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EVALUATION OF ANTI-ULCER ACTIVITY OF AQUEOUS & ETHANOLIC EXTRACT OF *OXALIS CORNICULATA* LEAF IN EXPERIMENTAL RATS

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ABSTRACT

The present study was performed to evaluate the anti-ulcer & anti-oxidant activity of aqueous & ethanolic extracts of *Oxalis corniculata* leaves in albino rats. The antiulcer activity of *oxalis corniculata* leaves aqueous & ethanolic extract (AEOC & EEOC respectively) was investigated by using ethanol induced gastric mucosal damage, pylorus ligated gastric ulcers. Evaluation was done on both models comparing with reference standard omeprazole (20mg/kg p.o.) The parameters taken to assess anti-ulcer activity for pylorus ligation method were volume of gastric secretion, pH, free acidity, total acidity and ulcer index. Ulcer index was common parameter studied in both models, further effect on lipid peroxidation namely Malondialdehyde [MDA] content and antioxidant enzymes viz. superoxide dismutase [SOD] and catalase [CAT] levels were studied in ethanolic induced mucosal damage model. These results may further suggest that ethanolic extract was found to possess antiulcerogenic activity.

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Key Words

INTRODUCTION

Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Considering the several side effects (arrhythmia's, impotence, funaecomastia and haematopoeitic changes) of modern medicine, indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer. Plants provide an alternative strategy in search for new drugs. There is a rich abundance of plants reputed in traditional medicine known to possess antiulcer properties. It is likely that plants will continue to be a valuable source of new molecules which may, after possible chemical manipulation, provide new and improved antiulcer drugs.

Oxalis corniculata L. (Family: Oxalidaceae) is a small creeping perennial herb which forms roots at nodes. Leaves alternate, long-petiolate, trifoliate (clover-like in appearance), the leaflets obcordate with a conspicuous notched apex, each leaflet up to 2 cm long. This plant reported for Hypoglycemic, antihypertensive, chronotropic effect, uterine relaxant, antipsychotic, CNS-stimulant, antiyeast^[1].

Phytochemical investigations of *Oxalis corniculata* Linn. have revealed the presence of tannins, palmitic acid, a mixture of oleic, linoleic, linolenic and stearic acids^[2]. Methanolic and ethanolic extracts of this plant show the presence of carbohydrate, glycosides, phytosterols, phenolic compounds, flavanoids, proteins (12.5%), amino acids and volatile oil^[3]. Leaves contain tartaric acid and citric acids, calcium oxalate, flavones (acacetin and 7,4'-diOMe apigenin), glycoflavones (4'-OMe vitexin, 4'-OMeiso-vitexin and 3',4'-diOMe orientin), flavonols (3',4'-diOMe quercetin) and phenolic acids such as p- hydroxybenzoic, vanillic and syringic acids^[4].

However review literature revealed that majority of the antiulcer studies used ulcer models, the constituents like flavonoids, tannins, terpenes, steroids, Saponins, alkaloids is responsible for antiulcer effect^[5,6,7] therefore in the present study on the antiulcer effect of the aqueous and ethanolic extracts on pylorus ligation, and ethanol induced models was investigated in rats.

MATERIALS & METHODS

Plant material and preparation of the extract:

The plants were collected from area of Sangli District and it is authenticated by Dr. Mrs. U.S. Yadav. The leaves of plants were dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (200gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus with ethanol as a solvent and extracted till the solvent becomes colorless. The marc was dried and again extracted with distilled water to give aqueous extract. Both the extracts were evaporated under reduced pressure using rotary evaporator (Equitron Roteva) at a low temperature of 40-60°C until the extract turns syrupy and then the syrupy extract is transferred to an evaporating dish for drying at room temperature. Percentage yield of AEOC & EEOC was found to be 5 % & 4% w/w respectively.

Animals

Healthy Wistar adult male and female albino rats between 2 and 3 months of age and weighing about 150-200g were used for the study. Housed individually, in polypropylene cages, maintained under standard conditions (12-hr light: 12 -hr dark cycle; 25±3°C; 35-60% humidity), the animals were fed with standard rat pellet diet. . The experimental protocol has been approved by the Institutional Animal ethics Committee, Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra (Registered No. 843 / AC / 04 / CPCSEA), India.

