



Protective effect of *Corchorus capsularis* L. leaves on ethanol-induced acute gastric mucosal lesion in rats

Chiu-Fang LEE^{1) #}, Ching-Wen FAN^{2) #}, Ni-Na CHIANG¹⁾, Hsiou-Chuan CHANG²⁾, Chun CHEN²⁾, Yu-Syuan HUANG²⁾, Hui-Yun WANG²⁾, Wen-Chuan LIN³⁾ and Fu-An CHEN^{2) *}

¹⁾Kaohsiung Veterans General Hospital Pingtung Branch, Pingtung 912, Taiwan

²⁾Department of Pharmacy and Master Program, Tajen University, Pingtung 907, Taiwan

³⁾Taiwan Association of Chinese Medicine, Chiayi 600, Taiwan

ABSTRACT. In Taiwan, *Corchorus capsularis* L. has long been cultivated and the leaves are consumed as edible vegetable. This study is to investigate the protection effect of extract of *C. capsularis* leaves (ECC) on ethanol-induced acute gastric mucosal lesion (AGML) in rats. The results of phytochemical determination in ECC for total polyphenol, flavonoid and polysaccharide were 59.88 ± 0.61 mg/g, 86.39 ± 18.0 mg/g and 320.89 ± 6.99 mg/g, respectively. ECC showed significant activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging with IC_{50} of 0.25 mg/ml. *In vivo* studies, Sprague-Dawley (SD) rats were randomly divided into five groups: sham, vehicle (control) and low-, medium-, and high-dose ECC (LECC, MECC, HECC; 200, 400, and 1,000 mg/kg/day, respectively). ECC was able to decrease significantly the ulcer index (UI) caused by 80% ethanol in a dose dependent manner. There was no significant effect on growth trend and food intake rate after the administration of ECC in the experimental period. The serum lipid parameters in ECC groups revealed significant increase in glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT), and a decrease in malondialdehyde (MDA). Significant amelioration on pathological lesion score was found in ECC groups compared with the control group ($P < 0.05$). The overall results indicate that ECC has protective effects on ethanol-induced AGML in rats, which could be associated with its antioxidant activity.

KEY WORDS: acute gastric mucosal lesion (AGML), *Corchorus capsularis* L., gastroprotection

J. Vet. Med. Sci.

81(11): 1636–1642, 2019

doi: 10.1292/jvms.19-0054

Received: 24 January 2019

Accepted: 24 August 2019

Advanced Epub:

16 September 2019

A substantial number of people worldwide have been suffering from peptic ulcers which can be commonly classified into either gastric ulcers and duodenal ulcers [31]. The prevalence of peptic ulcers has been documented to be about 10% in the West and East [19]. In Taiwan, about 67% of peptic ulcer patients had no remarkable symptoms [33]. Peptic ulcers are caused by several conditions such as heavy drinking, smoking, emotional stress, caffeinated drinks, non-steroidal anti-inflammatory drugs (NSAIDs) and infection of *Helicobacter pylori* [11, 18]. Peptic ulcers result from an imbalance between the aggressive (acid, pepsin and *Helicobacter pylori*) and mucosal defensive (mucin, prostaglandin and mucosal secretion) factors [32]. In addition to the inhibition of aggressive factors, the strengthening of gastric mucosal protection provides one of the more effective approaches for the prevention and treatment of peptic ulcer disease [8, 9, 29]. Considering the fact that most medicines have their own limitations and potential adverse symptoms, natural herbal medicines are becoming increasingly proposed as an alternative source of treatment (for medicinal purposes).

Corchorus capsularis L., a perennial herb with alias White Jute and Ma Yi, has long been cultivated in Taiwan and the leaves are used as a foodstuff. The Zhong Yao Da Ci Dian (Chinese Materia Medica Grand Dictionary) recorded that *C. capsularis* root has antipyretic and antidiarrheal effect; leaves have hemostatic, myogenic and laxative effect; seeds have anesthetic, sedative and antipyretic effect; and the whole plant is used in the treatment of detumescence, and heatstroke [16]. The Illustration of Common Medicinal Plants in Taiwan recorded that *C. capsularis* contained β -sitosterol, β -sitosterol-D-glucoside, capsularone, corchorol, capsularol, glucose, galactose, arabinose, capsin, capsugenin-30-O- β -glucopyranoside and capsugenin. Antinociceptive and anti-inflammatory activities of *C. capsularis* chloroform extracts have been reported [37]. In Taiwan, leaves of *C. capsularis* have been popularly applied as an edible vegetable and was cited in folklore with gastroprotection. However, little research is known about

*Correspondence to: Chen, F. A.: fachen.tajen@yahoo.com.tw

#These authors contributed equally to this work.

(Supplementary material: refer to PMC <https://www.ncbi.nlm.nih.gov/pmc/journals/2350/>)

©2019 The Japanese Society of Veterinary Science



This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License. (CC-BY-NC-ND 4.0: <https://creativecommons.org/licenses/by-nc-nd/4.0/>)

the gastroprotective effect of the leaves of *C. capsularis*.

Alcohol consumption is acknowledged as an aggressive factor resulting in acute gastric mucosal lesion (AGML) [7, 15]. The mechanism of ethanol-induced AGML is a multi-factorial disease which oxygen species and free radicals provide the risk [26], and evidence indicates that pro-inflammatory cytokines, oxidative stress and apoptosis play crucial roles in interpretation of the cause [4, 21, 23, 25, 27]. Nevertheless, oxygen radical production with oxidative stress implicated in the lesion of gastric mucosal cell membranes via lipid peroxidation could play a significant role in the pathogenesis of ethanol-induced AGML [10, 11, 30]. *C. capsularis*, with antioxidant activity has been reported in a literature [3]. Therefore, the present study investigated the protective effect of *C. capsularis* leaves on ethanol-induced acute gastric mucosal damage in rats.

