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ANTI-ULCER AND ANTIOXIDANT ACTIVITIES OF THE LEAF AQUEOUS EXTRACT OF CORCHORUS OLITORIUS (TILIACEAE) IN RATS

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ABSTRACT

The present study was carried out to find the possible antiulcer mechanism of action of *C. olitorius*. Several models of gastric ulcers were induced in rats to evaluate the prophylactic (HCl/ethanol, indomethacin/HCl-ethanol, indomethacin and pylorus ligature) and the healing (Acetic acid and ethanol/aspirin) potential of the leaf queous extract of *Corchorus olitorius* (ECO). The gastric ulcerations, mucus production, pH, volume and acidity of the gastric juice were measured. Some parameters of oxidative stress (SOD and MDA) were measured in stomach simples obtained from the animals in the indomethacin model. Oral administration of ECO (100, 200 and 400 mg/kg) dose-dependently prevented ulcer formation by HCl/ethanol (4.65%, 39.70% and 46.17% of inhibition), indomethacin/HCl-ethanol (7.97, 34.95 and 45.85%), indomethacin (24.94, 48.83 and 58.44%) and pylorus ligature (36.93, 54.95 and 77.47%). The inhibitory effect of the extract against HCl/ethanol induced ulcer was not suppressed by the pre-treatment with indomethacin (20 mg/kg, *i.p.*). ECO reduced Shay-ligated gastric acid secretion from 81.20 mEq/l in the controls to 56.57, 53.96 and 49.421 mEq/l for the extract doses 100, 200 and 400 mg/kg, respectively. The ulcer-healing test showed a dose-dependent reduction of ulceration induced by acetic acid and ethanol/aspirin. The highest dose of extract (400 mg/kg) showed a highly significant ($p<0.001$) reduction of ulcer with corresponding healing rate of 94.08 and 33.75, respectively, for acetic acid and ethanol/aspirin induced ulcers. The prophylactic and healing actions of ECO were associated with significant increases in gastric mucus production. The levels of SOD were improved in rats treated with the extract. The antiulcer activity of ECO in rats was attributed to its ability to reduce acid secretion, to enhance mucosal defense and *in vivo* antioxidant status.

Key words: *Corchorus olitorius*, Gastric ulcer, Antiulcer activity, Antioxidant.

INTRODUCTION

Peptic ulcer is one of the world's major gastro-intestinal disorders, including both gastric and duodenal ulcers and affecting 10% of the world population (Zapata *et al.*, 2006). A peptic ulcer results from an imbalance between the aggressive (hydrochloric acid, pepsin, refluxed bile, leukotrienes, reactive oxygen species) and the protective (mucus-bicarbonate barrier, prostaglandins, mucosal blood flow, cell renewal and migration, non enzymatic and enzymatic antioxidants) factors in the

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stomach (Bandyopadhyay *et al*., 2002), (Bhattacharjee *et al.*, 2002), (Konturek and Konturek,1994), (Wallace,1996). Stress, smoking, nutritional deficiencies, ingestion of non steroidal anti-inflammatory drugs, hereditary predispositions and infection by *Helicobacter pylori* are all factors that can increase the incidence of gastric ulcers (Baros *et al.,* 2008). Complications of acute and sub-acute gastric ulcers usually heal without leaving any visible scar. However, absence of healing of chronic ulcers may result in complications such as obstruction, hemorrhage and perforation (Mohan, 2002).

Due to the multiple causes of gastric ulcers, numerous drugs are being used for their treatment notably: antiacids, anticholinergics proton pump inhibitors, H_2-receptor antagonists, prostaglandin analogues and anti-*Helicobacter* drug (Rang and Dale, 2003). However, gastric ulcer therapy faces nowadays a major drawback because most of the drugs currently available in the market show limited efficacy against gastric diseases and are often associated with severe side effects (Bandyopadhyay *et al*., 2002), (Lehne, 1998). In this context, the use of medicinal plants is in continuous expansion all over the world for the prevention and treatment of different pathologies (Lehne, 1998). In traditional medicine, various herbal preparations are being used for treating ulcers. Plant extracts are some of the most attractive sources of new drugs and have shown promising results for the treatment of gastric ulcers (Schemeda-Hirschmann and Yesilada, 2005).

Corchorus olitorius Linn. (Tiliaceae), generally known as "Jute", is an annual, much-branched herb, 90-120 cm tall with glabrous stems, leaves of 6-10 cm long and 3.5-5 cm broad, with pale yellow flowers and black trigonous seeds (Kirtikar and Basu, 1987). *C. olitorius* is an important green leafy vegetable in many tropical areas including Egypt, Sudan, India, Bangladesh, in tropical Asian countries such as the Philippines and Malaysia, as well as in tropical Africa, Japan, South America, the Caribbean and Cyprus. In West African countries particularly Ghana, Nigeria, Cameroon and Sierra Leone, where staple diets consist of starchy food-stuffs such as rice, cassava, maize and yams, leafy vegetables are used to complement such staple foods (Tulio *et al.,* 2002). *C. olitorius* is cultivated to provide bark for the production of fibers and its mucilaginous leaves are used in food as a vegetable (Meikle, 1977), (Abou Zeid, 2002).