Acute Oral Toxicity Study:

Acute toxicity studies for aqueous and ethanolic extract of *oxalis corniculata* were conducted as per OECD guideline 420 (modified, adopted 23rd march 2006) using Albino Wister mice. Each animal was administered ethanolic and aqueous extracts solution by oral route. The test procedure minimizes the number of animals required to estimate the oral acute toxicity of chemical and in addition estimation of LD50^[8].

Experimental design:

Albino wistar rats were divided into six groups of six animals each. Group I received vehicle control (1% w/v CMC, 1ml/100 g) whereas Group-II and III, received aqueous extract of the leaves of *Oxalis corniculata* (L.)

(AEOC) (200 and 400 mg/kg body weight, *p.o* respectively). Group-IV and V, received ethanolic extract of the leaves of *Oxalis corniculata* (L.) (EEOC) (200 and 400 mg/kg body weight, *p.o* respectively). Group VI received standard drug (Omeprazole – 20 mg / kg. *p.o*).

Antiulcer activity by pylorus ligation method

Extracts of *oxalis corniculata* leaves were screened for antiulcer activity by pyloric ligation method [9,10,11]. In this method albino rats were fasted in individual cages for 24 hr care was taken to avoid coprophagy. *Oxalis corniculata* aqueous & ethanolic extracts of leaves, standard drug and control vehicle was administered 30min prior to pyloric ligation under light ether anaesthesia. Under light ether anesthesia, the abdomen was opened and the pylorus was ligated. The abdomen was then sutured. At the end of 4 hr. after ligation the animals were sacrificed with excess of anaesthetic ether. Abdomen was opened & oesophageal end (cardiac end) of the stomach was tied. The entire stomach was removed from the body of the animal. Gastric juice was collected into graduated centrifugation tube and was centrifuged at 1000 rpm for 10 min and gastric volume was noted. The pH of the gastric juice was recorded by pH meter. Then the centrifuged supernatant contents were subjected to analysis for free and total acidity. Open the stomach along the greater curvature and washed with running water to observation of ulcers in glandular portion of the stomach. The number of ulcers per stomach was noted and severity of the ulcers scored microscopically with the help of hand lens (10X) and scoring was done.

Ethanol induced gastric mucosal damage

Animals were fasted for about 16 hrs before the experiment, but were allowed free access to water. One ml of absolute alcohol was administered orally to the rats. In the treatment groups the rats were given the drug 1 hr prior to the administration of ethanol. After 2 hrs of ethanol treatment, animals were sacrificed; stomach was removed and cut along the greater curvature and

examined for lesions. Severity was determined by measuring ulcer index.

In this model, extent of damage due to free radicals were also tested to determine the lipid peroxidation viz. Malondialdehyde (MDA) [12] and antioxidant markers were estimated such as Superoxide dismutase (SOD) [13,14] and Catalase (CAT) [15,16] in stomach, tissue homogenate.

Measurement of Ulcer Index

The stomach was washed with saline and the lesion was examined under a 10 x dissecting microscope, Ulcer index of each animal was calculated by adding the values and their mean values were determined by the following scoring system

(i) Normal coloured stomach – 0, (ii) red coloration – 0.5, (iii) spot ulceration – 1, (iv) hemorrhagic streak – 1.5, (v) ulcers – 2, (vi) perforations – 3.

Statistical analysis

The results were expressed in terms of mean \pm SEM for 6 animals in each group. Significance was determined by one way analysis of variance followed by Dunnet's test [17] and $P < 0.01$ is considered as significant.

Result

Acute toxicity study in which the animals treated with the aqueous & ethanolic extracts at a higher dose of 5000mg/kg did not manifest any significant abnormal signs, behavior changes, body weight changes or macroscopic findings at any time of observation, hence the dose of 200 and 400 mg / Kg were taken for the study.

Antiulcer activity by pylorus ligation method

EEOC treated animals has showed significant reduction in ulcer index. EEOC 400 mg / kg and Omeprazole treated showed significant reduction ($P < 0.01$) in gastric volume, total and free acidity and increase in GI pH and when compared with the control groups [Table No.1].

Table No. 1: Effects of Aqueous (AEOC) and Ethanolic (EEOC) extracts of *Oxalis Corniculata* leaves against Pylorus ligation induced gastric ulcers in rats.

