GASTROENTEROLOGY

Gastroprotective effects of Corchorus olitorius leaf extract against ethanol-induced gastric mucosal hemorrhagic lesions in rats

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Abstract

Background and Aim: Corchorus olitorius is a medicinal plant traditionally utilized as an antifertility, anti-convulsive, and purgative agent. This study aimed to evaluate the gastroprotective effect of an ethanolic extract of C. olitorius against ethanol-induced gastric ulcers in adult Sprague Dawley rats.

Methods: The rats were divided into seven groups according to their pretreatment: an untreated control group, an ulcer control group, a reference control group (20 mg/kg omeprazole), and four experimental groups (50, 100, 200, or 400 mg/kg of extract). Carboxymethyl cellulose was the vehicle for the agents. Prior to the induction of gastric ulcers with absolute ethanol, the rats in each group were pretreated orally. An hour later, the rats were sacrificed, and gastric tissues were collected to evaluate the ulcers and to measure enzymatic activity. The tissues were subjected to histological and immunohistochemical evaluations.

Results: Compared with the extensive mucosal damage in the ulcer control group, gross evaluation revealed a marked protection of the gastric mucosa in the experimental groups, with significantly preserved gastric wall mucus. In these groups, superoxide dismutase and malondialdehyde levels were significantly increased (P < 0.05) and reduced (P < 0.05), respectively. In addition to the histologic analyses (HE and periodic acid-Schiff staining), immunohistochemistry confirmed the protection through the upregulation of Hsp70 and the downregulation of Bax proteins. The gastroprotection of the experimental groups was comparable to that of the reference control medicine omeprazole.

Conclusions: Our study reports the gastroprotective property of an ethanolic extract of C. olitorius against ethanol-induced gastric mucosal hemorrhagic lesions in rats.

Introduction

Alcohol is associated with various types of gastric mucosal damage, including gastritis and peptic ulcer diseases. Other etiologies of these pathologies include stress, smoking, dietary inadequacies, and diseases, as well as haphazard administration of non-steroidal anti-inflammatory drugs.1 Different models have been proposed to induce lesions in the gastric trunk.2,3 Ethanol exerts its dangerous effects either by directly producing reactive metabolites, which, together with free radical species, alter the structure and function of a number of cellular proteins, or by supporting other pathways that maintain high levels of oxidative damage.4 Ethanol-aggravated gastric mucosal wounds are linked to widespread damage to mucosal capillaries and increased vascular permeability.5 Mucosal capillary necrosis, vascular congestion, and thrombosis in the subepithelial microvasculature are other pathologies that may develop from a gastric mucosal obstruction. In addition to the direct negative consequences of ethanol on the gastric mucosa, other elements are considered to contribute to the pathogenesis of the wound.6

Several plants, such as Polygonum chinense, Carica papaya, Polygonum minus, and Corchorus olitorius, have been reported to have antifluecker properties.7-10 C. olitorius, generally known as “Jute,” is a member of Tiliaceae family and is a widespread vegetable found in Egypt, Sudan, Malaysia, Philippines, tropical Africa, South America, and the Caribbean.8 Jute is also referred to as long-fruited jute, tossa jute, jute mallow, and jew’s mallow.11 Jute contains large amounts of all of the amino acids except...
methionine, which is present in low concentrations. The plant has a high protein content in its supplementary leafy species, which serve as the main source for dietary protein in several tropical countries. The young leaves of C. olitorius L. are edible and are used as an ingredient for an ethnic soup in Egypt. This plant is also rich in potassium, calcium, phosphorous, iron, ascorbic acid, and carotene. A phenolic extract of C. olitorius exhibited antioxidant activity through the radical generator-initiated peroxidation of linoleic acid. Furthermore, the role of phenolic antioxidants in the activities of this vegetable was suggested from their activity patterns. C. olitorius has demulcent, diuretic, purgative, and tonic properties, and it is served as a lactagogue. The leaves showed therapeutic effects against cystitis, dysuria, fever, and gonorrhea. Ingestion of this plant mixture helps to stimulate appetite and vitality. It is an ingredient in facial creams, lotions, hair tonic, and hand creams and has been reported to have diuretic, antipyretic, analgesic, and antimicrobial activity. Jute leaves contain flavonoids, and vitamin C. This study was performed to assess the gastroprotective properties of C. olitorius against absolute ethanol-induced acute hemorrhagic lesions in rats.