The crop is an excellent source of vitamins A and C, fiber, minerals including calcium and iron and other micronutrients. *C. olitorius* is extensively consumed as a "healthy vegetable" in Japan, because it contains abundant carotenoids, vitamin B_1, B_2, C and E, and minerals (Matsufuji, 2001). Jute contains high levels of all essential amino acids except methionine which is at marginal concentrations. It has high protein levels and is, along with other leafy species, the main source of dietary protein in many tropical countries (Tulio *et al.,* 2002). The leaves are used in ethnomedical practices to treat ache and pain, dysentery, malaria, enteritis, fever, gonorrhea, pectoral pains and tumors (Yoshikawa *et al.*, 1997).

The seeds are used as a purgative and the leaves as demulcent, diuretic, febrifuge and in chronic cystitis and dysuria (Farah *et al.*, 2006). The seeds have been reported to possess estrogenic activities as well as contain high content of hydrogen cyanide and several cardiac glycosides (Sharaf *et al*., 1979). The ethanolic extract of *C. olitorius* seeds extract of has great potentials as anti-diabetic remedy (Egua *et al.,* 2013). The polyphenolic extract has been reported to have anti-obesity effect (Wang *et al.,* 2011). The extract has been reported to suppress transformation of the aryl hydrocarbon receptor induced by dioxins (Nishiumi *et al*., 2005). The petroleum ether extract of *C. olitorius* leaves presented a good antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Yersinia enterocolitica* . The ethyl acetate and water extract presented a good antifungal activity against *Geotrichum candidum* and *Botrytis cinerea* (İlhan *et al*., 2007). Its aqueous extract has also been reported to have protective effect in arsenic-induced myocardial injury (Das *et al*., 2010).

The wound healing activity and the antioxidant activity of the leaf methanolic extract of *C. olitorius* were demonstrated (Barku *et al*., 2013). It is however known that plants which heal wounds and possess antioxidant activities could have anti-ulcerogenic properties. In this sense, the work of Batran *et al* in 2013 showed the gastro-protective property of an ethanolic extract of *C. olitorius* against ethanol-induced gastric mucosal hemorrhagic lesions in rats. Moreover, oral administration of the aqueous extract of *C. olitorius* inhibited gastric acid production (Owolyele *et al.,* 2014).

The genesis of gastro-duodenal ulcers being multi-factorial has led to advances in the discovery of novel and more efficient anti-ulcer therapies with the introduction of a larger number of experimental methods to evaluate their anti-ulcer activity as well as their mechanism of action (Lahiri and Palit, 2012). It is in the respect of this, that we proposed in the present work various animal models of gastric ulcers used to test the anti-ulcer and anti-oxidant activities of the aqueous extract of *C. olitorius* so as to elucidate its possible mechanism of action.

MATERIALS AND METHODS

Plant Collection

The fresh leaves of *C. olitorius* were collected in July 2013, in Yaounde Centre Region of Cameroon. The botanical identification was done at the National Herbarium, Yaounde, by Tadjoteu Fulbert, by comparison with existing herbarium specimen N° HNC/7422.

Preparation of plant extract

The dried ground leaves were extracted in water by boiling 500 g in 5 liters of water for 15 minutes. The extract solution was filtered using Whatman filter paper N° 3. The resulting solution was lyophilized to obtain 100 g of a brown solid (20 % yield). The extract dissolved readily in distilled water was used as the vehicle in the subsequent experiments.

Experimental animals

Male albino Wistar rats (180 ± 20 g) raised in the animal house of the Higher Teachers Training College, University of Yaoundé I, were used. They were fed with a standard laboratory diet (supplied by SPC Ltd., Bafoussam, Cameroon) and given tap water *ad libitum*. Prior authorization for the use of laboratory animals in this study was obtained from the Cameroon National Ethics Committee (Reg. no. FWAIRB00001954). The use, handling, and care of animals were done in adherence to the European Convention (Strasbourg, 18.III.1986) for the protection of vertebrate animals used for experimental and other purposes (ETS-123), with particular attention to Part III, articles 7, 8, and 9.

Anti- ulcer tests

HCl/ethanol-induced gastric lesions

The ulcer induction method described by Hara and Okabe in 1985 was used. The rats were deprived of food for 48 h prior to experimentation but the animals had free access to tap water. The test rats received the plant extract (100, 200 and 400 mg/kg) by oral route 1h before they were given the necrotizing solution of HCl/ethanol (150 mM HCl in 60 % v/v ethanol). Positive and negative control rats received sucralfate (50 mg/kg) and distilled water (1 ml/200 g) respectively, in place of the extract. They were sacrificed 1h later using ether. The abdomen of each rat was opened and the stomach removed. The ulcers produced in the glandular region of each stomach were measured and scored as early described (Tan *et al*., 1996). The ulcer index (UI), percent inhibition (% I) and percentage of ulcerated surface (% US) were calculated.

HCl/ethanol-induced gastric lesions in rats pre-treated with indomethacin

The effect of pre-treatment with indomethacine on the preventive effect of the extract on HCl/ethanol-induced gastric lesions was studied following the method described by Sun *et al* in 1992. All rats received indomethacin (20 mg/kg) by intra-peritoneal route. 1h later, the test rats received the plant extract (100, 200 and 400 mg/kg) while the positive and negative control rats received sucralfate (50 mg/kg) and distilled water (1ml / 200g) respectively by oral route. An hour later, all the animals were given orally 1 ml of HCl/ethanol solution. The rats were then sacrificed after 1h using ether and the stomach examined for gastric lesions.

