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Evaluation of Anti-Ulcer Activity of Aqueous and Ethanolic Extract of Whole Plant of *Clitoria ternatea* in Albino Wistar Rats

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ABSTRACT

The objective of the present study was to evaluate antiulcer potential of aqueous and ethanolic extract *Clitoria ternatea* in different experimental induced ulcer models in rats. In the present study different extracts [ethanolic (200 mg/kg and 400 mg/kg) and aqueous (200 mg/kg and 400 mg/kg) extract of whole plant were examined in Pylorus ligation and Indomethacin induced gastric ulcer in rats. Various parameters like volume of gastric acid secretion, pH, total acidity, ulcer index and antioxidant parameters were determined and compared between extract treated, standard and vehicle control group animals following ulcer induction. Among different dose of alcoholic extract, high dose showed significant antiulcer activity in Pylorus ligation and Indomethacin induced ulceration. The result of present study concluded that the alcoholic extract of whole plant of *Clitoria ternatea* has antiulcer activity in Pylorus ligation and Indomethacin induced gastric ulcer model in rats. The extract containing flavonoids show antiulcer activity, indicating the flavonoids component of the extract to be responsible for the activity of the extracts.

Keywords: Antiulcer, Pylorus ligation, Indomethacin, Ulcer index, *Clitoria ternatea*.

INTRODUCTION

Peptic ulcer disease (PUD) is one of the most common gastrointestinal disorders, which causes a high rate of morbidity particularly for the population of non-developed countries. Peptic ulcer occurs in the part of the gastrointestinal tract which is exposed to gastric acid and pepsin, i.e., the stomach and duodenum. The etiology of peptic ulcer is not clearly known but it results probably due to an imbalance between the aggressive (acid, pepsin and *H. pylori*) and the

defensive (gastric mucus and bicarbonate secretion, prostaglandins, nitric oxide, innate resistant of the mucosal cells) factors. Hyper secretion of gastric acid is a pathological condition, which occurs due to uncontrolled secretion of hydrochloric acid from the parietal cells of the gastric mucosa through the proton pump H⁺ K⁺ ATPase. Even the normal rate of acid secretion may cause ulceration in the breached mucosa when some gastro protective factors are imbalanced. [1] Various classes of synthetic antiulcer drugs used for its treatment like, H₂-blockers, M₁ blockers etc, are associated with danger of drug interaction, adverse effect and increased incidence of relapse during ulcer therapy. Therefore, search for an ideal antiulcer drug continues and has also been extended to herbal drugs for their easy availability, better protection, low cost and lesser toxicity. [2-3]

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Clitoria ternatea, known by a number of names including butterfly pea, winged leaved Clitoria, Mezereon, Girikarnikaa, Aasphota, Kakkanam, Aparajita and Koyal is a plant in Fabaceae family. It is a perennial herbaceous plant with elliptic and obtuse leaves. It grows as a vine or creeper, doing well in moist neutral soil. It is grown as an ornamental plant and as a revegetation species (e.g., in coal mines in Australia), requiring little care when cultivated. This plant is also used to improve soil quality roots due to nitrogen fixation. *Clitoria ternatea* is widely used in various ayurvedic formulation and its various parts like root and root bark, flowers, whole plant have traditionally been proved for various ailments like purgatives, ascites, diuretics, and bronchitis, anxiolytic, antidepressant, anticonvulsant, colouring agent in food preparation, migraine, memory enhancer, antimicrobial, hepatoprotective, and nootropic. [4-11] Moreover *Clitoria ternatea* contain a lactone aparajitin, taraxerol, flavanoids, phenol glycoside, alkaloid, P-hydroxy cinnamic acid polypeptide, hexacosanol, anthoxanthin, clitorin, cyanin chloride, palmitic, stearic, oleic, linoleic, linolenic acids, protein and amino acids. It was hypothesized that *Clitoria ternatea* might possess gastroprotective effect due to presence of flavanoids. The objective of the present study was to investigate the gastroprotective effect of the different extract of the whole plant of *Clitoria ternatea* using pylorus ligation and Indomethacin induced ulcer model.