Methods

Chemicals. All of the chemicals used in this study were obtained from Sigma-Aldrich (USA). Omeprazole was acquired from the University Malaya Medical Centre Pharmacy and was used as the reference drug. It was dissolved in carboxymethyl cellulose (CMC) and administered orally to the rats at a dose of 20 mg/kg body weight (5 mL/kg) as previously described.

Plant specimens and extract preparation. C. olitorius leaves were obtained from Ethno Resources Sdn Bhd (Selangor, Malaysia) and were confirmed by comparing them to the voucher sample at the Herbarium of Rimba Ilmu, Institute of Science Biology, University of Malaya, Kuala Lumpur. The desiccated leaves were ground into powder and soaked in 95% ethanol. The ethanol mixture was then evaporated using an Eyela rotary evaporator (Sigma-Aldrich, St. Louis, MO, USA). The dry extract was dissolved in CMC (0.25% w/v) and then administered orally to rats at doses of 50, 100, 200, and 400 mg/kg body weight (5 mL/kg body weight) according to a previously published study.

Acute toxicity. Mature male and female Sprague Dawley rats (6–8 weeks old, weight 150–180 g) were acquired from the animal house at the Faculty of Medicine, University of Malaya, Kuala Lumpur [Ethics No. RM 07/05/2008/MMA (a) (R)]. They received normal rat pellets with water ad libitum. A severe toxicity experiment was performed to determine the maximum prescribed dose of leaf extract that did not kill the animals according to a previous study with some modifications.

Gastroprotective experimental design

Experimental animals. Healthy, mature Sprague Dawley male rats were provided by the Experimental Animal House, Faculty of Medicine, University of Malaya [Ethic No. RM28/09/2006/MAA (R)]. The rats were randomly separated into seven groups containing six rats per group. The rats (200–225 g) were kept individually in wide mesh wire bottom cages to prevent coprophagia during the test.

Gastric ulcer induction by ethanol. The animals were fasted for 24 h prior to the test but were allowed to drink water until 2 h prior to the test. Gastric ulcers were aggravated through the gastric intubation of absolute ethanol (5 mL/kg) as previously described.

Measurement of gastric juice acidity. The gastric content was collected and centrifuged at 4000 rpm for 10 min. The supernatant was used to determine the hydrogen ion concentration by titration with NaOH solution (0.1 N), and it was measured with a digital pH meter (Hanna instruments, Ann Arbor, MI, USA).

Macroscopic appearance of gastric mucosa. Ulcers associated with the gastric mucosa presented as stretched bands of hemorrhagic lesions similar to the extended axis of the stomach. The total area of the lesions of each stomach was used to calculate the ulcer region (UA) as follows:

\[
(I\%) = \left[ \left( \frac{UA_{\text{treated}} - UA_{\text{control}}} {UA_{\text{control}}} \right) \times 100 \%ight] 
\]

This equation was used as previously described with minor changes. The restraining percentage (I %) was calculated using the formula described by Njar et al. with minor adjustments.

Content measurement of gastric wall mucus. The glandular regions of the stomach were removed from the rats, measured, and evaluated to measure the content of the gastric wall mucus. The gastric wall mucus was evaluated in accordance with the method of Corne et al.

Antioxidant activity

Preparation of stomach homogenate. Glandular gastric tissue was carefully rinsed with ice-cold saline. Using a homogenizer (Polytron, Heidolph RZR 1, Schwabach, Germany), the homogenate was prepared on ice in potassium phosphate buffer (10% w/v), 50 mmol, pH 7.8) containing mammalian protease inhibitors. The homogenate was centrifuged at 4500 rpm for 30 min at 4°C.

Measurement of superoxide dismutase (SOD). SOD activity was measured as previously described.

Measurement of membrane lipid peroxidation. The rate of lipoperoxidation in the gastric mucus membrane was determined through the measurement of membrane lipid peroxidation (malondialdehyde [MDA]) using the Bradford assay.

Histology of gastric lesions

HE staining. Samples of gastric tissue were fixed in 10% buffered formalin and processed in a paraffin tissue-processing
The data were analyzed using one-way ANOVA with SPSS 20 software. All the values are expressed as the mean ± standard error of mean. The mean difference is significant when compared with the ulcer control group. The data were analyzed using one-way ANOVA with SPSS 20 software.

CMC, carboxymethyl cellulose; GWM, gastric wall mucus.

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the submucosal layer. The rats pretreated with C. olitorius demonstrated significantly improved protection of the gastric mucosa with decreased total ulcer area, edema, and leukocyte infiltration into the submucosal layer (Fig. 2).