MATERIALS AND METHODS

Materials

Fresh *C. capsularis* leaves harvested in summer were purchased from local store (Da-Duo-Fu Co.) in Taichung (Taiwan). Folin-Ciocalteu reagent, DPPH (1,1-diphenyl-2-picrylhydrazyl) and ethanol were bought from Sigma Aldrich (Merck KGaA, Darmstadt, Germany). All the other chemicals were analytical reagent grade.

Preparation of *C. capsularis* extract

Fresh *C. capsularis* leaves (443.31 g) were dried through free-drying by a lyophilizer (Panchun, Taipei, Taiwan) and then ground to powders using an electrical blender (Rong Tsong, Taipei, Taiwan). The ground powders were obtained by extraction with distilled water for 4 hr using a reflux extraction apparatus (Angu, Kaohsiung, Taiwan). The aqueous extract solution was filtered through filter paper in a filter funnel. The extract of *C. capsularis* leaves (ECC) was obtained through solvent evaporation and freeze-drying procedures. After that, the ECC was then stored in an electronic dry cabinet (Taiwan Dry Tech Corp., Taipei, Taiwan) for the following experiment.

Analysis of phytochemical compositions in ECC

The phytochemical determination in ECC was conducted including total polyphenols, total flavonoids and polysaccharides. Total polyphenols in ECC were estimated spectrophotometrically using Folin-Ciocalteu reagent based on a colorimetric oxidation/reduction reaction [12, 24]. In brief, 10 mg of ECC was dissolved with 10 ml of acetone solution (acetone: H₂O=6:4). 0.2 ml of ECC solution was mixed with 7.5% Na₂CO₃ and 1.0 ml of Folin-ciocalten reagent were added to each well of a 96-well microplate (Thermo scientific, Denmark) and mixed thoroughly. After a 30 min incubation period at room temperature, color change was measured with a spectrophotometer at 765 nm using an ELISA reader (Spectra MAX190, Molecular Devices, San Jose, CA, U.S.A.). The total polyphenol content was expressed as microgram of gallic acid equivalent (GAE). For total flavonoid determination, the formation of chelatic colorimetric compounds when reacted with aluminium chloride was used to measure total flavonoids [12, 14]. In brief, 0.5 ml diluted ECC solution was prepared by adding 10 ml 80% methanolic solution to 0.1 g ECC. The solution was mixed with 0.15 ml of 10% aluminium chloride and 2 ml of 4% sodium hydroxide, and adjust volume to 5 ml with distilled H₂O. After standing for 15 min at room temperature, color change was measured with a spectrophotometer (Hitachi, Tokyo, Japan) at 510 nm. Total flavonoid content was expressed in milligrams per gram rutin equivalent (RE). The polysaccharide content in ECC was measured by the phenol-sulfuric acid method [12, 22]. Briefly, 1 ml of ECC was mixed with 1 ml of 5% phenol solution and 5 ml of concentrated sulfuric acid. After shaking the mixtures in a waterbath for 30 min at 30°C, the mixtures were added to each well of a 96-well microplate and mixed thoroughly. Color change was measured with a spectrophotometer at 490 nm using an ELISA reader. Total polysaccharide concentration was expressed as galactose equivalents.

Determination of DPPH free radical scavenging activity of ECC

The antioxidant activity of ECC was measured with a DPPH free-radical scavenging assay [6, 12]. The stock solution of ECC (4 mg/ml) was prepared and diluted with methanol to make sample solution at different concentrations. Aliquot of 50 μ l of each dilution was added into the 96-well microplate. Measurement of O.D. value by a microplate reader, at 490 nm after addition of 150 μ l working solution of DPPH (250 μ M) into each well for 30 min. 50% inhibitory concentration (IC₅₀) of ECC on DPPH free radical scavenging activity was calculated.

Test animals

A total of 40 male Sprague Dawley (SD) rats were bought from the BioLASCO (Taipei, Taiwan) and housed under standard laboratory conditions in a 12 hr each of light and dark cycle and temperature (22 \pm 2°C) controlled animal facility. Standard animal food (Fwusow Industry Co., Ltd., Taichung, Taiwan) and sterilized water were available *ad libitum*. Rats were procured 1 week prior to testing to allow them to acclimatize to the laboratory environment and diet before the experiments. This study was permitted by the appropriate animal care and use committees of Tajen University (Pingtung, Taiwan): approval No. IACUC-102-07.

