

EVALUATION OF ANTIULCER ACTIVITY OF METHANOLIC EXTRACT OF  
*ANDROGRAPHIS ECHIOIDES*

Ashok Kumar U\*, Manjunath C, Thaminzhmani T, Gnana Shekaran D, Ravi Kiran Y ,  
Brahmaiah Y

Department Of Pharmacology, Bharathi College Of Pharmacy, Bharathi Nagara, Maddur Tq.,  
Mandya Dt., Karnataka, India-571422.

**Summary**

The objective of this study was to investigate the antiulcer activity of leaves of *Andrographis echioides* N. (Acanthaceae) in rats. Fresh whole plant of *Andrographis echioides* was extracted with 95% of methanol by soxhlet apparatus. Antiulcer activity of the methanol extract at two concentrations 200 and 400 mg/kg were evaluated in rats using ethanol and pylorus ligation induced ulcer models. Famotidine as Standard drug. The evaluated parameters are ulcer index, acid volume, pH and total acidity. The extract (200 mg/kg & 400 mg/kg) showed significant (P<0.001) reduction in acid volume, total acidity and ulcer index as compared to control. This present study indicates that *Andrographis echioides* whole extract extract have potential anti ulcer activity in all the models.

**Keywords:** Antiulcer, Ethanol, Pylorus Ligation, Famotidine

**Abbreviations:** Carboxy methyl cellulose (CMC), *Angrographis echioides* methanol extract (ACMC)

Corresponding author

**Ashok Kumar Ummadisetty\***, M.Pharm,

Department of Pharmacology,

Bharathi College of Pharmacy,

Bharathinagara,

Mandya Dist

Karnataka, India- 571 422.

E-mail: ashok.ummadisetty@gmail.com

## Introduction

Gastric ulcers are mucosal lesions that result from an imbalance between aggressive factors such as acid and pepsin, and defensive mechanisms like gastric mucous, high mucosal blood flow and high mucosal turnover rate that work towards maintenance of mucosal integrity<sup>1</sup>. Another factor that has been implicated in the pathogenesis of gastric ulcers is oxidative stress in the gastric mucosa. Studies have shown a positive correlation between increased free radicals and the extent of gastric ulceration in experimental animals<sup>2-4</sup>. Agents that are currently available for the treatment of gastric ulcers act by either reducing gastric acid secretion (H<sub>2</sub> blockers, proton pump inhibitors, anti muscarinic agents), acting as physical barriers (sucralfate, colloidal bismuth subcitrate), or increasing the mucous and bicarbonate secretion (prostaglandin analogues, carbenoxolone). Even though these agents are effective in healing of gastric ulcers, continued use is required to prevent recurrence. Continued use of these agents can in turn lead to a plethora of side effects ranging from dryness of mouth to achlorhydria, atrophic gastritis, osteodystrophy and encephalopathy<sup>5</sup>.

The plant *Andrographis echioides*. Nees is an annual herb, distributed through India and Sri Lanka. It belongs to family *Acanthaceae*. The herb is useful for the treatment of dysentery, diabetes, swelling and also used for liver diseases and jaundice<sup>6</sup>. Plants belonging to the genus *Andrographis* have been shown to contain, a diterpenoid lactone and flavanoids. The group of flavanoids is famous for its anti-inflammatory, anti-allergic, antithrombotic vasoprotective, protection of gastric mucosa. Leaves of this plant as resulted in the isolation of two new flavones, echioidinin<sup>7</sup> and echioidin<sup>8</sup>. The plant also reported to contain a new flavonone, dihydroechioidinin<sup>15</sup> and two known flavones, skullcapflavone 2- methyl ether and skullcapflavone 2- o – glucoside<sup>9</sup>. Aerial parts of *Andrographis echioides* is reported to have anti-inflammatory, analgesic, antipyretic, hepatoprotective, antioxidant properties<sup>10</sup> and antimicrobial activity. The antiulcer activity of *Andrographis echioides* has not yet been studied. Hence the aim of the present investigation was to evaluate the antiulcer activity of methanolic extract of *Andrographis echioides*.

## Materials and methods

### Plant material

The whole plants were collected and authenticated by Dr. K. Madava Chetty, Assistant professor, department of botany, Tirupathi, AndhraPradesh, India and voucher specimen was deposited in institutional herbarium (voucher no 971).