MATERIALS AND METHODS

Drugs and chemicals

All the drugs and chemicals used were of analytical grade. Ethanol, anesthetic ether (Kabra Drugs Ltd.) and Omeprazole (Dr Reddy's Pharmaceutical limited, Mumbai) were used in this study.

Plant material and preparation of extracts

The whole plant of *Clitoria ternatea* were collected from UAS, GKVK, Bangalore and authenticated by Dr. Vasundhara M, Professor Department of Horticulture, GKVK, Bangalore.

Extraction

The whole plants were dried on filter paper sheets under shade at room temperature until changing of color of filter papers and milled into coarse powder. 200 g of powder material placed was extracted with 70% ethanol in a Soxhlet apparatus for 8-12 h. Solvent were removed at temperature below 50°C in an oven. The residue (extract) of respective plant material was stored at 4°C for further experimental studies. Aqueous extract were prepared by taking 100g of the powdered plant material with 500 ml of distilled water in a Soxhlet apparatus for 8-12 h. The filtrate was then concentrated and the extract was stored at 4°C for further experimental studies.

Preliminary Phytochemical Screening

Aqueous and Ethanolic extract of *C. ternatea* were subjected to preliminary phytochemical screening for the detection of various plants constituents like

Tannins, carbohydrates, Saponins, Flavanoids, Glycosides, Proteins, Alkaloids and Phenols.

Animals

Inbred Wister albino rats weighing between 150-200 g were housed in a group of 5 to 6. All rats were feed with pelleted diet (Pranav Agro Industries Ltd, Sangli, India) and *water ad libitum*. Institutional Animals Ethics Committee (IAEC) approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA, Department of Animal Welfare and Government of India.

Pylorus ligation induced gastric ulceration in rats

Pyloric ligation of the stomach was done according to method of Shay *et al.* [12] with slight modification. Albino rats of either sex were divided into six groups of six animals each. Animals were fasted for 24 h before the study, but had free access to water. Animals in the control group received only 0.1% of Tween 80 (10 ml/kg orally). Aqueous and Ethanolic extract of *C. ternatea* at 200 and 400 mg/kg, (*p.o.*) for each extract were given to the animals in the treatment group. Omeprazole (10 mg/kg) was used as a standard. After 1h of drugs treatment, they were anaesthetized with the help of anaesthetic ether; the abdomen was opened by a small midline incision below the lipid process. Pyloric portion of the stomach was slightly lifted out and ligated according to method of Shay *et al.* avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall was closed by interrupted sutures. Rats were sacrificed by an over dose of anaesthetic ether after four hours of pylorus ligation.

The abdomen was opened, the stomach was removed, and its content drained into a graduated centrifuge tube and centrifuged at 3000 rpm for 10 min. From the supernatant, aliquots (1 ml of each) were taken for the determination of pH, and total acidity. Each stomach was examined for lesions in the fore stomach portion and indexed according to severity. [13-14]

Determination of total acidity and pH of gastric juice

Pipetted out 1 ml of supernatant liquid and diluted it to 10 ml with distilled water. Acidity was determined by titration with 0.01 N NaOH using phenolphthalein as an indicator. The pH of the solution was noted using digital pH meter. The total acidity is expressed as mEq/L and calculated by the following formula: [15]

$$\text{Acidity} = \frac{\text{Vol. of NaOH} \times \text{Normality of NaOH} \times 100}{0.1} \text{ mEq/L}$$

Macroscopic evaluation of stomach

The stomachs were opened along the greater curvature, rinsed with saline to remove gastric contents and blood clots and examined by a 10x magnifier lens to assess the formation of ulcers. The number and degree of erosions and ulcers were scored in 0-5 levels as described in the (Cantarella *et al.*, 2005, 2007). [16-17]