Protective evaluation of ECC against ethanol-induced acute gastric mucosal lesion in rats

The ethanol-induced acute gastric mucosal lesion was carried out according to the method as described previously [5]. The male SD rats (7 weeks old) were randomly allotted into 5 groups (Sham; control; LECC 200 mg/kg/bw; MECC 400 mg/kg/bw; HECC 1,000 mg/kg/bw) of 8 animals each and fasted for 24 hr before the experiment, but had free access to water. Groups of animals

received daily ECC (200 or 400 or 1,000 mg/kg) or vehicle, the sterilized water (10 ml/kg, control) as gastric gavages. After 7 days, 4 groups of animals were then subjected to 80% ethanol (10 ml/kg) treatment with exception of the sham group. After 4 hr, all groups of animals were euthanized with CO₂ inhalation and the stomachs were removed. The removed rat stomachs were incised along the greater curvature and rinse with normal saline to remove blood clots and gastric debris contents. The morphology of each stomach was photographed using a digital camera (Nikon, Tokyo, Japan). The AGML areas in glandular stomach of each rat were calculated based on the software of a USB Microscope M2 (Myguard, Taoyuan, Taiwan). Semi-quantitative analysis of the gastric lesion was examined macroscopically and classified according to a scoring system. The ulcer index (UI) is percentage of lesion area in relation to total stomach area. Gastroprotection (%) was calculated according to: % gastroprotection=(UIC-UIT) × 100/UIC, where UIC is ulcer index in control and UIT is ulcer index in test.

Antioxidant effect of ECC on ethanol-induced acute gastric mucosal lesion

In addition, the blood from hepatic veins was collected for biochemical and antioxidant analyses including glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA). SOD activity was measured using reagent kit (SD 125; Randox Laboratories, Antrim, U.K.). GPx activity was measured by using commercial kit (RS 504; Randox Laboratories). CAT activity was carried out using the Beers and Sizer's method. In brief, take 54.6 μl of enzyme and dilute it to 3 ml with PBS (200 unit/ml). The solution was prepared with 30 μl of dilution enzyme, 570 μl ddH₂O and 300 μl sample solution. The data was obtained at 240 nm after 3 min of reaction.

In case of MDA analysis, a sample solution containing homogenate (2 g/10 ml), Tris-HCl (pH 7.2), FeCl₂ (4 mM) and ascorbic acid (0.1 mM) was prepared and shaken to ensure it was well mixed. Then the sample solution was bathed at 37°C water for 1 hr. Each sample solution was mixed with 500 μl of 0.1 N HCl and 200 μl of 9.8% SDS. 900 μl of pure water was added into the sample solution and then mixed well with 2 ml of 0.6% TBA. After incubation at 9°C for 1 hr, the solution was then cooled to room temperature for 5–10 min. After addition of 5 ml n-butanol and mixed well, centrifugation at a speed of 3,000 rpm was carried out at 25°C for 25 min. Aliquot of 200 μl supernatant was spiked into a 96-well microplate and the absorbance was measured at 532 nm by a ELISA reader.

Histopathological evaluation of ethanol-induced acute gastric mucosal lesions

Stomachs were fixed in a 10% buffered formalin solution. The fixed stomachs were embedded in paraffin wax and processed in a paraffin tissue processing machine (Leica, Nussloch, Germany). Sections of the stomachs were made to a thickness of 5 μm and stained with hematoxylin and eosin (H & E) for microscopic examination (Nikon). The evaluation of histoscore on gastric mucosal lesions histopathology in the glandular stomach was deliver to 5 levels: 1= minimum (<1%); 2= slightly (1–25%); 3= medium (26–50%); 4= medium/seriously (51–75%); 5= seriously (76–100%).

Statistical analysis

Data are expressed as mean ± SD. Statistical comparisons were analyzed by one-way ANOVA and subsequently the Duncan test was performed using a SPSS statistic software, version 10.0, SPSS Inc., Chicago, IL, U.S.A. *P*<0.05 was considered statistically significant.

RESULTS

The phytochemical compositions and the free radical scavenging activity of ECC

C. capsularis leaves aqueous extract with 19.5% yield was obtained in preparation. The extract's phytochemical analysis of total polyphenols, total flavonoids, polysaccharides and the DPPH free radical scavenging activity were investigated in this study. The phytochemical compositions of ECC are listed in Table 1. Phytochemical compositions for total polyphenol, flavonoid and polysaccharide in ECC were 59.88 ± 0.61 mg GAE/g, 86.39 ± 18.0 mg RE/g and 320.89 ± 6.99 mg/g, respectively. Table 1, polyphenols and flavonoids' content shows similar levels in ECC. The polysaccharides content had approximately quintuple more than that of polyphenols and flavonoids. It seems that polysaccharides are the abundant constitute in ECC. In the antioxidant activity performance of ECC, DPPH free radical scavenging activity assay method was applied to the free radical scavenging activity of ECC. The results revealed that ECC possessed significant DPPH free radical scavenging activity in a concentration-dependent manner (Table 2).

Table 1. The analysis of phytochemical composition in extract of *Corchorus capsularis* leaves (ECC)

| Ingredients | Amount |
|-----------------|----------------------------------|
| Polyphenols | 59.88 ± 0.61 mg GAE/g |
| Flavonoids | 86.39 ± 18.0 mg RE/g |
| Polysaccharides | 320.89 ± 6.99 mg/g ^{a)} |

Data are presented as the mean ± SD of three independent experiments. a) *P*<0.01 as compared with polyphenols and flavonoids. GAE: Gallic acid equivalent; RE: Rutin equivalent.

Table 2. DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity of extract of *Corchorus capsularis* leaves (ECC)

| ECC (mg/ml) | DPPH inhibition (%) ^{a)} |
|-------------|-----------------------------------|
| 0.8 | 84.02 ± 1.05 |
| 0.6 | 79.30 ± 0.17 |
| 0.4 | 77.79 ± 0.40 |
| 0.2 | 46.54 ± 1.34 |
| 0.1 | 28.46 ± 1.40 |

a) The free radical scavenging activity was evaluated as the DPPH scavenging percentage based on the reduction of the absorbance at 490 nm in the presence of ECC for 30 min. Data are presented as the mean ± SD (n=3).