### Preparation of extracts

Powdered leaves of of *Andrographis echioides* (500g) was extracted (soxhlet) with 95% methanol in 1:10 w/v ratio for 72hrs. Qualitative chemical analysis revealed that the extract contains alkaloids, carbohydrates steroids, glycosides, flavonoids and reducing sugars<sup>11,12</sup>.

### **Experimental animals**

Healthy adult albino rats of Wistar strain weighing 150-200g of either sex were used for this study. The animals were obtained from animal house, Bharathi College of Pharmacy, Bharathi nagara, Karnataka, India. The animals were maintained under controlled conditions of temperature ( $23 \pm 2^\circ\text{C}$ ), humidity ( $50 \pm 5\%$ ) and 12-h light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water *ad libitum*. Animals were habituated to laboratory conditions for 48 h prior to experimental protocol to minimize if any of non-specific stress. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Bharathi College of Pharmacy, Bharathinagara, Karnataka (BCP/IAEC/05/10-11), according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

### **Chemicals**

All the drugs and chemicals were of analytical grade. Famotidine (Osaka), Ethanol (Research lab) were used.

### **Assessment of Anti-Ulcer Activity**

#### **a) Ethanol induced ulcers:**

Four groups of albino Wistar rats (n=6) were selected. In this model, Group 1 served as normal control received 0.5 ml of carboxy methyl cellulose, *p.o.*, and group 2 received Famotidine (3 mg/kg, *p.o.*), whereas groups 3 and 4 animals received methanol extract of *Andrographis echinoides* (200 and 400 mg/kg, *p.o.* respectively). Animals were fasted overnight prior to start of the experiment, and water *ad libitum* 30 min after treatment, all rats received 1ml of absolute ethanol to induce gastric ulcer. After 1 h the animals were sacrificed by cervical dislocation, the stomachs were removed and opened along the greater curvature. Stomachs were gently rinsed with water to remove gastric contents and the mean ulcer index was calculated<sup>13</sup>.

#### **b) Pylorus ligation induced ulcers:**

Four groups of albino Wistar rats (n=6) were selected. In this model, Group 1 served as normal control received 0.5 ml of Carboxy methyl cellulose, *p.o.*, and group 2 received Famotidine (3 mg/kg), whereas groups 3 and 4 animals received methanolic extract of *Andrographis echinoides* (200 and 400mg/kg, respectively). Animals were fasted overnight prior to start of the experiment, and water *ad libitum*. Pyloric ligation was applied by ligating the pyloric end of the stomach of rats under Phenobarbital anaesthesia (35 mg/kg) after 30 min of methanol extract of *A. echinoides* and Famotidine treatments. Animals were allowed to recover and stabilize in individual cage and were deprived of water during postoperative method. After 6 h of surgery, rats were sacrificed with excess ether and gastric juice was collected for performing gastric secretion study and ulcer scoring was done in stomach<sup>14</sup>.

**Determination of ulcer index:**

The tracing of the stomach boundary and the ulcerated area on the transparent film was placed on top of a graph paper. The total surface area of the stomach and the lesions was determined in mm<sup>2</sup> from the graph paper. The ratio of total surface area and the total ulcerated area was determined and scoring of the ulcer index was done according to the method described by Ganguly<sup>14</sup>. Percentage protection was calculated in the drug treated groups against control using the formula:

$$\% \text{ protection} = (1 - \text{ulcer index in test} / \text{ulcer index in control}) \times 100$$

**Determination of free acid volume**

Instead of phenolphthalein indicator, the Topfer's reagent was used. Aliquot of gastric juice was titrated with 0.01N NaOH until canary yellow colour was observed. The volume of 0.01N NaOH consumed was noted. The free acidity was calculated by the same formula for the determination of total acidity.

**Determination of pH**

An aliquot of 1ml gastric juice was diluted with 1ml of distilled water and pH of the solution was measured using pH meter.

**Determination of total acidity**

An aliquot of 1ml gastric juice diluted with 1ml of distilled water was taken into a 50 ml conical flask and two drops of phenolphthalein indicator was added to it and titrated with 0.01N NaOH until a permanent pink colour was observed. The volume of 0.01N NaOH consumed was noted. The total acidity is expressed as mEq/L by the following

Formula:

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

**Statistical analysis:**

The data of results obtained were subjected to statistical analysis and expressed as mean  $\pm$  SEM. The data were statistically analyzed by one-way analysis of variance (ANOVA) and  $p < 0.01$  was considered to be significant and  $p < 0.001$  was considered to be more significant.

