



## ANTIULCER ACTIVITY OF *ABUTILON INDICUM* (L.), SWEET, LEAF EXTRACT USING DIFFERENT EXPERIMENTAL MODELS

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### ABSTRACT

*Abutilon indicum* (L.), sweet, belong to the family Malvaceae extensively used in traditional system of medicine for various ailments such as fever, dysentery, mouth wash and it is also used in the treatment of ulcer. The antiulcer activity was performed using models such as aspirin + pylorus ligation, ethanol induced and acetic acid induced ulcer model. The effect of the extract on volume of gastric content, pH, total and free acidity using the aspirin + pylorus ligation model were also evaluated. From the result, it was observed that the treatment with *Abutilon indicum* leaf extract significantly reduced the ulcer index ( $P < 0.001$ ) in alcoholic and aqueous extracts compared to that of control group in aspirin + pylorusligation, alcoholic and acetic acid induced ulcer model at a dose of (400 mg/kg, *p. o*). Famotidine at a dose of (20 mg/kg) was used as standard drug. The model of gastric acid secretion show a reduction in volume of gastric content, increased in pH, free and total acidity. The present study reveals that *Abutilon indicum* leaf extract displays gastro-protective activity.

**Keyword :** *Abutilon indicum*, Antiulcer, Pylorus ligation

### INTRODUCTION

Ulcer is the result of imbalance between aggressive and defensive factors. Secretion of too much acid and pepsin can damage the stomach lining causing ulcer. To regain the balance, different therapeutic agents including plant extract are used. The leaf of *Abutilon indicum* has been reported to be used in the treatment of various disorders like fever, ulcer and externally for wounds. Traditionally, roots of *Abutilon indicum* are used in urine hemorrhagic discharge, bark for febrifuge and as antihelmetic. Leaves are reported to

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have hepatoprotective and hypoglycemic activity<sup>1-5</sup>.

Since no scientific reports are available on the antiulcer property, the present study was aimed to investigate the antiulcer activity of the petroleum ether, chloroform, alcoholic and aqueous extract of *Abutilon indicum* using various models.

## EXPERIMENTAL

### Plant materials

Leaves of *Abutilon indicum* were collected from in and around Bidar and Shivamogga district of Karnataka, India during the month of may 2008. The plant was authenticated by Shri. S. B. Kamlakar, Professor and HOD of Botany, Sayadry College Shivamogga.

### Preparation of extract

Fresh plant leaves of *Abutilon indicum* were collected, washed with tap water. Then these were shade dried, powdered mechanically and applied for successive extraction using Soxhlet apparatus with solvents petroleum ether (4.48 %), chloroform extract (2.52 %), alcohol extract extract (14.29%) and aqueous extract (7.63 %) yields, respectively.

### Phytochemical screening

Preliminary phytochemical study showed positive test for steroids and triterpenoids in petroleum ether extract, steroids and tannins in chloroform extract, triterpenoids, tannins and flavonoids in alcoholic extract and flavonoids in aqueous extract<sup>6</sup>.

### Animals

The animal experiment was initiated only after approval of Animal Ethical clearance. The animals were procured from Central Animal House, National College of Pharmacy Shivamogga. Albino rats of wistar strain of either sex weighing between 150 to 200 gm were used and are housed in standard cages at room temperature and provided with food and water *ad libitum*. The animals were deprived of food for 24 h before experiment.

### Aspirin + pylorus ligation-induced ulcer

*Abutilon indicum* leaf extract, aspirin and standard antiulcer drug, famotidine were prepared in tween 80 (1 % v/v) as vehicle and administered orally once daily. The animals

were divided into six groups, consisting of six each. Group one received aspirin alone (200 mg/kg, *p. o.*). Groups two received standard drug famotidine only (20 mg/kg, *p. o.*) and group 3, 4, 5 and 6 received *Abutilon indicum* leaf extract orally at a dose of 400 mg/kg, respectively for 7 days. From 5 to 7 days, animals of all the groups received aspirin orally as an aqueous suspension at a dose of 200 mg/kg, 2 h after the administration of respective drug treatment. Animals in all the groups were fasted for 18 h after the respective assigned treatment and were anaesthetized with unaesthetic ether. The abdomen was opened by a small midline incision below the xiphoid process and pylorus portion of stomach was lifted out and ligated. Precaution was taken to avoid traction to the blood supply. The stomach was sutured with interrupted sutures. Four hours after pylorus ligation, the rats were sacrificed and the stomach was removed. The gastric contents were collected, centrifuged at 3000 rpm for 10 min and the supernatant volume and pH were recorded with a digital pH meter. The total acidity was determined by titration with 0.01 N NaOH using digital burette and phenolphthalein as an indicator and free acidity using Topfer reagent. The free and total acidity were expressed as  $\mu$  equiv. /100 g/4 h. The stomach was then incised along the greater curvature and observed for ulcers. The number of ulcers was counted using a magnifying glass and the diameter of the ulcers were measured using vernier calipers. The following arbitrary scoring system was used to grade the incidence and severity of lesions : (a) score 10 = denuded epithelium; (b) score 20 = petechial and flank haemorrhages; (c) score 30 = one or two ulcers; (d) score 40 = multiple ulcers; (e) score 50 = perforated ulcer.