ECC at concentration of 0.8 mg/ml was able to eliminate higher than 80% of DPPH free radicals with IC_{50} about 0.25 mg/ml. *C. capsularis* has been reported as having antioxidant activity [2]. As a result, the antioxidant activity observed with ECC was possibly derived from polyphenols, flavonoids, and polysaccharides in ECC.

Protection effect of ECC on ethanol-induced AGML

Effect of ECC on the body weight and daily intake of rats in the experimentation period is shown in Fig. S1. The results revealed that there was no significant effect on growth trend and daily food intake rate after the administration of ECC in the experimentation period. Ethanol-induced AGML in rats has been widely applied as an animal model to evaluate the gastro-protective activity of test sample [5, 35, 36]. The result of ECC on ethanol-induced AGML in rats is shown in Fig. 1. In macroscopic findings, 80% ethanol caused severe lesion of the stomach in the control group (Fig. 1Ab) with apparent black hemorrhagic lesion areas as compared with the sham group (Fig. 1Aa). As shown in ECC groups, ulcer areas had been dose-dependently decreased after LECC-, MECC- and HECC-treatment (Fig. 1Ac–Ae) as compared with the control group (Fig. 1Ab). Obviously, ECC was able to ameliorate effectively the severity of ethanol-induced AGML. Quantitative data for the ethanol-induced ulcer index (UI) is shown Fig. 1B. As shown in Fig. 1B, ethanol was capable of inducing AGML in rats by comparison to that of the sham group. In addition, a significant decrease in ethanol-induced UI was found in all ECC groups. The ethanol-

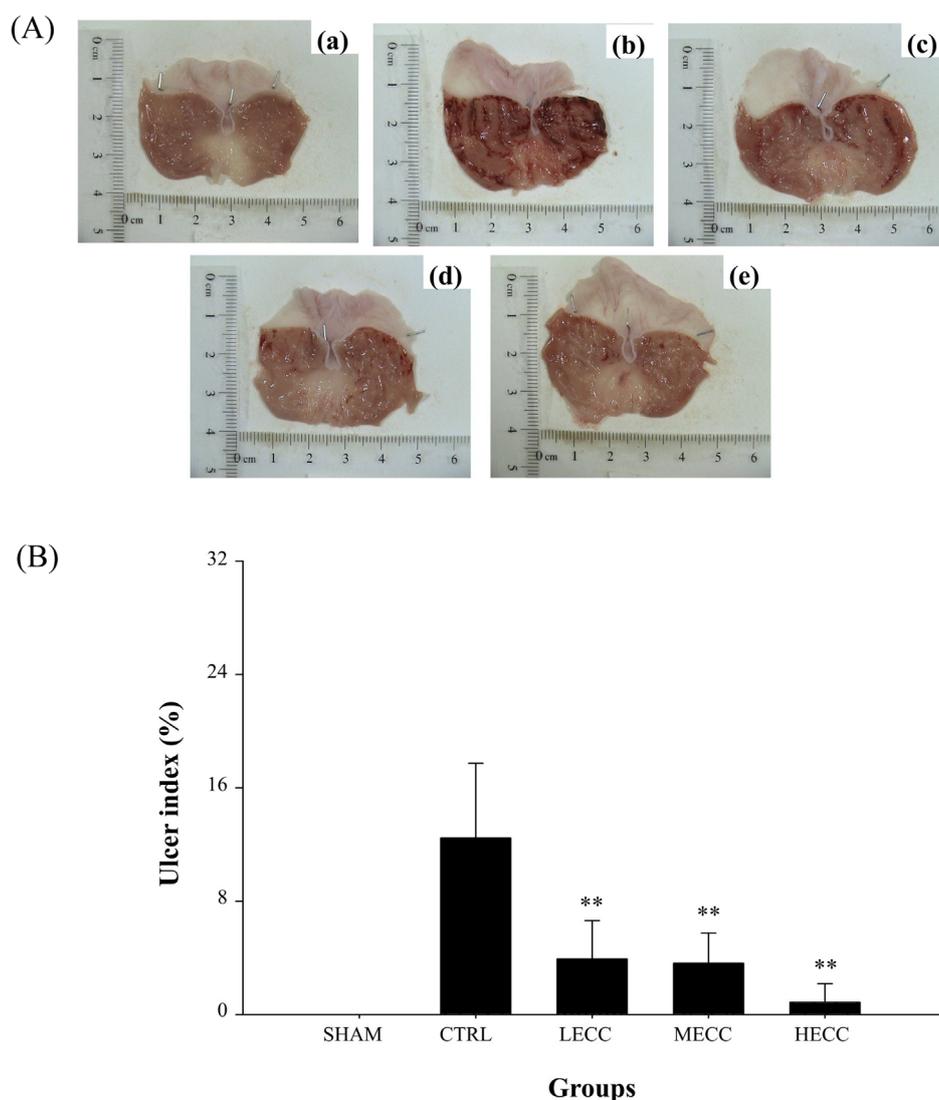


Fig. 1. (A) Macroscopic appearance of extract of *Corchorus capsularis* leaves (ECC) on ethanol-induced acute gastric mucosal lesions (AGML) in rats. (a) SHAM: Normal; (b) CTRL (Control): 80% ethanol + vehicle; (c) Low-dose of ECC (LECC): 80% ethanol + ECC 200 mg/kg; (d) Medium-dose of ECC (MECC): 80% ethanol + ECC 400 mg/kg. (e) High-dose of ECC (HECC): 80% ethanol + ECC 1,000 mg/kg. (B) Effect of ECC on ethanol-induced AGML. Each value represents mean \pm SD (n=8). ** P <0.01 denotes a statistical difference between ECC group and control group. Five groups: SHAM (Normal), CTRL (80% ethanol + vehicle), LECC (80% ethanol + ECC 200 mg/kg), MECC (80% ethanol + ECC 400 mg/kg) and HECC (80% ethanol + ECC 1,000 mg/kg).