PAS of mucosal glycoproteins. There was increased PAS staining of the gastric mucosa in the pretreated groups compared with the ulcer control group, which indicated an increase in the glycoprotein content of the gastric mucosa. However, the pretreated group showed increased PAS staining induced by ethanol, as shown in Figure 3.

Immunohistochemical evaluation. The immunohistochemical results showed that the groups pretreated with C. olitorius extract had increased Hsp70 protein expression levels. Hsp70 protein expression in the ulcer control group was lower compared with the pretreated groups (Fig. 4).

Table 2  Effects of Corchorus olitorius on the antioxidant activity of SOD and the MDA levels in the stomach homogenate of rats

| Animals                  | MDA (µM/g Tissue) | SOD (U/g) |
|--------------------------|-------------------|-----------|
| CMC (Normal control)     | 12.37 ± 0.64      | 8.49 ± 0.34 |
| CMC (Ulcer control)      | 25.35 ± 1.66      | 3.53 ± 0.58 |
| Omeprazole (20 mg/kg)    | 13.40 ± 1.35*     | 7.56 ± 1.12 |
| C. olitorius (50 mg/kg)  | 15.13 ± 1.72*     | 5.14 ± 0.70 |
| C. olitorius (100 mg/kg) | 11.79 ± 1.02*     | 7.22 ± 1.40 |
| C. olitorius (200 mg/kg) | 7.27 ± 0.93*      | 15.17 ± 1.48* |
| C. olitorius (400 mg/kg) | 4.33 ± 0.81*      | 20 ± 1.28* |

*P < 0.001.

All the values are expressed as the mean ± standard error of the mean. The mean differences are significant when compared with the ulcer control group. The data were analyzed using one-way ANOVA with SPSS 20 software.

CMC, carboxymethyl cellulose; SOD, superoxide dismutase; MDA, malondialdehyde.
The immunohistochemical staining of Bax demonstrated that the rats in the pretreated groups had decreased Bax protein expression. As shown in the ulcer control group, ethanol resulted in increased Bax expression, whereas the pretreatment with the plant extract decreased the expression of this protein in the pretreated groups (Fig. 5).

Discussion

Neither hepatic nor renal toxicity was observed upon high doses of C. olitorius. Peptic ulcers occur upon the disruption of the normal equilibrium between aggressive factors, such as acid and pepsin, and defensive mechanisms, such as mucus production, bicarbonate, mucosal turnover, and blood supply (mucosal barrier). Gastric lesions generated by ethanol treatment appear as multiple hemorrhagic red bands of various sizes along the glandular stomach. Ethanol is generally used for inducing ulcer formation in rats because it results in severe gastric mucosal injury. Ethanol generates necrotic lesions in the gastric mucosa by exerting irreversible toxic effects and by decreasing bicarbonates and mucus production. Omeprazole is a proton pump inhibitor that is generally used to treat gastric acid discharge by inhibiting acid secretion into the gastric mucosa. Oxidative stress plays a vital role in the pathogenesis of various ailments, including gastric ulcers, as antioxidants help to protect the gastric mucosa against necrotic agents. In this study, C. olitorius was shown to be an antioxidant. It has been suggested that the gastroprotective effect exerted by this plant could be due to its antioxidant properties, which might neutralize the oxidative injury induced by absolute ethanol toxicity. Previous studies have shown that antioxidants may be connected to antiulcer activity through gastroprotection. The results of this study also showed that the plant extract...
could protect the gastric mucosa as well as reduce leukocyte infiltration into the gastric wall in rats. Teprenone treatment had a protective effect against mucosal lesions by slowing the rate of neutrophil infiltration into ulcerated gastric tissue. The reduction of neutrophil infiltration into ulcerated gastric tissue prevented the development of gastric ulcers in rats. The administration of absolute alcohol may significantly harm the gastric mucosa, resulting in increased neutrophils in the gastric mucosa. Wasman et al. showed that oral treatment with a P. minus aqueous leaf extract prior to ethanol administration reduced neutrophil infiltration into the gastric mucosa. Moreover, the Centella asiatica leaf extract reduced neutrophil infiltration into ulcerated gastric tissue and blocked the development of gastric ulcers in rats.