Indomethacin-induced gastric lesions

Gastric mucosal lesions were induced by the method described by Pillai and Santhakumari in 1984. After depriving the animal of food for 48h, the test rats received the plant extract (200 and 400 mg/kg) by oral route, while the positive and negative control rats received sucralfate (100 mg/kg) and distilled water (1 ml/200 g), respectively. 1h later, all the animals were given indomethacin (50 mg/kg), orally. They were sacrificed 5h later and the ulcers produced in the glandular region of the stomachs were measured and expressed according to the score described by Tan et al in 1996. Gastric tissue samples were also collected at the end of the experiment for the analyses of some oxidative stress parameters.

Pylorus-ligated gastric secretion and ulceration

The pylorus ligation method described Shay *et al* in 1945 was used. Following a 48 h fast, the test rats received the extract of *C. olitorus* (100, 200 and 400 mg/kg), while the positive and negative control rats received cimetidine (50 mg/kg) and distilled water (1 ml/200 g) respectively, by oral route. One hour later, laparotomia was performed under light ether anesthesia and the pylorus of each rat was tied followed by the closure of the abdominal incisions. The rats were sacrificed 6h later and the gastric juice produced by each was collected, centrifuged, and the volume measured. The ulcers formed in the glandular region of the stomachs were scored as previously described by Tan *et al* in 1996. The ulcer index (UI) and percent inhibition (%I) were determined.

Acetic acid-induced chronic ulcers

The method described by Takagi *et al* in 1969 was used. Briefly, laparotomia was performed under ether anesthesia on experimental rats after a 24h fasting. Fifty microlitres of 30 % glacial acetic acid were injected into the wall of the stomach corpus at the region of the lesser curvature and the stomach wall wiped using cotton wool soaked in a 9 ‰ NaCl solution. The abdominal incisions were stitched up and feeding was resumed. A disinfectant (Betadine) was applied daily to avoid infection. Four days after the operation, a control group (group 1) was sacrificed using ether and their stomach was opened in order to establish the degree of ulceration prior to the onset of treatment. The remaining rats were divided into five groups: group 2 (ulcerated controls) received 1 ml/200 mg of distilled water daily by gavage for 10 day, while groups 3, 4, and 5 were given 100, 200, and 400 mg/kg of the extract respectively. Group 6 rats were given 50 mg/kg of ranitidine (Azantac).

Food and water intakes were measured daily and on the final day the rats were sacrificed and ulcer indices and gastric mucus production were measured. Ulcer healing rates were calculated by comparing the ulcer status of extract- and ranitidine-treated rats with those of the ulcerated untreated controls. The degree of auto-healing was evaluated by comparing the untreated controls with the rats sacrificed four days after induction. The stomachs were fixed and stored in formaldehyde awaiting histological studies.

Ethanol/Aspirin-Induced Chronic Ulcers

A modification of the method of Sun-Hye *et al* in 2008 was used. Gastric ulcers were induced in rats, following a 48h fast, using ethanol (70 %; 20 ml/200 g) and aspirin (200 mg/kg) by oral route. 24 hours after induction, ethanol (15 %; 20 ml/200 g) and the aspirin (200mg/kg) were administered 4 times at a 24h interval to maintain the ulcers. 24 hours after the last administration of ethanol/aspirin, one group (control 1) of rats were scarified using ether and the remaining animals were divided into five groups and were treated once a day for 10 consecutive days as follow: group 1 (control 2) rats received 1 ml/200 g of vehicle; groups 2, 3 and 4 received 100, 200 and 400 mg/kg of plant extract respectively and group 5 received 50 mg/kg of ranitidine. All the animals were sacrificed using ether 24 hours after the last administration. The ulcers created where observed morphologically and ulcer index was estimated. The healing rates of ulcers were calculated by comparing ulcer index of rats treated by extract or ranitidine with those of the ulcerated untreated controls (control 2). Normal stomachs were also made for comparison. Haematoxylin and eosin stains of stomach sections were then performed following standard histological procedures described by Bayelet-Vincent in 2002 and the sections observed microscopically.

Mucus production Assessment

The mucus covering the stomach of each rat was gently scraped using a glass slide and the mucus was weighed carefully using a sensitive digital electronic balance (exact name and address of manufacturer) (Djabanguiri, 1969).

Measurement of gastric acidity

The gastric content was collected and centrifuged at 4000 rpm for 10 min to remove residual debris. The volume of the gastric juice was measured using a graduated test tube. 1 ml of centrifuged gastric contents 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 (exact name and address of manufacturer). The acid content was expressed as mEq/l (Tan *et al*., 1996).

Preparation of histological sections

Section of stomach walls perpendicular to the surface of each ulcer crater were made. Sections of normal stomach were also made for comparison. Haematoxylin and eosin stains of stomach sections were then performed following standard histological procedures described by Bayelet-Vincent in 2002 and the sections observed microscopically.

Measurement of *in vivo* antioxidant capacity

Gastric tissue samples obtained from the rats subjected to indomethacine-induced gastric lesions were taken and prepared for the measurement of different oxidative stress parameters: lipid peroxidation was assessed by measuring the levels of malondialdehyde (MDA) in gastric tissue samples (Wilbur *et al*., 1949), superoxide dismutase (SOD) activity was measured using a standard method (Misra and Fridovich, 1972) and tissue protein was measured using the Biuret method of protein assay (Henry *et al*., 1974).

Statistical analysis

Values in tables were given as arithmetic means ± standard error of the mean (SEM). The significance of differences between groups was analyzed by means of Analysis of Variance (ANOVA) followed by Dunnett's comparison tests using GraphPad Prism 5.03 software. $P < 0.05$ was considered significant.