Score Macroscopic gastric damage

- | | |
|-----|-------------------|
| 0.0 | No lesions |
| 0.5 | Diffuse hyperemia |

- 1.0 1 to 2 small erosions
 1.5 3 to 6 small erosions
 2.0 7 to 10 small erosions
 2.5 More than 10 small erosions
 3.0 1 marked erosion plus 0 to 4 small erosions
 3.5 1 marked erosion plus 5 or more small erosions
 4.0 2 marked erosions plus 0 to 4 small erosions
 4.5 2 marked erosions plus 5 or more
 5.0 3 or more marked erosions

Small < 2 mm; marked ≥ 2 mm

Mean ulcer score for each animal were expressed as ulcer index. Ulcer index (UI) was measured by using following formula:

$$UI = UN + US + UP \times 10^{-1}$$

Where, UI= Ulcer Index; UN = Average number of ulcers per animal;

US = Average number of severity score; UP = Percentage of animals with ulcers

Percentage inhibition of ulceration was calculated as follows:

$$\% \text{ Inhibition of Ulceration} = \frac{(\text{Ulcer index Control} - \text{Ulcer index Test})}{\text{Ulcer index Control}} \times 100$$

UN = average of number of ulcers per animals. US = average of severity score. UP = percentage of animals with ulcers.

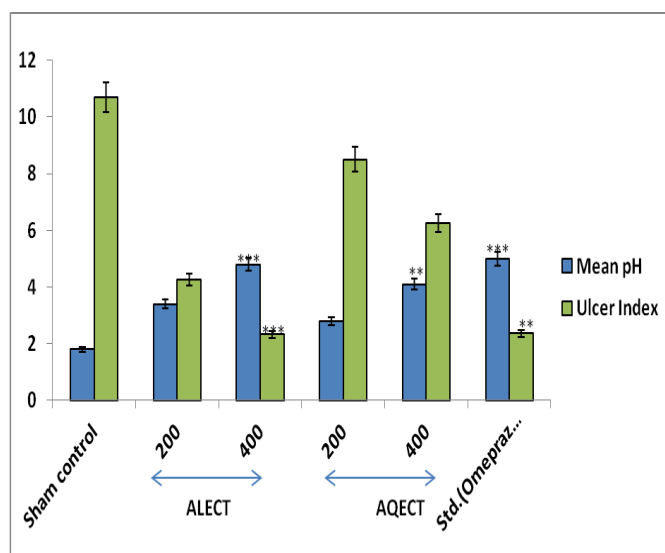
Indomethacin induced ulcer

Albino rats of either sex were divided into six groups of six animals each. Animals were fasted for 24 h before the study, but had free access to water. Animals in the control group received only vehicle 10 ml/kg orally. Aqueous and Ethanolic extract of *C. ternatea* at 200 and 400 mg/kg, were administered orally for each extract were given to the animals in the treatment group. Omeprazole (10 mg/kg) orally was used as a standard. Indomethacin (25 mg/kg body weight) was administrated orally to all animals 10 min prior to treatment. After 6 h of drugs treatment, rats were sacrificed by an over dose of anaesthetic ether and their stomach was removed. The contents of the stomach were drained into a glass tube. The volume of the gastric juice was measured and centrifuged at 2000 rpm for 10 min. From the supernatant, aliquots (1 ml of each) were taken for the determination of pH, and total acidity. [14]