induced UI of the sham, control, LECC, MECC and HECC were 0.0 ± 0.0 , 12.5 ± 5.27 , 3.92 ± 2.70 , 3.64 ± 2.11 and 1.87 ± 1.31 , respectively. These results indicate that ECC was able to protect rat stomach from ethanol-induced AGML.

Histopathological evaluation of ethanol-induced AGML

Histopathological evaluation of gastric lesions is shown in Fig. 2 and Table 3, pathological lesion scores were quantitated to estimate the severity of gastric lesions. In the sham group, gastric mucosa showed no acute degeneration, necrotic erosion, bleeding, and deep ulceration in the gastric tissues of the rats and the histoscore was 0.0 ± 0.0 . In the ethanol-treated control group, gastric mucosa showed acute degeneration, necrotic erosion, bleeding, and deep ulceration in the gastric tissues of the rats (the region above the dotted line) and the histoscore was 2.38 ± 0.70 . However, LECC, MECC and HECC reduced the necrotic erosion of gastric mucosa caused by ethanol induction and the histoscore was 1.13 ± 1.05 , 1.38 ± 0.70 and 1.00 ± 0.71 , respectively. These results indicate that ECC was able to decrease the histoscore on histopathological evaluation of ethanol-induced acute gastric mucosal lesions.

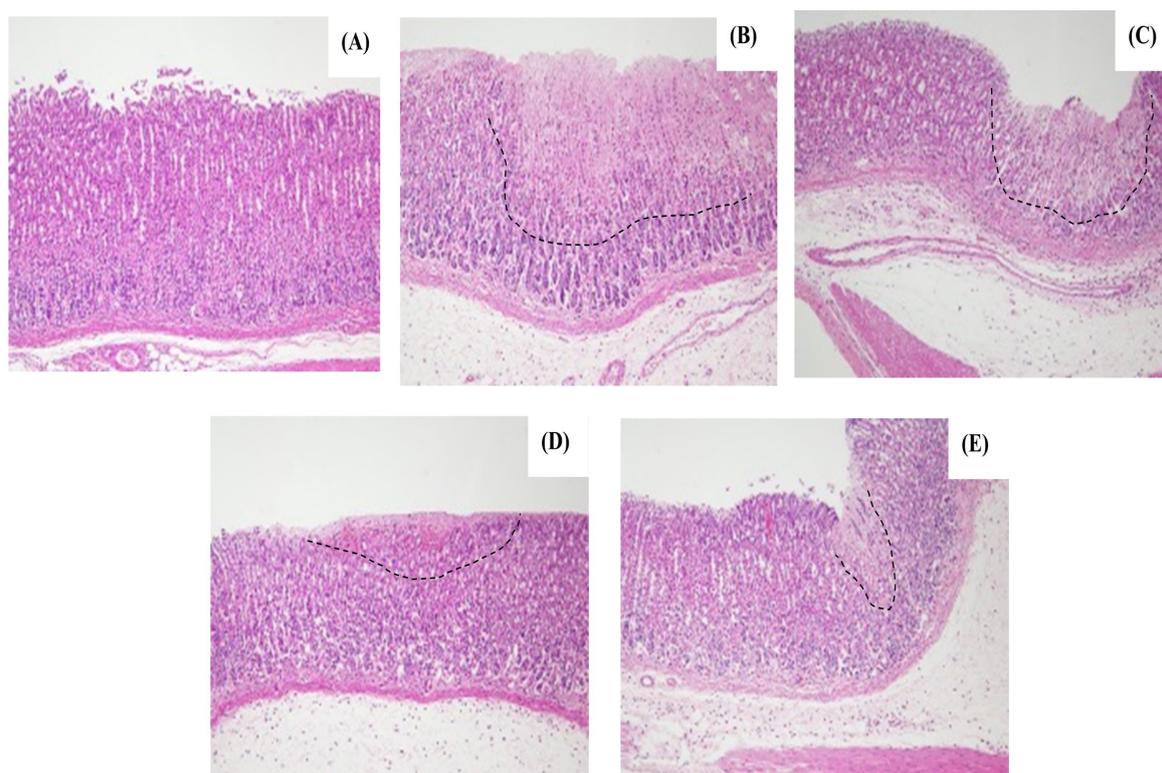


Fig. 2. Histopathological evaluation of extract of *Corchorus capsularis* leaves (ECC) on ethanol-induced acute gastric mucosal lesions (AGML) in rats. Dash-lines indicated the location of damage area. (A) SHAM: Normal; (B) CTRL (Control): 80% ethanol + vehicle; (C) Low-dose of ECC (LECC): 80% ethanol + ECC 200 mg/kg; (D) Medium-dose of ECC (MECC): 80% ethanol + ECC 400 mg/kg; (E) High-dose of ECC (HECC): 80% ethanol + ECC 1,000 mg/kg (40 \times , H&E stain).

