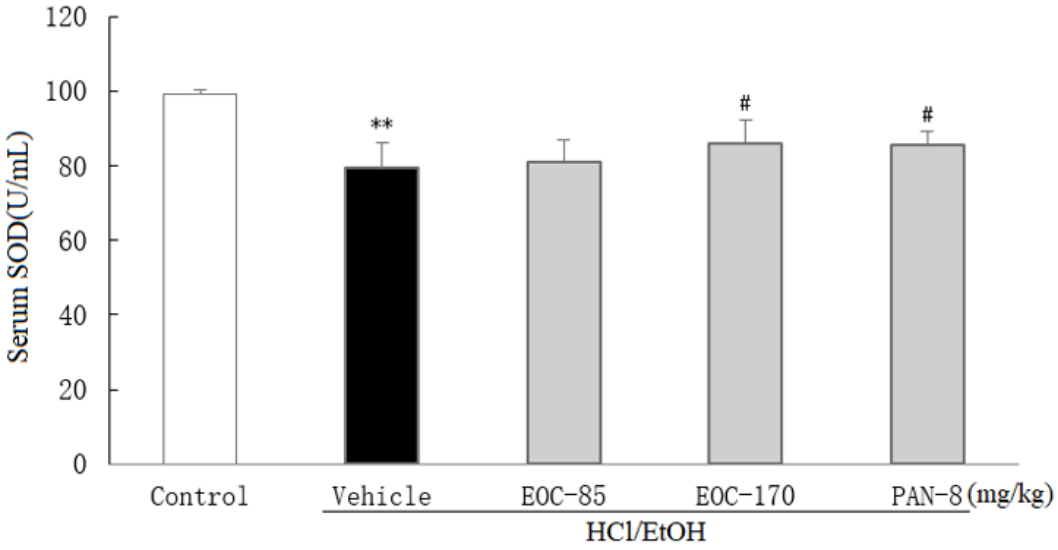
The MDA levels in the gastric tissues increased significantly in the vehicle group (23.15 ± 2.12 vs 36.88 ± 6.83 nmol/g tissue, $P < 0.01$) compared to the control group, while pretreatment with 85 mg/kg EOC or 8 mg/kg PAN significantly decreased MDA in these tissues. The MDA values of the two groups were 20.49 ± 5.79 and 18.58 ± 6.23 nmol/g tissue, respectively, which were significantly different from those of the vehicle group ($P < 0.01$).



(a)



(b)



(c)

Figure 2. Effect of EOC on SOD and MDA in gastric tissue (a, b) and SOD in serum (c) from HCl/EtOH-induced gastric ulcer models of mice. Control (no treatment); vehicle (2% Tween® 80, 10 mL/kg, p.o.); EOC (85, 170 mg/kg, p.o.), PAN (8 mg/kg, p.o.). Results are expressed as mean ± S.E.M., $^{**}P < 0.01$ compared to the control group and $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared to the vehicle-treated group.

DISCUSSION

CRP has been used in traditional Chinese medicine for more than two thousand years. Numerous species of CRP are available in the market and four of them are listed in the Pharmacopoeia of the People's Republic of China [7]. Reports have shown considerable differences in the CRP yields and % composition of d-limonene in EOC from different cultivars and regions [21]. In the present study, the yield of EOC obtained from a sample in the Jiangxi province of China was 1.87%, and GC/MS analysis revealed that the most abundant component of the sample was d-limonene (92.3% in the total essential oil), which is higher than that reported in previous literature [8]. Recent study showed that CRP is a safe, nongenotoxic herbal medicine [22]. Limonene is commonly used as flavoring in food and drink, and it has been designated as a chemical with low toxicity [23]. Due to the abundance *Citrus* resources in the Jiangxi province, exploitation of CRP and EOC for health-related products or natural medicines can be of commercial value.

Considering that an ulcer can be clinically induced by different etiologies and mechanisms, various animal models of acute gastric mucosal injuries are used to evaluate the anti-ulcer activities of potential drugs or compounds. The results obtained from this study showed that EOC possessed good anti-ulcer activity against experimental acute gastric ulcers induced by HCl/EtOH and indomethacin, with comparable ulcer inhibition doses in each model. The rat model of acetic acid-induced gastric ulcer is the standard model for screening of new anti-ulcer drugs, because the pathological features and healing mechanisms of these ulcers are closely similar with PUD in humans [24]. The rat models were used to investigate chronic ulceration and the study results showed that the area of ulceration was significantly reduced after treatment with EOC, and ulcer healing reached 63%, indicating that EOC significantly promoted ulcer healing.