Groups	Gastric volume	pH	Total acidity	Free acidity
Control	4.51±0.19	1.56±0.094	105.16±3.06	83.16±3.21
Std. (Omeprazole 20mg/Kg)	2.23±0.18**	3.50±0.21**	32.33±2.84**	24.33±1.70**
AEOC 200 mg/Kg	4.05±0.11 ^{ns}	1.77±0.04 ^{ns}	45.16±2.94**	38.83±2.96**
AEOC 400 mg/Kg	3.43±0.21**	2.12±0.37 ^{ns}	41.33±2.83**	35.66±2.97**
EEOC 200 mg/Kg	2.71±0.17**	2.32±0.21 ^{ns}	46.33±3.26**	41±3.12**
EEOC 400 mg/Kg	2.38±0.28**	2.71±0.25**	43.5±3.13**	38.16±3.00**

Results are expressed as mean ± S.E.M. (n = 6). Statistical comparison was performed by using ANOVA coupled with Dunnet's test. * P < 0.05, ** P < 0.01 were consider statistically significant when compared with control group, (ns=non significant).

Effect of *Oxalis corniculata* on Ethanol induced gastric mucosal damage.

In control animal oral administration of absolute ethanol produced characteristic lesions in the glandular portion of rat stomach. EEOC has shown significant inhibition of ulcer at the doses of 200mg/kg and 400mg/kg respectively in comparison to control. Ethanol-induced mucosal damage was significantly and dose dependently reduced by pre-treatment of the animals with EEOC.

Table No. 2: Ulcer Index and % protection of AEOC and EEOC in Pylorus ligation and ethanol induced gastric ulcers in rats.

Groups	Pylorus ligation model		Ethanol induced model	
	Ulcer Index	% protection	Ulcer Index	% protection
Control	13.33±0.6280	-	28.75±1.716	-
Std. (Omeprazole 20mg/Kg)	3.66±0.6009**	66.66	9.16±1.531**	68.139
AEOC 200 mg/Kg	9.33±0.6412**	23.54	21±1.176**	26.95
AEOC 400 mg/Kg	6.83±0.4944**	33.94	17.75±1.124**	38.26
EEOC 200 mg/Kg	6.16±0.6412**	35.19	18.41±0.9075**	35.96
EEOC 400 mg/Kg	5.66±0.4410**	44.34	12.33±0.9888**	57.10

Results are expressed as mean ± S.E.M. (n = 6). Statistical comparison was performed by using ANOVA coupled with Dunnet's test. * P < 0.05, ** P < 0.01 were consider statistically significant when compared with control group.

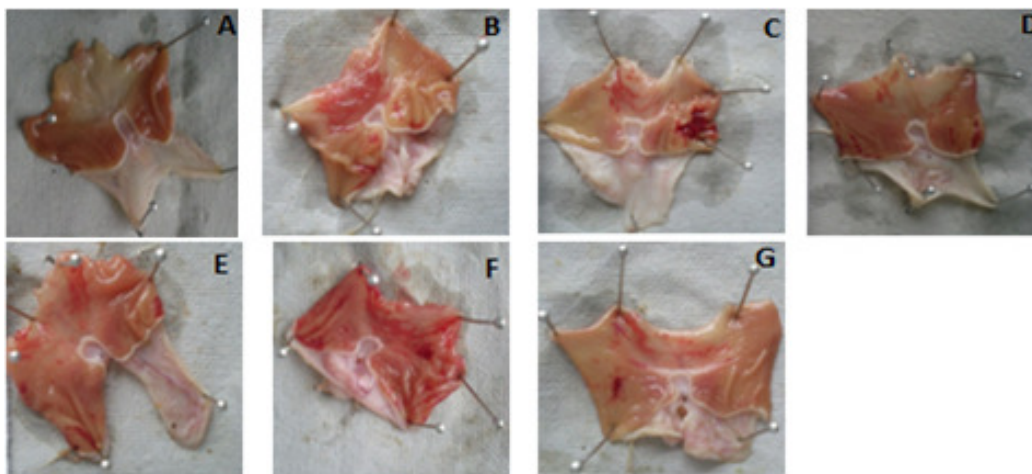
Effect of extracts on antioxidant enzymes:

The level of lipid peroxide (MDA) was reduced significantly (Table 3) in the gastric tissue in Omeprazole & extract treated groups as compared to ulcer control group. EEOC (200mg/kg) & AQOC (400mg/kg) has shown significant results when compared to ulcer control group. Table 3 shows that oral administration of extracts & drug to the experimental group of rats significantly increased the catalase activity & SOD activity as compared to the untreated ulcerated group.