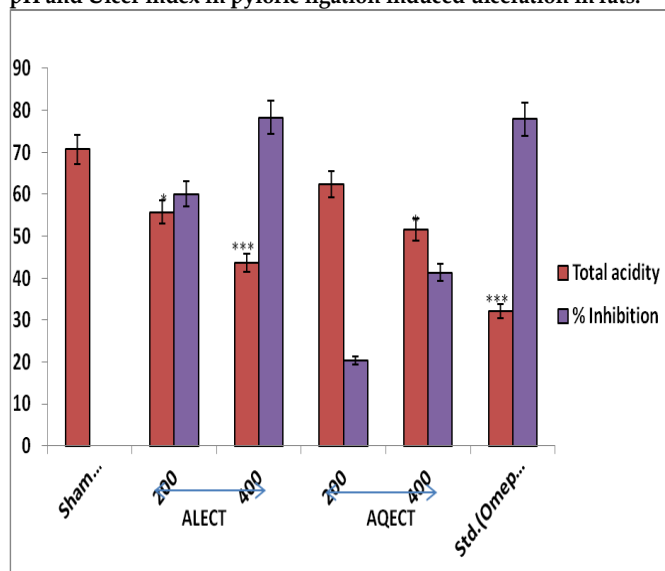
10% v/v Formalin was injected into the totally ligated stomach for storage overnight. The next day, the stomach were opened along the greater curvature, then washed in warm water, and examined under a 3 fold magnifier. The ulcer index was determined as described above.

Statistical analysis

Values were expressed as mean ± SEM from 6 animals. Statistical differences were evaluated using a One-way analysis of variance (ANOVA) followed by Dunnet's t-test. Results were considered to be statistically significant at $P < 0.05$.



Graph 1: Effect of ethanolic and aqueous extracts of *C. ternatea* on pH and Ulcer index in pyloric ligation induced ulceration in rats.



Graph 2: Effect of ethanolic and aqueous extracts of *C. ternatea* on total acidity and % inhibition of ulcer in pyloric ligation induced ulceration in rats.

Table 1: Phytochemical Screening of ethanolic and aqueous extracts of the whole plant of *Clitoria ternatea*

S. No.	Phytoconstituents	Ethanolic extracts of whole plant of <i>Clitoria ternatea</i>	Aqueous extracts of whole plant of <i>Clitoria ternatea</i>
1	Tannins	+	+
2	Carbohydrates	+	+
3	Saponins	+	+
4	Flavonoids	+	+
5	Glycosides	+	+
6	Proteins	+	+
7	Alkaloids	+	+
8	Phenols	+	+

(+) sign indicates presence of phytoconstituents in the extract.

RESULTS

Preliminary Phytochemical Screening

The phytochemical screening of the alcoholic and aqueous extracts of the whole plant of *Clitoria ternatea* revealed the presence of tannins, carbohydrates, Saponins, flavonoids, glycosides, proteins alkaloids, and phenols.

Table 2: Effect of ethanolic and aqueous extracts of *C. ternatea* on pH, total acidity, Ulcer index and % inhibition of ulcer in pyloric ligation induced ulceration in rats.