In the present study, we noted a flattening of the mucosal folds, which suggests that this plant could reduce gastric motility. It has been reported that modifications in gastric motility can affect the growth of experimental gastric lesions. A Jasminum sambac leaf extract shielded the gastric mucosa by flattening the folds. This flattening was suggested to increase the mucosal region exposed to necrotizing agents and decrease the quantity of gastric irritants on the rugal crest. This result was consistent with the gastroprotective effect of a cupper complex. The PAS histochemistry showed distinctive carmine staining of the stomach areas that release mucopolysaccharides. The group pretreated with 400 mg/kg C. olitorius showed high levels of mucus in the gastric glands. Mucus production is one of the major mechanisms of local gastric mucosal defense. Several factors are known to control ulcer prevention. Mucus and bicarbonate production may be vital for ulcer prevention because the mucus/bicarbonate layer serves to shield recently produced cells from acidic and peptic injury. Hsp70 is a 70-kDa protein from the heat shock protein family that is found in mammalian cells. It is the most preserved and most abundantly manufactured protein that responds to various types of stress, such as heat, toxic agents, infection, and proliferation. Bax (a pro-apoptotic protein) promotes cell death, whereas Bcl-2 (an anti-apoptotic protein) inhibits this process. In stress-induced ulcers, apoptosis occurs as a result of the disparity between the Bcl-2 family of anti-apoptotic proteins and the apoptotic Bax.

Figure 3 Glycoprotein secretion (periodic acid-Schiff [PAS] staining) of the gastric tissue in rats (20×). (a) Normal control group. (b) Ulcer control group. (c) Omeprazole-treated group. (d, e, f and g) Groups pretreated with 50, 100, 200 and 400 mg/kg Corchorus olitorius extract, respectively.
The susceptibility of a cell to apoptosis depends on the balance between apoptosis-promoting and apoptosis-suppressing factors. These proteins protect cellular homeostatic processes from ecological and physiological damage by promoting the formation of standard proteins as well as either restoring or eliminating aberrantly formed proteins. Hsp70 proteins protect cells from oxidative stress and heat shock. Ethanol-produced reactive oxygen species usually function by reducing the expression of Hsp70 and enhancing the expression of Bax. Hsp70 prevents these partially denatured proteins from aggregating and activates pathways that allow them to refold. The increased expression of Hsp70 observed in this study suggests that the plant extract preserved the gastric tissues via upregulating Hsp70. Our results showed significant Hsp70 expression in the groups pretreated with *C. olitorius*. Hsp70 exerts its cytoprotective effects by protecting mitochondria and interfering with the stress-induced apoptotic program. Immunoistochemical staining of Bax in the gastric mucosa of rats pretreated with the *C. olitorius* extract and omeprazole showed a reduction in Bax protein levels, whereas Bax expression in the ulcer control group was increased. The immunohistochemistry results of our study were consistent with the previous study on an aqueous leaf extract of *P. chinense*. In conclusion, *C. olitorius* was shown to protect the gastric mucosa against ethanol-induced damage. This defense was characterized by a reduction of the area of ulcerated regions in the gastric wall and a decrease in edema and leukocyte infiltration into the submucosal layers. The rats pretreated with the plant extract showed increased Hsp70 expression and downregulated Bax expression. The increase in PAS staining of the gastric mucosa of the pretreated rats suggests an increase in glycoprotein content. *C. olitorius* reversed the reduction in PAS staining induced by ethanol treatment. The measurement of SOD activity and MDA levels in the gastric tissue homogenates revealed that pretreatment with this plant markedly increased SOD activity and reduced the levels of lipid peroxidation (MDA). The data corroborate the long-established anecdotes reported about this herb and provide a new therapeutic option for the treatment of gastric ailments.
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References