Table No. 3 : Effects of AEOC and EEOC on antioxidant enzymes and lipid peroxidation in ethanol induced gastric mucosal damage in rats.

Groups	CAT U / mg of Protein	SOD U / mg of Protein	MDA μ mole / mg of protein
Normal Control	3.47±0.1112	35.67±0.3324	0.84±0.0290
Ulcer Control	0.33±0.02692**	19.75±0.2332**	2.60±0.1307**
AEOC 200 mg/Kg	0.41±0.0052 ^{ns}	22.67±0.2803**	0.933±0.010 ^{ns}
AEOC 400 mg/Kg	1.09±0.0764**	27.42±0.3281**	1.33±0.09765**
EEOC 200 mg/Kg	0.42±0.0038 ^{ns}	23.58±0.3708**	1.50±0.0952**
EEOC 400 mg/Kg	1.18±0.1120**	28.40±0.2689**	0.961±0.01731 ^{ns}
Std. (Omeprazole 20mg/Kg)	2.55±0.1169**	32.87±0.4102**	0.826±0.01629**

Results are expressed as mean ± S.E.M. (n = 6). Statistical comparison was performed by using ANOVA coupled with Dunnet's test. * P < 0.05, ** P < 0.01, ulcer control group was compared with normal control group and drug, extract treated groups were compared with ulcer control group.

Figure 1 : Images of ethanol induced mucosal damage model A) Normal Control, B) Standard (Omeprazole - 20 mg / Kg), C) Ulcer Control, D) AEOC - 200 mg / Kg, E) AEOC - 400 mg / Kg, F) EEOC - 200 mg / Kg, G) EEOC - 400 mg / Kg.

DISCUSSION

In this work, we have studied anti-ulcerogenic activity of *Oxalis corniculata* leaves in models including Antiulcer activity by pylorus ligation method & Ethanol induced gastric mucosal damage, where ulcerogens produce ulcer is either due to the effect on acid secretion or on cytoprotection.

Review of the available literature revealed that majority of the antiulcer studies used pylorus ligation, aspirin and ethanol induced ulcer models. The different constituents like flavonoids, tannins, terpenes, steroids,

Saponins, alkaloids and glycosides have been reported to be responsible for anti ulcer activity.^{[18] [19]}

Ulcers caused by chemical agents such as ethanol shows effect on mucosal damage to capillary endothelium and release of arachidonate metabolites, leukotrienes C4 / D4 (LTC4 / D4) and platelet activating factor (PAF)^[20]. Ethanol administration to rats causes gastric injury which is associated with the production of oxygen free radical leading to increased lipid peroxidation resulting in increased MDA level^[21]. That is ethanol produces oxidative stress. In the present study it was found that

the level of SOD, catalase were decreased by administration of ethanol, whereas, the administration of *Oxalis Corniculata* extract resulted in a significant increase in the SOD and CAT levels. SOD is an important endogenous antioxidant enzyme that acts as the first line defense system against ROS which scavenges superoxide radicals.

Pylorus ligation induced ulcers are due to auto digestion at the gastric mucosa and breakdown of the gastric mucosal barrier [22]. In case of pyloric ligation, ulcer formation is mainly due to the stasis at the gastric juice and stress [23]. Anti secretory activity of the extracts was noticed in pylorus ligation induced ulcer model. There was decrease in gastric volume and reduction in free and total acidity in the animals treated with aqueous and ethanolic extracts.

CONCLUSION

Aqueous and ethanolic extracts of *Oxalis corniculata* at dose 400mg/kg, has reduced ulcer incidence when compared to the control group as evident by decrease in ulcer index in above the two models. There was decrease in gastric volume and reduction in free and total acidity in the animals treated with aqueous and ethanolic extracts in case of antiulcer activity in pylorus ligation method. The extracts of *Oxalis corniculata* were found to possess antiulcer activity due to the presence of flavonoids and tannins in them.

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