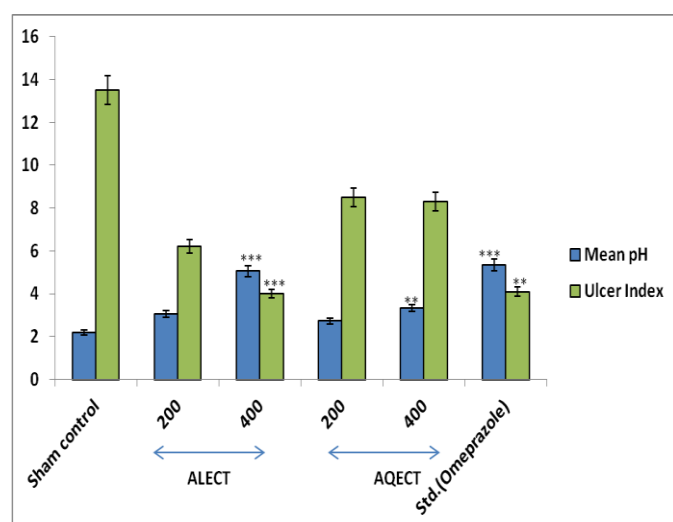
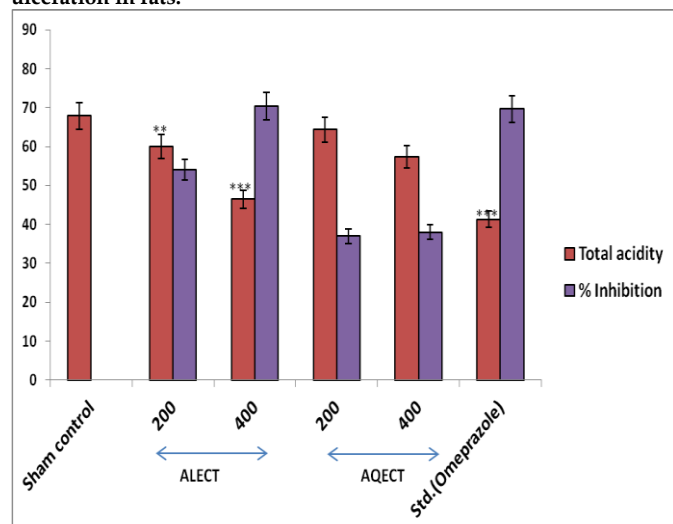
Groups	Treatment	Mean pH	Mean total acidity	Mean ulcer index	% Inhibition of ulcer
G1	Control (0.1% of Tween 80) 10 ml/kg p.o.	1.8 ± 0.05	70.73 ± 2.63	10.69 ± 2.51	
G2	ALECT (200mg/kg p.o.)	3.4 ± 0.25	55.7 ± 4.64*	4.27 ± 0.18	60.05%
G3	ALECT (400mg/kg p.o.)	4.8 ± 0.28**	43.68 ± 2.56***	2.32 ± 0.12***	78.29%
G4	AQECT (200mg/kg p.o.)	2.8 ± 0.15	62.36 ± 3.33	8.51 ± 0.26	20.39%
G5	AQECT (400mg/kg p.o.)	4.1 ± 0.16**	51.61 ± 3.37*	6.27 ± 0.21	41.34%
G6	Omeprazole (10 mg/kg p.o.)	5.0 ± 0.17***	32.11 ± 2.12***	2.36 ± 0.16**	77.29%

Values are expressed as mean ± S.E.M. n = 6. Significant values were compared with* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ Pylorus ligation control Vs treated groups using One way ANOVA followed by Dennett's test.

Table 3: Effect of ethanolic and aqueous extracts of *C. ternatea* on pH, total acidity, Ulcer index and % inhibition of ulcer in Indomethacin induced ulceration in rats.

Groups	Treatment	Mean pH	Mean total acidity	Mean ulcer index	% Inhibition of ulcer
G1	Control (0.1% of Tween 80) 10 ml/kg p.o.	2.20 ± 0.18	67.86 ± 1.14	13.50 ± 0.56	
G2	ALECT (200mg/kg p.o.)	3.05 ± 0.15	60.00 ± 2.36**	6.2 ± 0.32	54.0%
G3	ALECT (400mg/kg p.o.)	5.05 ± 0.11***	46.47 ± 1.53***	4.00 ± 0.36***	70.37%
G4	AQECT (200mg/kg p.o.)	2.73 ± 0.30	64.38 ± 1.37	8.5 ± 0.49	37.0%
G5	AQECT (400mg/kg p.o.)	3.33 ± 0.17**	56.38 ± 1.50	8.3 ± 0.42	38.0%
G6	Omeprazole (10 mg/kg p.o.)	5.35 ± 0.14***	41.32 ± 0.83***	4.1 ± 0.32**	69.62%

Values are expressed as mean ± S.E.M. n = 6. Significant values were compared with* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ Indomethacin control Vs treated groups using One way ANOVA followed by Dennett's test.