RESULTS

Anti-ulcer activity

Oral administration of the HCl/ethanol solution produced characteristic lesion in the glandular portion of rat stomachs. The aqueous extract of *C. olitorius* produced a dose-dependent inhibition of gastric ulceration of 4.65, 39.70 and 46.17% at the doses of 100, 200 and 400 mg/kg respectively. Sucrafate (50 mg/kg) also inhibited the formation of gastric lesion formation (65.11% inhibition). Treatment with *C. olitorius* extract or sucralfate was associated with increased mucus production compared with the control (Table 1).

The inhibitory effect of the extract and sucralfate against HCl/ethanol induced gastric ulcers were not suppressed by pre-treatment with indomethacin (Table 2). Mucus productions increased in a dose-dependent manner when increasing doses of *C. olitorius* were given to the rats. Sucralfate promoted lower mucus production Mucus productions increased in a dose-dependent manner when increasing doses of *C. olitorius* were given to the rats. Sucralfate promoted lower mucus production compared with the control following indomethacin pre-treatment.

The oral administration of indomethacin induced acute damage in the rat glandular stomach. The extract of *C. olitorius* at 100, 200 and 400 mg/kg prevented significantly ($p<0.05$) and dose-dependently the

development of gastric lesions, corresponding to inhibition percentages of 24.94 (100 mg/kg), 48.83 (200 mg/kg) and 58.44 (400 mg/kg). Omeprazol (60 mg/kg) showed the highest inhibition of lesion formation (74.54%) (Table3).

Tables 4 and 5 show the results of pylorus ligation on gastric lesion formation and gastric secretion respectively. Pyloric ligation of rats in the control group produced pointed lesions or raised inflammation. Ulcer index of the control group (4.44 ± 0.25) was significantly decreased to 2.00 ± 0.93 and 1.00 ± 0.63 when extract of *C. olitorius* was administered at the doses of 200 and 400 mg/kg respectively, leading to inhibition percentages of 54.95 and 77.47. Omeprazol at the dose of 50 mg/kg produced a significant ($p<0.05$) decrease in ulcer index (0.50 ± 0.31) compared with the control (4.44 ± 0.25) leading to an inhibition percentage of 88.74 (Table 4). Increasing doses of extract reduced gastric acid secretion from 81.20 ± 8.82 mEq/l in the controls to 53.96 ± 4.84, and 49.42 ± 4.97 mEq/l respectively for the doses 200 and 400 mg/kg, but had no significant effects on the volume of the gastric juice (Tables 5).

The ulcer healing test showed significant healing of chronic acetic ulcers following 10 days of treatment with *C. olitorius.* Ulcer surface areas reduced from 40.2 mm^2 in the 4-day (in the controls) to 16.8, 10, and 1.8 mm^2, respectively, for the rats receiving 100, 200 and 400 mg/kg of the extract. The highest dose of extract (400 mg/kg) showed a highly significant ($p<0.001$) reduction of ulcer with corresponding healing rate of 94.08%. Control rats that were sacrificed on day 4, post operation had deep well-defined gastric ulcer craters representing an ulcerated area of 40.20 ± 2.13 mm^2. In the control rats that were given the vehicle during 10 days post operation, ulcer area dropped to 30.40 mm^2 indicating a healing rate of 24.38%. However, this auto-healing was accompanied by a low degree of mucus production (50.22 mg) compared with the 4-day controls (68.00 mg). On the contrary, treatment with the plant extract was associated with an increase in mucus production, up to 162.90 mg ($p<0.001$) for the 400 mg/kg dose. A similar increase in mucus production (121.2 mg) was observed with ranitidine although it generated a healing rate of 35.53% (Table 6).

On the fourth day after induction of chronic gastric ulcers, stomach tissue (in the control 1) presented a mucosa destruction reaching the muscular layer, then the presence of edema and sclerotic block (Figure 1, photo 2). Ten days after (photo 3), the muscular layer returned to normal with a persistence of mucosa destruction, sclerotic block and leukocyte infiltration. *Corchorus olitorius* aqueous extract induced dose-dependent normalization of the stomach tissue. *Corchorus olitorius* extract (400 mg/kg) and ranitidine (50 mg/kg) induced almost complete healing of the stomach tissue (Figure 1).

Table 7 shows the healing effect of *C. olitorius* on gastric ulcers induced by oral administration of ethanol/aspirin in rats. In the 5-day controls, ulcer index was reduced from 6.92 to 5.54 in the vehicle controls, indicating auto-healing of 19.94%. Following two weeks of treatment with *C. olitorius*, ulcer index reduced from 5.54 in the vehicle controls to 3.67 for the rats receiving the highest dose of the extract (400 mg/kg) with corresponding healing rate of 33.75%. A healing rate of 20.57% was recorded for Ranitidine (50 mg/kg). Ranitidine (50 mg/kg) and extract (400 mg/kg) promoted significantly higher levels of mucus production (150.1 and 152.1 mg) during the treatment period compared to the vehicle controls (109.3 mg/kg).

Anti-oxidant Activity

Table 8 shows the *in vivo* anti-oxidant capacity of ECO. The extract dose-dependently (100, 200 and 400 mg/kg) increased concentration of superoxide dismutase (57.38, 58.46 and 63.74 U/g organ) in treated rats compared with the controls (33.8 U/g organ). In contrast, the level of malondialdehyde was decreased slightly in rats treated with the extract compared with the controls.