Table 3. Inhibition effects of extract of *Corchorus capsularis* leaves (ECC) supplementation on % histoscore in ethanol-induced acute gastric mucosal lesion experiment

| | Histoscore | Inhibition (%) |
|------|-------------------|----------------|
| SHAM | 0.00 ± 0.00 | - |
| CTRL | 2.38 ± 0.70 | 0.0 |
| LECC | 1.13 ± 1.05^a | 52.5 |
| MECC | 1.38 ± 0.70^a | 42.0 |
| HECC | 1.00 ± 0.71^a | 58.0 |

Each value represents mean \pm SD (n=8). a) $P < 0.01$ denotes a statistical difference between ECC group and control group. Five groups: SHAM (Normal), CTRL (80% ethanol + vehicle), LECC (80% ethanol + ECC 200 mg/kg), MECC (80% ethanol + ECC 400 mg/kg) and HECC (80% ethanol + ECC 1,000 mg/kg).

Table 4. Effects of extract of *Corchorus capsularis* leaves (ECC) on biochemical and antioxidant activity

| Molecules | SHAM | CTRL | LECC | MECC | HECC |
|-----------------------|---------------|-----------------------------|----------------------------|----------------------------|----------------------------|
| SOD (U/mg protein) | 46.80 ± 3.20 | 39.42 ± 8.68 ^{d)} | 42.30 ± 4.54 | 41.52 ± 8.30 | 46.69 ± 2.97 ^{a)} |
| CAT (U/mg protein) | 9.24 ± 2.14 | 5.47 ± 2.14 ^{c)} | 8.44 ± 1.88 ^{a)} | 10.89 ± 3.98 ^{a)} | 9.26 ± 1.79 ^{a)} |
| GPx (U/mg protein) | 13.35 ± 0.45 | 11.62 ± 0.36 ^{d)} | 13.01 ± 0.42 ^{b)} | 13.22 ± 0.49 ^{b)} | 12.45 ± 0.82 ^{b)} |
| MDA (nmol/mg protein) | 0.109 ± 0.030 | 0.170 ± 0.060 ^{d)} | 0.119 ± 0.020 | 0.102 ± 0.020 | 0.100 ± 0.01 |

Each value represents mean ± SD (n=8). a) $P < 0.05$ and b) $P < 0.01$ denotes a statistic difference between ECC group and CTRL group. c) $P < 0.05$ and d) $P < 0.01$ denotes a statistic difference between CTRL group and SHAM group. Five groups: SHAM (Normal), CTRL (80% ethanol + vehicle), LECC (80% ethanol + ECC 200 mg/kg), MECC (80% ethanol + ECC 400 mg/kg) and HECC (80% ethanol + ECC 1,000 mg/kg). *Superoxide dismutase: SOD, catalase: CAT, glutathione peroxidase: GPx, malondialdehyde, MDA.

Antioxidant effect of ECC on ethanol-induced AGML

Following the experiment, the blood from hepatic veins was collected for biochemical and antioxidant analyses including glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) and the results are shown in Table 4. As shown in Table 4, ethanol-induced AGML in the vehicle control group significantly decreased antioxidant enzyme activities at SOD, CAT, and GPx levels, and significantly increased the MDA levels in comparison with that of the sham group ($P < 0.05$). In the case of the ECC-treating groups, some of ECC groups significantly reduced the ethanol-induced decrease in levels of antioxidant enzymes ($P < 0.05$), in which HECC was capable of significantly elevating the levels of antioxidant enzymes to near normal levels ($P > 0.05$).

DISCUSSION

Leaves of *C. capsularis* are a popular edible vegetable in Taichung city located in central Taiwan and was cited in folklore with gastroprotection. This present study investigates the protection effect of extract of *C. capsularis* leaves (ECC) on ethanol-induced AGML in rats. Alcohol consumption is an aggressive factor resulting in gastric mucosal lesion [7, 15]. The mechanism of ethanol-induced AGML is a multi-factorial disease which associated mainly with oxygen species and free radicals [26]. It has been reported that *C. capsularis* contain ingredients including flavonoids, saponins, tanins and triterpenes but no alkaloids [37]. Literatures revealed that antioxidant ingredients like polyphenols, flavonoids and polysaccharides with antioxidant activity exist ubiquitously in natural plant materials [1, 17, 28]. Several studies have demonstrated that polysaccharides, polyphenols and flavonoids in natural plant extracts with free radical scavenging activity are associated with preventing ethanol-induced gastric mucosal damage [13, 20, 34]. We have demonstrated that ECC contain phytochemical components as polysaccharides, polyphenols and flavonoids. In addition, ECC was able to scavenge significantly DPPH radical in a concentration-dependent manner. The presence of polysaccharides, polyphenols and flavonoids in ECC with potent free radical scavenging activity may be involved in the protective effect of ethanol-induced AGML in rats.

In conclusion, we have demonstrated that ECC possesses potent protective effects on ethanol-induced AGML in rats, which may be associated with polysaccharides, polyphenols and flavonoids in ECC with free radical scavenging activity. Finding new therapeutic sources for the pharmaceutical industry is very important. In addition, providing valid, effective, low-priced and easily produced plant extracts are important. *C. capsularis* extract may be a potential anti-ulcer agent in the near future.