Ulcer index (UI) was then calculated from the above scorings as follows :

$$UI = UN + U_s + U_p \times 10^{-1} \quad \dots(1)$$

where UN is the average of number of ulcers per animal,  $U_s$  is the mean severity of ulcer score and  $U_p$  is the percentage of animals with ulcer incidence.<sup>7-11</sup>

### **Absolute alcohol-induced ulcer**

The animals were divided into six groups, consisting of six each. Group one received alcohol alone (1 mL of absolute alcohol, *p. o.*). Group two received standard drug famotidine 20 mg/kg and Groups 3, 4, 5 and 6 received *Abutilon indicum* leaf extract orally at the dose of 400 mg/kg body weight, respectively. *Abutilon indicum* leaf extract and famotidine were administered orally 30 min before the oral administration of 1 mL of absolute alcohol. Sixty minutes later, the animals were sacrificed and their stomachs excised and gastric contents were aspirated. Stomachs were removed and kept immersed in 10% formalin for 5 min. Each stomach was incised along the greater curvature and

examined for linear haemorrhagic lesions in the glandular region. The length (mm) of each lesion was determined at 10X magnification with pair of dividers and each length was summed per stomach. The sum of length (mm) of all lesions for each stomach was used as the ulcer index (UI). Stomach was again immersed in 10% formalin for 24 h and histopathological examinations were carried out and later the slides were photographed. The percentage inhibition was calculated by the following formula<sup>7, 12-13</sup>.

$$\% \text{ Inhibition} = (\text{UI Control} - \text{UI Treated}) / \text{UI Control} \times 100 \quad \dots(2)$$

### Acetic acid-induced gastric ulcers

Rats were starved for 24 h prior to the experiment and were divided into six groups, one for control and the other five for drug treatment. Under light ether anesthesia, laparotomy was performed through a midline gastric incision. After exposing the stomach, 0.05 mL of 30% acetic acid solution was injected into the subserosal layer in the glandular part of the anterior wall. The stomach was bathed with saline to prevent adhesion to the external surface of the ulcerated region. The vehicle, extract and standard drug was administered orally for 14 days beginning one day after surgery. Body weight was recorded daily through out the experiments to evaluate the possible chronic toxicity induced by *Abutilon indicum* leaf extract. On 14<sup>th</sup> day, the animals were sacrificed at proper intervals to assess the healing processes of the ulcer. The stomach was removed and the gastric lesions were evaluated by examining the inner gastric surface with a dissecting binocular microscope. Subsequently, the ulcer area (mm<sup>2</sup>) and curative rate percentage were determined.<sup>7, 11-15</sup>

### Statistical analysis

Statistical analysis was performed using ANOVA, the significance of difference was accepted at P<0.001 data's are presented as mean  $\pm$  S. E. M.

## RESULTS AND DISCUSSION

Gastric and duodenal ulcers are illness that affects a considerable number of people in the world. Stress, smoking, nutritional deficiencies and ingestion of non-steroidal anti-inflammatory drug grows the gastric ulcer and also caused by Helico bacteria pylori spiral shaped bacteria found in the stomach<sup>12, 13</sup>.

For treatment of ulcer, number of drugs are available in the market. These are H<sub>2</sub> receptor antagonist, famotidine, cimetidine, but these drugs produce adverse effect like nausea, vomiting, tiredness in addition to other effects have promoted search for new drugs

devoid of these adverse effects<sup>3</sup>.

Peptic ulcer results due to over production of gastric acid. aspirin + pylorus ligation induced ulcer (Table 1) occurs because of an increase in acid pepsin accumulation due to distraction.