Table 1: Effect of the leaf aqueous extract of *C. olitorius* on the HCl/ethanol-induced gastric lesions in rats.

Treatment	Dose (mg/kg)	N	Ulcer index (mean ± SEM)	% inhibition	Mucus production (mg) (mean ± SEM)
Control	-	5	6,02 ± 1.01		54.87 ± 7.45
C. olitorius	100	5	5.74 ± 0.30	4.65	119.25 ± 24.16***
C. olitorius	200	5	3.63 ± 0.99	39.70	127.51 ± 8.64***
C. olitorius	400	5	3.24 ± 0.88*	46.17	146.01 ± 13.59***
Sucralfate	100	5	2,10 ± 0.60**	65.11	88.43 ± 7.14*

*Statistically different relative to control; *p<0.05; **p<0.01; ***p < 0.001; N, number of rats. The values are expressed as mean ± SEM*

Table 2. Effect of the leaf aqueous extract of *C. olitorius* on the HCl/ethanol induced gastric lesions in rats pre-treated with indomethacin.

Treatment	Dose (mg/kg)	N	Ulcer index (mean ± SEM)	% inhibition	Mucus production (mg)
Control	-	5	6.15 ± 0.86		54.87 ± 7,45
C. olitorius	100	5	5.66 ± 0.76	7.97	106.93 ± 9.25***
C. olitorius	200	5	4.00 ± 0.15*	34.95	120.93 ± 6.86***
C. olitorius	400	5	3.33 ± 0.34**	45.85	132.40 ± 21.87***
Sucralfate	50	5	3.52 ± 0.46*	42.76	67.7 ± 14.70

*Statistically different relative to control; *p<0.05; **p<0.01; ***p < 0.001; N, number of rats. The values are expressed as mean ± SEM*

Table 3. Effect of theleaf aqueous extract of *C. olitorius* on indomethacin induced gastric lesions in rats.

Treatment	Dose (mg/kg)	N	Ulcer index (mean ± SEM)	% inhibition	Mucus production (mg)
Control	-	5	3.85 ± 0,18	-	35.67 ± 5.92
C. olitorius	100	5	2.88 ± 0,34	24.94	68.14 ± 7.41*
C. olitorius	200	5	1.97 ± 0,54**	48.83	85.25 ± 2.79***
C. olitorius	400	5	1.60 ± 0,28**	58.44	95.02 ± 7.48***
Omeprazol	60	5	0.98 ± 0,30***	74.54	71.62 ± 6.40**

*Statistically different relative to control; *p<0.05; **p<0.01; ***p<0.001; N, number of rats. The values are expressed as mean ± SEM.*

Table 4. Effect of the leaf aqueous extract of *C. olitorius* on pylorus-ligated gastric ulceration in rats.

Treatment	Dose (mg/kg)	N	Ulcer index (mean ± SEM)	% inhibition	Mucus production (mg)
Control	-	5	4.44 ± 0.25	-	69.13 ± 6.72
C. olitorius	100	5	2.80 ± 0.85	36.94	92.38 ± 4.00*
C. olitorius	200	5	2.00 ± 0.93*	54.95	100.13 ± 2.85*
C. olitorius	400	5	1.00 ± 0.61**	77.47	114.06 ± 3.94**
Omeprazol	60	5	0.50 ± 0.31**	88.74	98.66 ± 8.90*

*Statistically different relative to control; *p<0.05; **p<0.01; N, number of rats. The values are expressed as mean ± SEM.*

Table 5. Effect of the leaf aqueous extract of *C. olitorius* on gastric secretion in pylorus-ligated rats.

Treatment	Dose (mg/kg)	*N*	Gastric contents (ml)	pH of gastric juice	Gastric acidity (mEq/l)
Control	-	5	4.32 ± 0.55	2.64 ± 0.15	81.20 ± 8.82
C. olitorius	100	5	3.21 ± 0.61	3.81 ± 0.11**	56.57 ± 5.70*
C. olitorius	200	5	2.86 ± 0.59	3.93 ± 0.21**	53.96 ± 4.84*
C. olitorius	400	5	2.26 ± 0.55	4.09 ± 0,03**	49.42 ± 4.97**
Omeprazol	60	5	3.66 ± 0.57	6.17 ± 0.40***	32.5 ± 4.11***

*Statistically different relative to control; *p<0.05; **p<0.01; ***p<0.001; N, number of rats. The values are expressed as mean ± SEM.*

Table 6. Healing effect of the leaf aqueous extract of *C. olitorius* on chronic acetic acid-induced gastric ulcers in rats.

Treatment	Dose (mg/kg)	N	Ulcerated area (mm^2)	% ulcerated area	% healing	Mucus production (mg)
Control 1	-	5	40.20 ± 2.13	5.96		68.00 ± 3.74
Control 2	-	5	30.40 ± 4.06	4.50	24.38	50.22 ± 5.81
C. olitorius	100	5	$16.80 \pm 2.82^{*}$	2,49	44.74	$104.50 \pm 6.43^{***}$
C. olitorius	200	5	$10.00 \pm 4.47^{***}$	1.48	67.11	$134.60 \pm 8.45^{***}$
C. olitorius	400	5	$1.80 \pm 0.20^{***}$	0.27	94.08	$162.90 \pm 15.85^{***}$
Ranitidine	50	5	19.60 ± 3.27	2.90	35.53	$121.20 \pm 2.56^{***}$

*Control 1 (ulcerated rats sacrificed 4 days after acetic acid ulcer induction); Control 2 (ulcerated rats given vehicle for 10 days following ulcer induction). Statistically different relative to control 2; *p<0.05; ***p<0.001; N, number of rats. The values are expressed as mean ± SEM.*

Table 7. Healing effect of the leaf aqueous extract of *C. olitorius* on chronic Ethanol/Aspirin -induced gastric ulcers in rats.