**Graph 3: Effect of ethanolic and aqueous extracts of *C. ternatea* on Mean pH and Ulceration index of ulcer in Indomethacin induced ulceration in rats.****Graph 4: Effect of ethanolic and aqueous extracts of *C. ternatea* on total acidity and % inhibition of ulcer in pyloric ligation induced ulceration in rats.**

Effect of different doses of ethanolic and aqueous extracts of the whole plant of *C. ternatea* on pylorus ligation-induced gastric ulceration

Effect of ethanolic and aqueous extracts of the whole plant of *C. ternatea* on pyloric ligation induced ulceration is shown in Table 2. The pyloric ligation has caused the accumulation of gastric secretions with pH 1.8 ± 0.05 in a control group. The total acidity of the gastric secretions was found to be 70.73 ± 2.63 mEq/L in a control group. Pretreatment with the extracts of *C. ternatea* extract, significantly ($P < 0.05$) elevated the pH of the gastric fluid up to 3.4 ± 0.25 and 4.8 ± 0.28 at dose of 200 mg/kg and 400 mg/kg of ethanolic extract and 2.8 ± 0.15 , 4.1 ± 0.16 at dose of 200 mg/kg and 400 mg/kg of the aqueous extract. In addition, total acidity were also reduced significantly ($P < 0.05$) in a dose dependant manner. Further it was observed that pyloric ligation has caused gastric ulcerations and pretreatment with ethanolic and aqueous extract of *C. ternatea* extract has reduced them significantly ($P < 0.05$) in a dose dependent manner. In this model, percentage inhibition of ulceration was found to be 60.05, 78.29 for ethanolic extract and 20.39, 41.34 for aqueous extract at a dose of 200 and 400 mg/kg respectively. The gastroprotection offered by the test extracts was comparable to that of the standard drug, Omeprazole (10 mg/kg) which offered 77.29% inhibition of ulceration. Hence the ethanolic extract of *C. ternatea* has shown maximum protection against pylorus ligation induced gastric ulcer.

Effect of different doses of ethanolic and aqueous extracts of the whole plant of *C. ternatea* on Indomethacin-induced gastric ulceration

Effect of alcoholic and aqueous extracts of the whole plant of *C. ternatea* on Indomethacin induced ulceration is shown in Table 3. The pyloric ligation has caused the accumulation of gastric secretions with pH 1.8 ± 0.05 in a control group. The total acidity of the gastric secretions was found to be 70.73 ± 2.63 mEq/L in a control group. Pretreatment with the extracts of *C. ternatea* extract, significantly ($P < 0.05$) elevated the pH of the gastric fluid up to 3.4 ± 0.25 , 4.8 ± 0.28 at dose of

200 mg/kg and 400 mg/kg of ethanolic extract and 2.8 ± 0.15 , 4.1 ± 0.16 at dose of 200 mg/kg and 400 mg/kg of the aqueous extract. In addition, total acidity were also reduced significantly ($P < 0.05$) in a dose dependant manner. Further it was observed that pyloric ligation has caused gastric ulcerations and pretreatment with ethanolic and aqueous extract of *C. ternatea* extract has reduced them significantly ($P < 0.05$) in a dose dependent manner. In this model, percentage inhibition of ulceration was found to be 60.05, 78.29 for ethanolic extract and 20.39, 41.34 for aqueous extract at a dose of 200 and 400 mg/kg respectively. The gastroprotection offered by the test extracts was comparable to that of the standard drug, Omeprazole (10 mg/kg) which offered 77.29% inhibition of ulceration. Hence the

ethanolic extract of *C. ternatea* has shown maximum protection against pylorus ligation induced gastric ulcer.

DISCUSSION

Peptic ulcer is the erosion in the lining of the stomach which involves an imbalance between the aggressive (acid, pepsin and *H. pylori*) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, nitric oxide, innate resistant of the mucosal cells) factors. To regain the balance, different therapeutic agents including plant extracts may be used.