Reactive oxygen species (ROS) play an important role in the pathogenesis of gastrointestinal mucosal injuries induced by most of the ulcerative factors [25,26]. In response to the effect of various noxious agents, a massive generation of ROS in gastric tissues occurs, in which, its concentration increases much more than in other tissues or blood [27]. SOD is a well-known superoxide radical scavenger that can catalyze the dismutation of ROS to non-toxic H_2O_2 and O_2 [28]. Antioxidant enzyme systems such as SOD and catalase (CAT) are the first line of defense against ROS [29]. The present study showed that SOD levels in gastric tissues were much higher than those in the serum, suggesting a higher level of oxidative stress and local antioxidant concentration in the gastric mucosa.

HCl/EtOH-induced gastric ulcers in animals are a good preclinical model since the model includes two common factors that can induce PUD in humans [30]. High concentrations of alcohol can induce massive production of ROS in gastric mucosal tissue [31]. When excessive generation of ROS exceeds the ability of ROS scavenging in the epithelial cells, these species cause oxidative stress and depletion of antioxidant enzymes such as SOD, finally resulting in damage of the gastric mucosa [32,33]. ROS, combined with

polyvalent unsaturated fatty acids of cell membranes, causes lipid peroxidation resulting in the formation of MDA. MDA can thus be considered an indicator of increase in lipid peroxidation and inflammatory process [34,35]. It is known that *citrus* essential oils have scavenging effects on free radicals in *vitro* [12]. Owing to this scavenging activity, our studies aimed to investigate the effect of these oils in vivo. The results of the studies performed showed that, the administration of HCl/EtOH significantly decreased SOD activity and increased oxidative stress and MDA levels in the gastric tissues of mice. These findings revealed severe oxidative stress and lipid peroxidation in the gastric epithelial cells. EOC pretreatment significantly increased the activity of the antioxidant enzyme, SOD, and decreased the levels of the inflammatory marker, MDA, indicating that EOC decreased gastric mucosal damage by preserving vital antioxidant enzyme activities and inhibiting lipid peroxidation due to scavenging ROS.

In this study, EOC at the lower dose of 85 mg/kg exerted the best antioxidant effect in gastric tissue, and there was a dose-effect relationship in the gastroprotective effects of EOC against acute gastric injures estimated using ulcer index, indicating the anti-ulcer effect of EOC also involves other mechanisms. Moraes TM and coauthors [15,16] showed that the essential oils from *Citrus aurantium* (250 mg/kg) or limonene (245 mg/kg) increased PGE_2 levels in the gastric mucosa and didn't display any evidence of toxicity during the 14-day for acetic acid-induced gastric ulcer in rats. It is considered that EOC at a dose of 255 mg/kg used in mice or 170 mg/kg used in rats is safe and effective, however, lower doses of EOC might be more valuable in the chronic treatment of peptic ulcer due to its good antioxidant and anti-inflammatory effects. The effect of the antacid (PAN) on SOD and MDA was similar with that of two doses of EOC, suggesting that the mechanism of gastric mucosal damage induced by gastric acid involves ROS and oxidative reactions.

CONCLUSION

To the best of our knowledge, this is the first report that has quantified the content of d-limonene in EOC from the Jiangxi province of China up to 92.3% of the total essential oil. The study demonstrated that EOC possessed gastroprotective and healing effects by using various animal models of gastric ulcers, which provided chemical and pharmacological evidence for the traditional and folk therapeutic applications of CPR. EOC successfully increased SOD activity and decreased MDA levels. Therefore, the underlying mechanism behind the anti-ulcerogenic properties displayed by EOC is mainly attributed to its potent antioxidant and anti-inflammatory properties in the gastric mucosa. However, in-depth studies should be carried out to clarify the efficacy and mechanism of lower doses of EOC in chronic treatment of gastric ulcer.

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