1. Huang JQ, Sridhar S, Hunt RH. Role of Helicobacter pylori infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis. Lancet 2002; 359: 14–22.
2. Nakashita M, Suzuki H, Miura S et al. Attenuation of acetic acid-induced gastric ulcer formation in rats by glucosylceramide synthase inhibitors. Dig. Dis. Sci. 2013; 58: 354–62.
3. A. Ketuly K, A. Hadi AH, Golbabapour S et al. Acute toxicity and gastroprotection studies with a newly synthesized steroid. PLoS ONE 2013; 8: e59296.
4. Kato S, Kawase T, Alderman J, Inatomi N, Lieber CS. Role of xanthine oxidase in ethanol-induced lipid peroxidation in rats. Gastroenterology 1990; 98: 203–10.
5. Szabo S, Trier JS, Brown A, Schnoor J. Early vascular injury and increased vascular permeability in gastric mucosal injury caused by ethanol in the rat. Gastroenterology 1985; 88: 228–36.
6. Bujanda L. The effects of alcohol consumption upon the gastrointestinal tract. Am. J. Gastroenterol. 2000; 95: 3374–82.
7. Wasman S, Mahmood A, Salehhuddin H, Zaha A, Salma I. Cytoprotective activities of Polygonum minus aqueous leaf extract on ethanol-induced gastric ulcer in rats. J. Med. Plant Res. 2010; 4: 2658–65.
8. Fawusi MOA. Quality and compositional changes in Corchorus olitorius as influenced by N fertilization and post-harvest handling. Sci. Hortic. 1983; 21: 1–7.
9. Kirtikar KR, Basu BD. Indian Medicinal Plants, Vol. 3, 2nd edn. Dehra Dun: International Book Distributors, 1987.
10. Ismail IF, Golbabapour S, Hassandarvish P et al. Gastroprotective activity of Polygonum chinense aqueous leaf extract on...
ethanol-induced hemorrhagic mucosal lesions in rats. *Evid. Based Complement. Alternat. Med.* 2012; 404012.

Tindall H. *Vegetables in the Tropics*. Hound mills, Basingstoke, Hampshire: The Macmillan press Ltd., 1993, 553.

Zeghichi S, Kallithraka S, Simopoulos AP. Nutritional composition of molokhia (*Corchorus olitorius*) and stammagathi (*Cichorium spinosum*). *World Rev. Nutr. Diet.* 2003; 91: 1–21.

Azuma K, Nakayama M, Koshioka M et al. Phenolic antioxidants from the leaves of *Corchorus olitorius* L. *J. Agric. Food Chem.* 1999; 47: 3963–6.

Farah W, Nazaratalwamwarina R, Fatimah C. The in vitro antibacterial activity of *Corchorus olitorius* extracts. *Int. J. Pharmaceut.* 2006; 2: 213–15.

Pal DK, Mandal M, Senthilkumar GP, Padhiairi A. Antibacterial activity of *Cuscuta reflexa* stem and *Corchorus olitorius* seed. *Fitoterapia* 2006; 77: 589–91.

Khan M, Bano S, Javed K, Mueed MA. A comprehensive review on the chemistry and pharmacology of Corchorus species-A source of cardiac glycosides, triterpenoids, ionones, flavonoids, coumarins, steroids and some other compounds. *J. Sci. Ind. Res.* 2006; 65: 283–98.

Zakaria ZA, Sulaiman MR, Jais AM et al. The antinociceptive activity of *Muntingia calabura* aqueous extract and the involvement of L-arginine/nitric oxide/cyclic guanosine monophosphate pathway in its observed activity in mice. *Fundam. Clin. Pharmacol.* 2006; 20: 365–72.

Furumoto T, Wang R, Okazaki K. Resources IoLA, ed. *Guide for the Care and Use of Laboratory Animals*. Washington: National Academies Press, 1996.

Indran M, Mahmood AA, Kuppusamy UR. Protective effect of *Carica papaya* L. leaf extract against alcohol induced acute gastric damage and blood oxidative stress in rats. *West Indian Med. J.* 2008; 57: 323–6.

Kauffman GL Jr, Grossman MI. Prostaglandin and cimetidine inhibit the formation of ulcers produced by parenteral salicylates. *Gastroenterology* 1978; 75: 1099–102.

Njar VCO, Adesanwo JK, Raji Y, Angolenate M. Methyl angolenate: the antiulcer agent from the stem bark of *Entandrophragma angolense*. *Planta Med.* 1995; 61: 91–2.

Corne SJ, Morrissey SM, Woods RJ. Proceedings: a method for the quantitative estimation of gastric barrier mucus. *J. Physiol.* 1974; 242: 116P–7P.

Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin. Chem.* 1988; 34: 497–500.

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 1976; 72: 248–54.

Behmer A, Tolosa E, Neto A. Manual de práticas para histologia normal e patológica. *São Paulo, Brazil: Edart-Edusp.* 1976.

McManus JFA. Histological and histochemical uses of periodic acid. *Biotechnic & Histochemistry* 1948; 23: 99–108.

Piper D, Stiel D. Pathogenesis of chronic peptic ulcer, current thinking and clinical implications. *Med. Prog.* 1986; 2: 7–10.

Hajrezai M, Golbabapour S, Hassanardvish P et al. Acute toxicity and gastroprotection studies of a new Schiff base derived copper (II) complex against ethanol-induced acute gastric lesions in rats. *PLoS ONE* 2012; 7: e51537.