In the present study *Clitoria ternatea* is used to evaluate the anti-ulcerogenic potential in pylorus ligation and Indomethacin induced ulcer in rats.



Pylorus ligation Control



ALECT (200mg/kg)



ALECT (400mg/kg)



AQECT (200mg/kg)



AQECT (400mg/kg)



Std. Omeprazole (10 mg/kg)

Fig. 1: Macroscopic evaluation of stomach ulcer in pyloric ligation induced ulceration in rats.

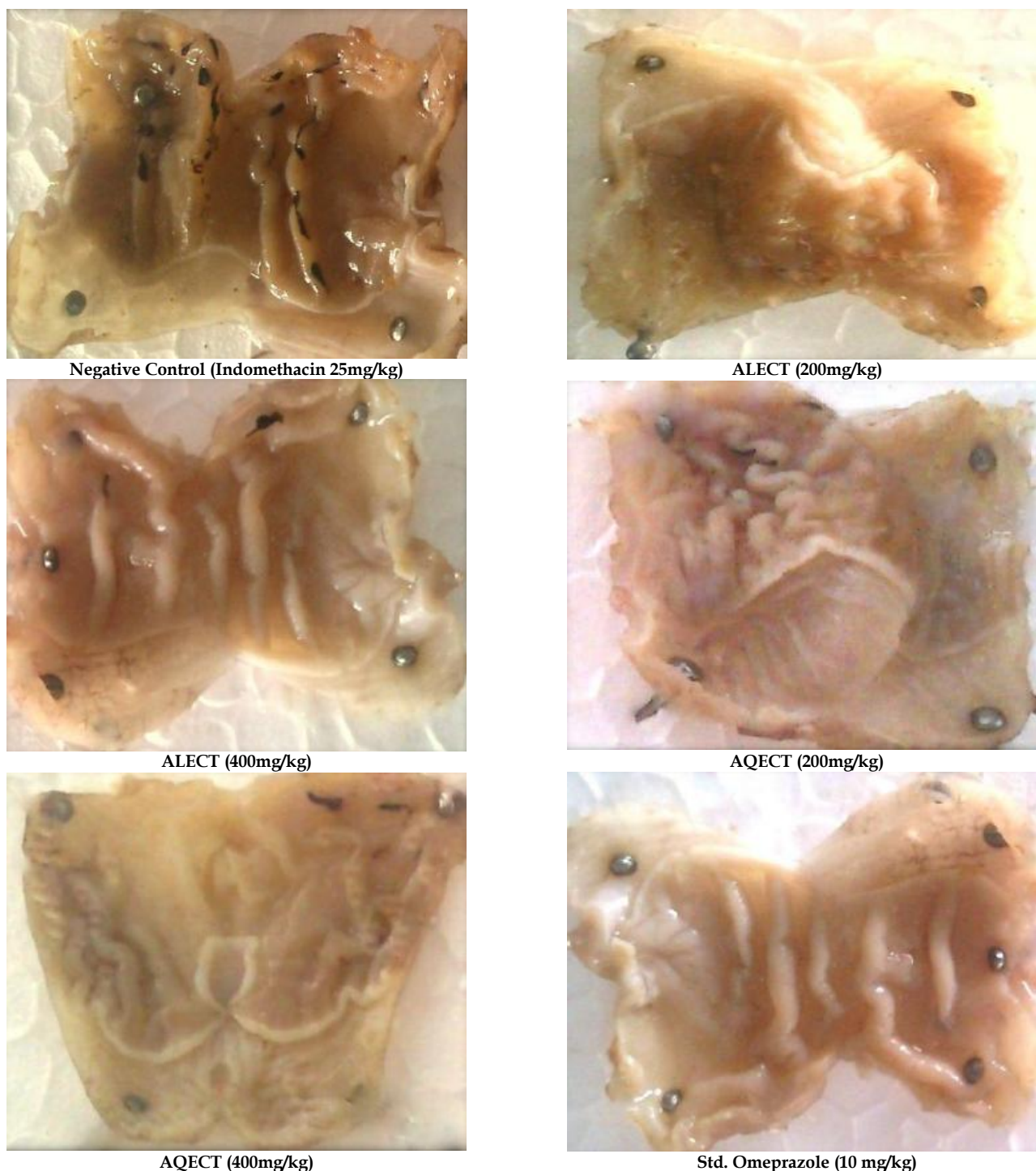


Fig. 2: Macroscopic evaluation of stomach ulcer in Indomethacin induced ulceration in rats.

Qualitative phytochemical analysis of the alcoholic and aqueous extracts of the whole plant of *C. ternatea* showed the presence of carbohydrates, flavonoids, saponins, alkaloids, and tannins.

Anti-secretory activity of ALECT and AQECT in this model is evident from its significant reduction in acid secretory parameters viz. total acidity. Moreover, these results showed that the antiulcer activity of this was not only related to a local neutralization of gastric content but also it has significant Systemic effect.^[18]

As the extract of *C. ternatea* showed the positive result for the presence of flavonoids and it has reported that flavonoids have antiulcerogenic activity. So here may

be the ulcerogenic activity reduced because of the flavonoids, which shows significant result as compared to standard drug.

Non-steroidal anti-inflammatory drugs (NSAIDs) like Indomethacin are known to induce gastric damage, particularly due to inhibition of the cyclooxygenase pathway of arachidonic acid metabolism. It is currently believed that the ulcerogenic effects of the NSAIDs are due to inhibition of cyclooxygenase 1 (COX-1) and that its isoforms, cyclooxygenase 2 (COX-2), plays a pathological role in inflammation, pain and fever. Several studies shown that gastric mucosal prostaglandins (PGs), produced mainly by COX-1, play