**Table 1 : Effect of *Abutilon indicum* leaf extract on gastric secretion and ulcer index using aspirin + pylorus ligation ulcer model**

Treatment (Dose in mg/kg. p. o.)	pH of gastric con- tent	Gastric volume (mL /100g)	Free acidity ( $\mu\text{eq}$ / 100g/4h)	Total acidity ( $\mu\text{eq}$ /100g/4)	Ulcer index	% inhibit- tion
Control	2.74 $\pm$ 0.72	2.32 $\pm$ 0.1	53.33 $\pm$ 1.12	62.33 $\pm$ 1.37	30.05 $\pm$ 0.22	--
Famotidine (20)	4.77 $\pm$ 0.02	1.62 $\pm$ 0.04*	28.83 $\pm$ 2.04*	37.83 $\pm$ 2.04*	14.28 $\pm$ 0.19*	52.4
Pet. ether extract (400)	2.58 $\pm$ 0.11	2.30 $\pm$ 0.04	52.5 $\pm$ 3.12	61.5 $\pm$ 2.07	28.68 $\pm$ 0.82	4.75
Chloroform extract (400)	2.5 $\pm$ 0.02	2.28 $\pm$ 0.06	51.5 $\pm$ 1.05	60.8 $\pm$ 1.67	28.27 $\pm$ 0.37	5.93
Alcoholic extract (400)	4.32 $\pm$ 0.01*	2.25 $\pm$ 0.02	31.5 $\pm$ 2.07*	52.83 $\pm$ 2.23*	15.93 $\pm$ 0.26*	46.9
Aqueous extract (400)	4.61 $\pm$ 0.01*	1.96 $\pm$ 0.05*	29.0 $\pm$ 2.37*	42.33 $\pm$ 1.37*	15.32 $\pm$ 0.04*	49.2

Values are mean  $\pm$  S. E. M; n = 6 in each group, \*P < 0.001 compared to control group.

The present study showed that the alcoholic and aqueous leaf extract of *Abutilon indicum*, significantly (p<0.001) reduced gastric volume, free acidity and total acidity and increase in gastric pH (p<0.001) as compared to that of the control (untreated) and also significantly (p<0.001) reduction in ulcer index but no more effect in case of petroleum ether and chloroform extracts. Alcohol induced ulcer model administration of ethanol produced haemorrhagic gastric lesion in the gastric mucosa of the control group. *Abutilon indicum* leaf extract shows significant (p<0.001) reduction (Table 2) in the ulcer index in alcoholic and aqueous extract as compared to the control group, but petroleum ether and chloroform extracts did not show more effect. In acetic acid induced ulcer model treatment

of *Abutilon indicum*, leaf extracts in alcoholic and aqueous media show reduction (significant,  $p < 0.001$ ) in ulcer, which after 14 days of treatment, shows protection of ulcer (Table 3), where as petroleum ether and chloroform extracts did not show significant results.

**Table 2 : Effect of *Abutilon indicum* leaf extract on alcohol induced ulcer model.**

Treatment (Dose in mg/kg. p. o.)	Ulcer index	% inhibition
Control	28.86±0.53	--
Famotidine (20)	10.71±0.4*	62.88
Pet. ether extract (400)	27.78±0.42	3.74
Chloroform extract(400)	26.15±0.56	9.39
Alcoholic extract (400)	13.03±0.35*	54.85
Aqueous extract(400)	12.18±0.41*	57.79

Values are mean ± S. E. M; n = 6 in each group, \*P < 0.001 compared to control group.

**Table 3 : Effect of *Abutilon indicum* leaf extract on acetic acid induced ulcer model.**

Treatment (Dose in mg/kg. p. o.)	Ulcer index	% inhibition
Control	29.28 ± 0.36	--
Famotidine (20)	10.85 ± 0.52*	62.94
Pet. ether extract (400)	27.42 ± 0.44	6.35
Chloroform Extract (400)	26.88 ± 0.42	8.19
Alcoholic extract (400)	13.1 ± 0.46*	55.25
Aqueous Extract (400)	11.7 ± 0.76*	60.04

Values are mean ±S. E. M; n = 6 in each group, \*P < 0.001 compared to control group.

The present study showed alcoholic and aqueous extracts exhibit significant antiulcer activity. This activity may be due to presence of flavonoids, which are secondary

metabolites present in *Abutilon indicum* plant leaves. The study showed that flavonoids have antiulcer activity<sup>13</sup>. On the bases of this, it may be concluded that the leaf extracts posses significant activity due to presence of flavonoids.

Further work is under progress to find out, which active principle is responsible for this activity.

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