Treatment	Dose (mg/kg)	N	% ulcerated area	Ulcer index (mean ± SEM)	% healing	Mucus production (mg)
Control 1	-	5	25.39	6.92±0.53		105.4± 6.38
Control 2	-	5	23.94	5.54±0.17	19.94	109.3±11.82
C. olitorius	100	5	7.59	4.44± 0.41	19.86	144.7± 12.69
C. olitorius	200	5	6.64	3.70 ± 0.32**	33.21	146.7 ±6.17
C. olitorius	400	5	3.88	3.67±0.31**	33.75	152.1± 6.08*
Ranitidine	50	5	9.19	4.40 ± 0.31	20.57	150.1±17.18*

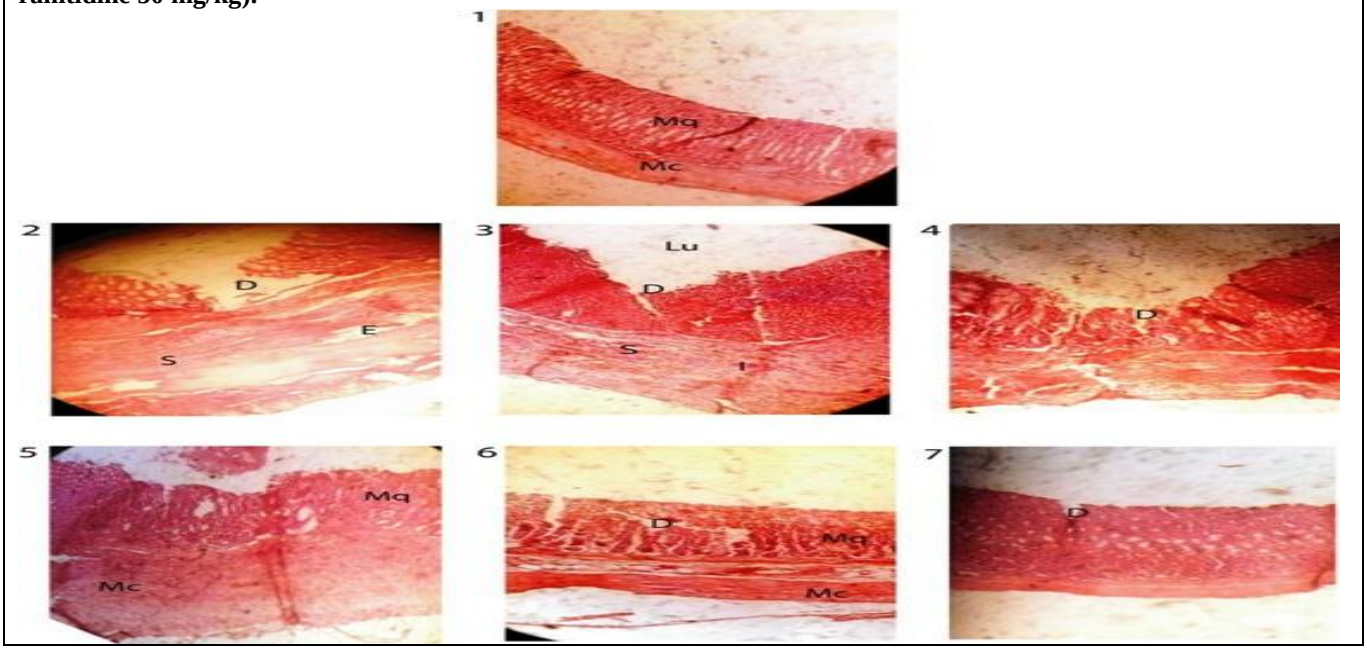
*Control 1 (ulcerated rats sacrificed 5 days after ethanol/aspirin induction); Control 2 (ulcerated rats given vehicle for 14 days following ulcer induction). Statistically different relative to control 2; *p<0.05; **p<0.01; N, number of rats. The values are expressed as mean ± SEM.*

Table 8. Antioxidant effect of the leaf aqueous extract of *C. olitorius* in rats subjected to indomethacin treatment.

Treatment	Dose (mg/kg)	N	Total Proteins (g/l)	Malondialdehyde (µmol/mg protein x10^{-6})	Super oxyde dismutase (U/g organ)
Normal rats	-	5	28.2 ± 1.92	1.11 ± 0,03	45.23 ± 3
Control	-	5	37.85 ± 2.67	1.32 ± 0.08	33.8 ± 2.5
C. olitorius	100	5	23.6 ± 3.08 **	1.19 ± 0.11	57.38 ± 8.54
C. olitorius	200	5	22.46 ± 2.37 **	1.12 ± 0.11	58.46 ± 6.54*
C. olitorius	400	5	19.95 ± 1.22 ***	0.95 ± 0.11	63.74 ± 3.94 *
Omeprazol	60	5	27.32 ± 2.96	1.09 ± 0.06	48.15 ± 5.32

*Statistically different relative to control; *p<0.05; ***p<0.001; N, number of rats. The values are expressed as mean ± SEM.*

Figure 1. Histological presentation of the chronic acetic acid-induced ulcers (Photo 1: normal rat; Photo 2: control 1; photo 3: control 2; photo 4: extract 100 mg/kg; Photo 5: extract 200 mg/kg; photo 6: extract 400mg/kg and photo 7: ranitidine 50 mg/kg).