an important role in maintaining gastric mucosal integrity and Indomethacin markedly decrease mucosal PGE₂ level. On the other hand, recent reports show that Indomethacin is a dual inhibitor of COX-1 and COX-2 because both thromboxanes and inflammatory PGE₂ synthesis are suppressed, and that inhibition of both isoform of these enzymes is required for the development of gastric erosions after NSAID administration. Indeed, endogenous PG deficiency alone did not induce visible gastric lesions and the pathogenesis of NSAID-induced gastric lesions also involves luminal acid, neutrophils activation and gastric hyper motility.^[19]

Indomethacin is known to cause ulcer especially in an empty stomach and mostly on the glandular (mucosal) part of the stomach by inhibiting prostaglandins synthetase through the cyclooxygenase pathway. Prostaglandins function to protect the stomach from injury by stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal turn over and repair. Suppression of prostaglandins by Indomethacin results in increased susceptibility of the stomach to mucosal injury and gastro duodenal ulceration^[20]. The extract was observed to significantly reduce mucosal damage in the Indomethacin induced ulcer model, suggesting the possible extracts mobilization and involvement of prostaglandin in the anti-ulcer effect of the extract.

It is well established that various antisecretory agent, such as H₂ inhibitor, Proton pump inhibitor prevent gastric lesions. Omeprazole has been reported to possess antioxidant, anti inflammatory and cytoprotective effect, which is responsible for its anti ulcerogenic activity. Hence in the present study Omeprazole has taken as a standard drug to compare the antiulcer activity of *C. ternatea* featuring its phytoconstituents having antioxidant and antisecretory activity. It can be concluded that *C. ternatea* can suppress chronic gastric damage induced by administration of Indomethacin and pyloric acid accumulation by virtue of its antioxidant phytoconstituents like flavonoids and tannins. The result indicates that *C. ternatea* exerts cytoprotective effect in addition to its gastric antisecretory activity that could be due to the presence of flavonoids responsible for its protective effect by maintaining an efficient gastric mucosal micro vascular supply.

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