**Results**

**Ethanol induced ulcer model:**

The results indicate that *A. echioides* methanol extract at the dose levels of 200 mg/kg and 400 mg/kg produced a significant decrease in the ulcer index (Fig: 3-4). The Results suggests that the ulcer index of *A. echioides* methanol extract (200mg/kg and 400mg/kg) was found to be  $5.21 \pm 0.63$  and  $7.43 \pm 0.35$  which was found to be significant when this data was compared with Standard i.e Famotidine (3 mg/ kg) with ulcer index  $3.01 \pm 0.28$ .

The results of acid volume determination of *A. echioides* methanol extract treated groups indicate that there was a more significant decrease in the volume of the gastric juice. The activity was comparable and equipotent as that of Famotidine ( $p < 0.001$ ) (Table no.1)

The results of gastric pH determination of *A. echioides* methanol extract treated groups indicate that there was a significant increase in the pH of the gastric juice. The activity was comparable and equipotent as that of Famotidine ( $p < 0.001$ ) (Table no.1).

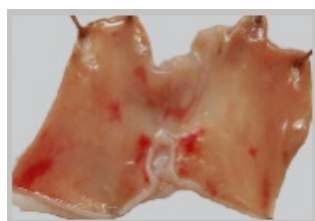
The results of total acidity estimation of gastric juice of *A. echioides* methanol extract treated groups indicate that there was a significant decrease in total acidity of the gastric juice. The activity was comparable and equipotent as that of Famotidine ( $p < 0.001$ ) (Table no.1).

**Pylorus ligation induced ulcers:**

The results suggests that *A. echioides* methanol extract at the dose levels of 200 mg/kg and 400 mg/kg produced more significant decrease in the ulcer index ( $p < 0.001$ ). The Results suggests that the ulcer index of *A. echioides* methanol extract (200mg/kg and 400mg/kg) was found to be  $7.35 \pm 0.02$  and  $11.21 \pm 0.09$  which was found to be significant when this data was compared with Standard i.e Famotidine (3 mg/ kg) with ulcer index  $5.21 \pm 0.35$  (Table no. 2)

The results of acid volume determination of *A. echioides* methanol extract treated groups indicate that there was a more significant decrease in the volume of the gastric juice. The activity was comparable and equipotent as that of Famotidine ( $p < 0.001$ ) (Table no.2)

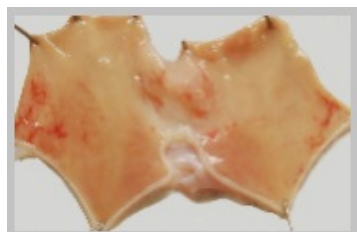
The results of gastric pH determination of *A. echioides* methanol extract treated groups indicate that there was a significant increase in the pH of the gastric juice. The activity was comparable and equipotent as that of Famotidine ( $p < 0.001$ ) (Table no.2).



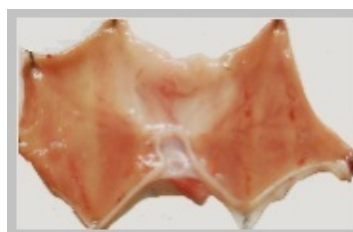
**Fig: 1: Ethanol induced control**



**Fig: 2: Ethanol induced std. Treated**



**Fig: 3: Ethanol induced 200mg/kg. test drug**



**Fig: 4: Ethanol induced 200mg/kg. test drug**

**Table 1.** Effect of *A. ehioides* methanolic extract (ACMC) on ethanol induced gastric ulcers

G. No	Treatment	Dose mg/kg	Parameters			
			Ulcer Index	Acid Volume (ml)	P <sup>H</sup>	Total Acidity (mEq/l)
I	CMC Suspension	1 m.l	11.43 ± 0.21	8.41 ± 0.16	2.96 ± 0.04	117 ± 0.39
II	Famotidine Standard	3	3.01 ± 0.28 <sup>***</sup>	2.41 ± 0.26 <sup>***</sup>	5.91 ± 0.57 <sup>***</sup>	63.10 ± 0.21 <sup>***</sup>
III	ACMC Lower dose	200	5.21 ± 0.63 <sup>***</sup>	4.7 ± 0.15 <sup>***</sup>	4.72 ± 0.03 <sup>***</sup>	72.10 ± 0.37 <sup>***</sup>
IV	ACMC Higher dose	400	7.43 ± 0.35 <sup>**</sup>	5.1 ± 0.10 <sup>**</sup>	3.97 ± 0.31 <sup>**</sup>	85.78 ± 1.24 <sup>**</sup>