DISCUSSION

The present study investigated on the prophylactic and healing activities of the aqueous extract of *C. olitorius* in several models of gastric damage in rats. The HCl/ethanol method was used to screen the extract for gastric cytoprotection against the mucosal irritant substance, and indomethacin was used to test if the cytoprotection was endogenous prostaglandin-linked. The ability of the extract to inhibit gastric acid secretion was tested against pylorus ligation-induced gastric lesions. Acetic acid and ethanol/aspirin methods were used to test the healing action of the extract in chronic ulcers. Oral

administration of ECO to the rats prevented the formation of gastric lesions induced by HCl/ethanol, indomethacin/HCl-ethanol, indomethacin and pylorus ligature. It also significantly enhanced the healing of chronic ulcers induced by acetic acid and ethanol/aspirin.

The pathogenesis of HCl/ethanol-induced gastric lesion involves the direct irritation of the stomach mucosa, reduction of mucosal resistance and erosion of the mucosal barrier. Products that have gastric protective effects against similar gastric irritant substances are said to possess cytoprotective potentials (Miller, 1982). Treatment with indomethacin causes gastric mucosal injury through a reduction of endogenous prostaglandin (PG) synthesis, inhibition of gastro-duodenal bicarbonate secretion, disruption of the mucosal barrier and reduction of mucosal blood flow (Flemstrom *et al*., 1982), (Selling *et al*., 1987). However, pre-treatment with indomethacin prior to HCl/ethanol solution did not affect the previously observed effect of ECO. Similar results have been interpreted by Sun *et al* in 1992 and Yamamoto *et al* in 1992 to mean that the cytoprotective action of the extract involves a direct mucosal protection similar to that of PGs and not related to endogenous PGs. PGs are known to prevent experimentally-induced ulcers and to protect the gastro-duodenal mucosa against various ulcerogens. Gastric mucosal protection by ECO may involve an enhancement of mucus production as was observed in all the models tested.

In addition to reduced gastric blood flow, reduced bicarbonate and PG secretion, indomethacin also inactivates gastric peroxidase to induce reactive oxygen-mediated gastric mucosal injury (Chattopadhyay *et al*., 2006). Indomethacin-induced oxidative damage by reactive oxygen species can be demonstrated by increased lipid peroxydation and thiol depletion which results in the formation of MDA. Indomethacin causes nearly a fivefold in hydroxyl radical ($^{\cdot}OH$) and significant of gastric mucosal peroxidase to elevate endogenous H_2O_2 and H_2O_2-derived $^{\cdot}OH$ (Chattopadhyay *et al*., 2006). Indomethacin also reduces the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione reduce S-transferase (Halici *et al*., 2005). However the role of these enzymes in the defense against the oxidative stress is well-known. SOD and catalase are the first line of cellular defense against oxidative injury (Mac Millan et *al*., 1998). SOD converts superoxide free radicals into H_2O_2 which is subsequently degraded by catalase (Favier, 2003), (Pincemail, 2005). The dose-dependent increases of SOD concentrations following ECO treatment are evidence of the extract-induced enhancement of the antioxidant status of the animals. Phytochemical screening of the aqueous extract of *C. olitorius* realized by Barku et al in 2013 revealed the presence of flavonoids, alkaloids, tannins and terpenoids, which are the phytochemical compounds with well-known antioxidant activity (Favier, 2003), (Vera-Arzave *et al*., 2012). This antioxidant activity was confirmed *in vitro* using 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) assay and Ferric Reducing Antioxidant power (FRAP) assay (Adedosu *et al*., 2013).

Ulcerogenesis in the case of pyloric ligature is due to the accumulated gastric juice in the stomach. The accumulated acid, in addition to its corrosive action on gastric glandular epithelium, provides the optimum pH (1.6-3.2) for the conversion of pepsinogen to pepsin. Both HCl and pepsin are important ingredients for the formation of pylorus-ligated ulcers (Shay *et al*., 1945). Agents that decrease gastric acid secretion and/or increase mucus secretion are efficient in protecting the ulcers induced by this method (Devaraj *et al*., 2007). In this study, the oral administration of the extract (100, 200 and 400 mg/kg) produced significant dose-dependent reductions in ulcer indices (36.94, 54.95 and 77.47 % inhibition), accompanied by significant dose-dependent increases in gastric mucus production (92.38, 100.13 and 114.06 mg) compared with the controls (69.13 mg). These results underline the protective role of mucus in the gastric mucosal. Moreover, gastric acid secretion reduced dose-dependently in extract-treated rats (56.57, 53.96 and 49.42 mEq/l) compared with the controls (81.20 mEq/l). Omeprazol treatment also significantly decreased gastric acidity to 32.5 mEq/l. The reduced gastric acidity measured after pylorus ligation suggests that the cytoprotective mechanism of action of the extract on gastric mucosa may involve direct inhibition of gastric secretion or a simple neutralization of the acid secreted by parietal cells. Gastric pH values obtained in all the experiments in response to extract administration ranged between 3.81 and 4.09. However, pepsin inactivation occurs at about pH 6. Between pH 4 and 6, pepsin is still stable but inactive (Vatier and Vallot, 1998). These results suggest that the extract may deactivate gastric pepsin and interfere with protein digestion.