REFERENCES

1. Abhijit, S., Tripathi, S. J., Bhagya, V., Shankaranarayana Rao, B. S., Subramanyam, M. V. and Asha Devi, S. 2018. Antioxidant action of grape seed polyphenols and aerobic exercise in improving neuronal number in the hippocampus is associated with decrease in lipid peroxidation and hydrogen peroxide in adult and middle-aged rats. *Exp. Gerontol.* **101**: 101–112. [Medline] [CrossRef]
2. Ademiluyi, A. O., Oboh, G., Aragbaiye, F. P., Oyeleye, S. I. and Ogunsuyi, O. B. 2015. Antioxidant properties and *in vitro* α -amylase and α -glucosidase inhibitory properties of phenolics constituents from different varieties of *Corchorus* spp. *J. Taibah Univ. Med. Sci.* **10**: 278–287.
3. Al-Snafi, A. E. 2016. The contents and pharmacological importance of *Corchorus capsularis*-A review. *IOSR J. Pharm.* **6**: 58–63.
4. Al Batran, R., Al-Bayaty, F., Jamil Al-Obaidi, M. M., Abdulkader, A. M., Hadi, H. A., Ali, H. M. and Abdulla, M. A. 2013. *In vivo* antioxidant and antiulcer activity of *Parkia speciosa* ethanolic leaf extract against ethanol-induced gastric ulcer in rats. *PLoS One* **8**: e64751. [Medline] [CrossRef]
5. Chen, F. A., Lee, S. C., Chao, H. R., Fu, W. C., Hsu, M. C., Horng, C. T., Wang, C. C., Matsui, H. and Agoramorthy, G. 2009. Effects of burdock extract preparation on gastric mucosal protection. *Asian J. Chem.* **21**: 3015.
6. Chen, F. A., Wu, A. B., Shieh, P., Kuo, D. H. and Hsieh, C. Y. 2006. Evaluation of the antioxidant activity of *Ruellia tuberosa*. *Food Chem.* **94**: 14–18. [CrossRef]
7. Chou, S. P. 1994. An examination of the alcohol consumption and peptic ulcer association—results of a national survey. *Alcohol. Clin. Exp. Res.* **18**: 149–153. [Medline] [CrossRef]
8. D'Souza, R. S. and Dhume, V. G. 1991. Gastric cytoprotection. *Indian J. Physiol. Pharmacol.* **35**: 88–98. [Medline]
9. Dharmani, P., Kuchibhotla, V. K., Maurya, R., Srivastava, S., Sharma, S. and Palit, G. 2004. Evaluation of anti-ulcerogenic and ulcer-healing properties of *Ocimum sanctum* Linn. *J. Ethnopharmacol.* **93**: 197–206. [Medline] [CrossRef]
10. Hernández-Muñoz, R., Montiel-Ruiz, C. and Vázquez-Martínez, O. 2000. Gastric mucosal cell proliferation in ethanol-induced chronic mucosal injury is related to oxidative stress and lipid peroxidation in rats. *Lab. Invest.* **80**: 1161–1169. [Medline] [CrossRef]
11. Horng, C. T., Chao, H. R., Lee, C. F., Hsueh, C. W. and Chen, F. A. 2012. Gastro Protective effect of Madeira vine against ethanol-induced gastric