Values are expressed as Mean + SEM (n=6 rats) P<0.05\*, P<0.01\*\* and P<0.001\*\*\* as compared to control group.

**Table 2.** Effect of *A. ehioides* methanolic extract (ACMC) on pylorus ligation induced gastric ulcers

G. No	Treatment	Dose mg/kg	Parameters			
			Ulcer Index	Acid Volume (ml)	P <sup>H</sup>	Total Acidity (mEq/l)
	CMC Suspension	1 m.l	15.01 ± 0.21	8.76 ± 0.16	3.21 ± 0.27	97.3 ± 0.7
	Famotidine Standard	3	5.21 ± 0.35 <sup>***</sup>	2.37 ± 0.51 <sup>***</sup>	6.23 ± 0.15 <sup>***</sup>	39 ± 0.23 <sup>***</sup>
	ACMC Lower dose	200	7.35 ± 0.02 <sup>***</sup>	3.57 ± 0.21 <sup>***</sup>	5.37 ± 0.21 <sup>***</sup>	56.09 ± 0.39 <sup>***</sup>
	ACMC Higher dose	400	11.21 ± 0.09 <sup>**</sup>	4.92 ± 0.15 <sup>**</sup>	4.78 ± 0.34 <sup>**</sup>	70 ± 0.66 <sup>***</sup>

Values are expressed as Mean + SEM (n=6 rats) P<0.05\*, P<0.01\*\* and P<0.001\*\*\* as compared to control group.

### Discussion

Peptic ulcer and gastritis have been associated with multipathogenic factors and could be due to disturbances in natural balances between the aggressive factors (e.g. of acid, bicarbonate, pepsin) and maintenance of the mucosal integrity through the endogenous defense mechanism (e.g. of defensive mechanisms of mucus, mucosal turnover and blood supply (mucosal barrier). Due to the reported side effects of available antiulcer drugs, focused have been shifted towards natural

products as the new sources of antiulcer agents. With the increasingly growing interest in natural medicine, various plants have been studied based on the traditional knowledge of their pharmacological properties and confirmed to be useful in treating and managing ulcer<sup>15,16</sup>. *A. echioides* whole methanolic extract for the first time here. The present study demonstrated the potential of ACMC to significantly reduced gastric ulceration as indicated by the reduction in ulcer index in the ethanol induced and pylorus ligation ulcer induced models.

*A. echioides* treated animals significantly decreased both the concentration of acid volume, total acidity and increased the pH, it is suggested that *A. echioides* can suppress gastric damage induced by aggressive factors. When compared the treatment of *A. echioides* whole extracts at the doses of 200 mg/kg, 400 mg/kg resulted in 200 mg/kg dose showed significant effect in increase of antiulcer activity.

The preliminary phytochemical analysis of *A. echioides* extract showed the presence of alkaloids, flavonoids, triterpenoids, carbohydrates and glycosides. The significant increase in the antiulcer activity of *A. echioides* could be attributed to the presence of flavonoids, alkaloids, tannins, saponin glycosides and phenolic compounds. Flavonoids are among the cytoprotective materials for which antiulcerogenic efficacy has been extensively confirmed. It is suggested that, these active compounds would be able to stimulate mucus, bicarbonate and the prostaglandin secretion and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen<sup>17</sup>. So the antiulcer activity of *A. echioides* may be attributed to its flavonoids content. The results of the present study suggest that the methanol extract of *A. echioides* whole plant may be beneficial in the treatment of gastric lesions. Further studies to identify the active moieties and elucidation of the mechanism of action are recommended.

### Conclusions

From the Data of results obtained it is evaluated that the plant *Andrographis echioides* possesses a significant antiulcer activity compare to the standard drug. The study also helped us to identify the therapeutic values of the common plants present around us.

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