The ulcer-healing test with acetic acid-induced chronic gastric ulcers showed a dose-dependent (100, 200 and 400mg/kg) enhancement of the healing process (44.74, 67.11 and 94.08 %) following 10 days of treatment with ECO.

This was associated with improved mucus production, which can protect the gastric mucosa against irritant stomach secretions (acid and pepsine) (Miller, 1982), (Bernier and Florent, 1986). Healing is a normal physiological process that proceeds through a series of coordinated cellular events, culminating in the restoration of the functional integrity of tissues (Anamika and Arti, 2000). Cellular proliferation plays an essential role in maintaining the integrity of the gastric mucosa (Polo *et al*., 2012). Other researchers observed that ulcer re-epithelialization is an essential process for gastrointestinal ulcer healing and without restoration of a continuous

epithelial barrier the mucosa would be vulnerable to mechanical or chemical injury and infections (Tarnawski *et al*., 2001). Histological observations revealed that treatment with ECO showed normalization of the mucosa. At the dose of 100 mg/kg we observed a proliferation of fibroblasts and epithelization. At the doses of 200 and 400 mg/kg we observed a disappearance of fibrosis, sclerosis and lymphocyte infiltration. These observations suggest that extract promoted ulcer healing up to the recovery stage possibly through increase in the concentration and movement of fibroblasts surrounding the ulcer region or facilitation of the proliferation of epithelial cells to the uncovered area (Shanbhag *et al*., 2006).

In humans and experimental models, peptic ulcer healing is delayed by non-steroid anti-inflammatory drugs (NSAIDS) but is accelerated by gastric acid inhibition, which enhances angiogenesis, cell proliferation, cell migration and maturation of the granulation tissue. Only highly effective gastric acid inhibition (by omeprazole, for example) reliably reverses NSAID-induced delay of gastric ulcer healing (Schmassmann, 1998). Prostaglandin analogs, mucosal defense agents (e.g. sucralfate) and various growth factors also significantly enhance healing of acetic acid ulcers (Okabe and Amagase, 2005). The extract (100, 200 and 400 mg/kg) dose-dependently inhibited gastric acid secretion (56.57, 53.96 and 49.42 mEq/l) compared with the controls (81.20 mEq/l), in pyloric ligature model.

These results suggest that cytoprotection and gastric acid inhibition by *C. olitorius* extract can promote ulcer healing through enhancement of angiogenesis, cell proliferation, cell migration and maturation of the granulation tissue. The aqueous extract of *C. olitorius* significantly reduced the formation of ulcers induced by indomethacin, increased the concentrations of SOD and decreased MDA concentrations in this model. Thus, the ability of the extract to improve the antioxidant status may also have been useful in the promotion of ulcer healing. The antioxidant effects of *C. olitorius* phytoconstituents may be implicated in the promotion of ulcer healing. The ulcer-healing test with ethanol/aspirin-induced gastric ulcers showed encouraging results after 14 days therapy with ECO.

The highest dose (400 mg/kg) of extract showed a very significant ($p \leq 0.01$) reduction of ulcer index with a healing rate of 33.75. This healing was accompanied by a high levels of mucus production (152.1 mg) compared with the vehicle controls (109.3 mg). Ulcerogenesis in this case is due to the combination of the aggressive action of ethanol and aspirin in the gastric mucosal. Pathogenesis of ethanol-induced gastric ulcers is complex. Ethanol may interact directly with the gastric mucosa or it may act through a more general mechanism affecting the release of hormones and the regulation of nerve functions involved in acid secretion (Bode and Bode, 1997), (Chari *et al*., 1993). Directly, ethanol disrobes the integrity of gastric mucosal barrier contributing to acid reflux into the subluminal layer of the mucosa and submucosa (Oh *et al*., 2005). Indirectly, ethanol releases the tissue-derived mediators such as histamine and leucotriene C_4. The action of these mediators on gastric microvasculature results in both mucosal and submucosal gastric mucous tissue destruction (Oates and Hakkinen, 1988). Aspirin, on its part, is a non-steroidal anti-inflammatory drug, like indomethacin, which reduces prostaglandin and bicarbonate secretion (Miller, 1982), (Flemstrom *et al*., 1982), (Selling *et al*., 1987). Thus, the reduction of prostaglandin synthesis increased susceptibility to mucosal injury and gastro-duodenal ulceration (Deore et al., 2011).

These mechanisms sufficiently explain the important level of ulceration observed in the rats in the 5-day control group (25.39 % of the ulcerated area). The significant reduction of the degree of ulceration in the rats treated with the extract, correlated with the increase in the production of mucus, confirms the implication of mucus in the healing mechanism of the ulcers caused by the aggressive substances.

CONCLUSION

This study demonstrated the anti-ulcer activity of the leaf aqueous extract of *C. olitorius* in various experimental models of gastric ulcer. The anti-ulcer property of the extract is probably due to numerous mechanisms notably the stimulation of mucus secretion by a mechanism similar to that of endogenous prostaglandins, the inhibition of acid secretion by parietal cells, the reinforcement of the *in vivo* antioxidant status by the increase in the concentration of SOD and the reduction of MDA. The results support the traditional use of the plant material in folk medicine for the management of the symptomatic complaints of peptic ulcers diseases.

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CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

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