- mucosal lesion in rat. *Asian J. Chem.* **24**: 765.
12. Horng, C. T., Huang, J. K., Wang, H. Y., Huang, C. C. and Chen, F. A. 2014. Antioxidant and antifatigue activities of *Polygonatum Alte-lobatum* Hayata rhizomes in rats. *Nutrients* **6**: 5327–5337. [Medline] [CrossRef]
 13. Jung, H. J., Choi, J., Nam, J. H. and Park, H. J. 2007. Anti-ulcerogenic effects of the flavonoid-rich fraction from the extract of *Orostachys japonicus* in mice. *J. Med. Food* **10**: 702–706. [Medline] [CrossRef]
 14. Kenny, O., Smyth, T. J., Hewage, C. M. and Brunton, N. P. 2013. Antioxidant properties and quantitative UPLC-MS analysis of phenolic compounds from extracts of fenugreek (*Trigonella foenum-graecum*) seeds and bitter melon (*Momordica charantia*) fruit. *Food Chem.* **141**: 4295–4302. [Medline] [CrossRef]
 15. Khazaei, M. and Salehi, H. 2006. Protective effect of *Falcaria vulgaris* extract on ethanol induced gastric ulcer in rat. *Iranian J. Pharmacol. Ther.* **5**: 43–40.
 16. Kim, C., Sin, M., Lee, K. and Ahn, D. 1999. Chinese Materia Medica Grand Dictionary 1st ed., Jeongdam Publishing Company, Seoul.
 17. Kondeva-Burdina, M., Shkondrov, A., Simeonova, R., Vitcheva, V., Krasteva, I. and Ionkova, I. 2018. *In vitro/in vivo* antioxidant and hepatoprotective potential of defatted extract and flavonoids isolated from *Astragalus spruneri* Boiss. (Fabaceae). *Food Chem. Toxicol.* **111**: 631–640. [Medline] [CrossRef]
 18. Kulshreshtha, M., Srivastava, G. and Singh, M. P. 2017. Pathophysiological status and nutritional therapy of peptic ulcer: An update. *Environ. Dis.* **2**: 76. [CrossRef]
 19. Lam, S. K. 2000. Differences in peptic ulcer between East and West. *Best Pract. Res. Clin. Gastroenterol.* **14**: 41–52. [Medline] [CrossRef]
 20. Lee, J. S., Oh, T. Y., Kim, Y. K., Baik, J. H., So, S., Hahm, K. B. and Surh, Y. J. 2005. Protective effects of green tea polyphenol extracts against ethanol-induced gastric mucosal damages in rats: stress-responsive transcription factors and MAP kinases as potential targets. *Mutat. Res.* **579**: 214–224. [Medline] [CrossRef]
 21. Luo, X. J., Liu, B., Dai, Z., Li, T. B., Li, N. S., Zhang, X. J., Yang, Z. C., Li, Y. J. and Peng, J. 2013. Expression of apoptosis-associated microRNAs in ethanol-induced acute gastric mucosal injury via JNK pathway. *Alcohol* **47**: 481–493. [Medline] [CrossRef]
 22. Majdoub, H., Mansour, M. B., Chaubet, F., Roudesli, M. S. and Maaroufi, R. M. 1790. 2009. Anticoagulant activity of a sulfated polysaccharide from the green alga *Arthrospira platensis*. *Biochim. Biophys. Acta* 1377–1381.
 23. Mei, X., Xu, D., Xu, S., Zheng, Y. and Xu, S. 2012. Novel role of Zn(II)-curcumin in enhancing cell proliferation and adjusting proinflammatory cytokine-mediated oxidative damage of ethanol-induced acute gastric ulcers. *Chem. Biol. Interact.* **197**: 31–39. [Medline] [CrossRef]
 24. Negi, P., Jayaprakasha, G. and Jena, B. 2003. Antioxidant and antimutagenic activities of pomegranate peel extracts. *Food Chem.* **80**: 393–397. [CrossRef]
 25. Park, S. W., Oh, T. Y., Kim, Y. S., Sim, H., Park, S. J., Jang, E. J., Park, J. S., Baik, H. W. and Hahm, K. B. 2008. *Artemisia asiatica* extracts protect against ethanol-induced injury in gastric mucosa of rats. *J. Gastroenterol. Hepatol.* **23**: 976–984. [Medline] [CrossRef]
 26. Repetto, M. G. and Llesuy, S. F. 2002. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. *Braz. J. Med. Biol. Res.* **35**: 523–534. [Medline] [CrossRef]
 27. Sangiovanni, E., Vrhovsek, U., Rossoni, G., Colombo, E., Brunelli, C., Brembati, L., Trivulzio, S., Gasperotti, M., Mattivi, F., Bosisio, E. and Dell’Agli, M. 2013. Ellagitannins from Rubus berries for the control of gastric inflammation: *in vitro* and *in vivo* studies. *PLoS One* **8**: e71762. [Medline] [CrossRef]
 28. Shang, H., Zhou, H., Duan, M., Li, R., Wu, H. and Lou, Y. 2018. Extraction condition optimization and effects of drying methods on physicochemical properties and antioxidant activities of polysaccharides from comfrey (*Symphytum officinale* L.) root. *Int. J. Biol. Macromol.* **112**: 889–899. [Medline] [CrossRef]
 29. Shanmukha, I. and Kumar, V. 2013. Protective effect of *Tecoma stans* leaf extract on Experimentally induced gastric ulcers in rats. *Int. J. Drug Dev. Res.* **5**: 231–236.
 30. Shetty, R., Kumar, V., Naidu, M. and Ratnakumar, K. 2000. Effect of *Ginkgo biloba* extract in ethanol induced gastric mucosal lesions in rats. *Indian J. Pharmacol.* **32**: 313–317.
 31. Siddique, R. A. H. 2014. Prevalence of peptic ulcer disease among the patients with abdominal pain attending the department of medicine in Dhaka Medical College Hospital, Bangladesh. *IOSR J. Dent. Med. Sci.* **13**: 5–20. [CrossRef]
 32. Vomero, N. D. and Colpo, E. 2014. Nutritional care in peptic ulcer. *Arq. Bras. Cir. Dig.* **27**: 298–302. [Medline] [CrossRef]
 33. Wang, F. W., Tu, M. S., Mar, G. Y., Chuang, H. Y., Yu, H. C., Cheng, L. C. and Hsu, P. I. 2011. Prevalence and risk factors of asymptomatic peptic ulcer disease in Taiwan. *World J. Gastroenterol.* **17**: 1199–1203. [Medline] [CrossRef]
 34. Wang, M., Konishi, T., Gao, Y., Xu, D. and Gao, Q. 2015. Anti-gastric ulcer activity of polysaccharide fraction isolated from mycelium culture of lion’s mane medicinal mushroom, *Hericium erinaceus* (higher basidiomycetes). *Int. J. Med. Mushrooms* **17**: 1055–1060. [Medline] [CrossRef]
 35. Yang, H. J., Kim, M. J., Kwon, D. Y., Kang, E. S., Kang, S. and Park, S. 2017. Gastroprotective actions of *Taraxacum coreanum* Nakai water extracts in ethanol-induced rat models of acute and chronic gastritis. *J. Ethnopharmacol.* **208**: 84–93. [Medline] [CrossRef]
 36. Yang, J., Zhou, W., Gu, Y., Dai, J., Li, X., Tai, P., Li, Y., Ma, X. and Zhang, Y. 2018. Protective effect of Pu-erh tea extracts against ethanol-induced gastric mucosal damage in rats. *Biomed. Rep.* **8**: 335–342. [Medline]
 37. Zakaria, Z. A., Sulaiman, M. R., Gopalan, H. K., Abdul Ghani, Z. D., Raden Mohd Nor, R. N., Mat Jais, A. M. and Abdullah, F. C. 2007. Antinociceptive and anti-inflammatory properties of *Corchorus capsularis* leaves chloroform extract in experimental animal models. *Yakugaku Zasshi* **127**: 359–365. [Medline] [CrossRef]