Available online at www.ijpsdr.com
International Journal of Pharmaceutical Sciences and Drug Research 2011; 3(2): 97-100

Research Article ISSN 0975-248X

Evaluation of Anti-Ulcer Activity of Methanolic Extract of *Abutilon indicum* Linn Leaves in Experimental Rats

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ABSTRACT
Gastric ulcer is one of the most prevalent gastrointestinal disorders, which affects approximately 5-10% of people during their life. In recent years, abundant work has been carried out on herbal medicine to clarify their potential efficacy in gastric ulcer prevention or management. Here, present study was carried out to investigate antiulcer activity of methanol extract of *Abutilon indicum* L. (Family: Malvaceae) leaves in pylorus ligated and ethanol induced ulceration in the albino rats. Preliminary methanol extract of *A. indicum* was subjected to the acute oral toxicity study according to the OECD guideline no. 425. Based on which, two dose levels i.e. 250 and 500 mg/kg were selected for the further study. In pylorus ligation induced ulcer model, various parameters were studied viz. gastric volume, pH, total acidity, free acidity, and ulcer index. Ulcer index and percentage inhibition of ulceration was determined for ethanol induced ulcer model. Ranitidine at 50 mg/kg was used as the standard drug. Pretreatment of methanol extract of *A. indicum* leaves showed significant ($P<0.05$) decrease in the gastric volume, total acidity and free acidity. However, pH of the gastric juice was significantly ($P<0.05$) increased only at higher dose, 500 mg/kg. It showed also significant ($P<0.05$) decrease in number of ulcers and ulcer score index in pylorus ligation and ethanol induced ulceration models. The methanol extract of *A. indicum* leaves possess significant antiulcer properties in a dose dependent manner. In conclusion the antiulcer properties of the extract may be attributed to the presence of phytochemicals like flavonoids (quercetin), alkaloids and tannins present in the plant extract with various biological activities.

Keywords: Antiulcer activity, *A. indicum*, Pylorus ligation, Ulcer index.

INTRODUCTION

Peptic ulcer disease is one of the most common gastrointestinal disorders, which causes a high rate of morbidity particularly in the population of non-industrialized countries. [1] Peptic ulcer occurs due to an imbalance between the aggressive (acid, pepsin and *Helicobacter pylori*) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors. [2] In Ayurveda, peptic ulcer mostly refers to *Amlapitta* or *Parinamasula. Amlapitta* is a disease of the gastrointestinal tract, especially of the stomach. *Amlapitta* literally means, pitta leading to sour taste. [3] Number of drugs including proton pump inhibitors, prostaglandins analogs, histamine receptor antagonists and cytoprotective agents are available for the treatment of peptic ulcer. But most of these drugs produce several adverse reactions including toxicities and even may alter biochemical mechanisms of the body upon chronic usage. [4] Hence, herbal medicines are generally used in such cases when drugs are to be used for chronic periods. Several natural drugs have been reported to possess anti-ulcerogenic activity by virtue of their predominant effect on mucosal defensive factors. [5-6]

Abutilon indicum L. commonly known as "Atibala" in Sanskrit gives excessive tonic strength. [7] Atibala is a stronger diuretic and heart tonic. [8] *A. indicum* reported in the Siddha system as a remedy for jaundice, piles, ulcer, leprosy, rakttapitta dosha and blood purifier. [9-10] Chemically it contains flavonoids (quercetin), saponins, alkaloids, tannins and phenolic compounds. [11-12] The pharmacological activities previously reported are analgesic, larvicidal, hepatoprotective, and hypoglycemic properties. [13-16]

Recent screening with plants has revealed many compounds like flavonoids, alkaloids, saponins, terpenoids, monoterpenoids (linalool), glycoproteins, polysaccharides, tannins, essential fatty acids, phenolic compounds and vitamins having pronounced antioxidant, antineoplastic, anti-

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ulcer, anti-inflammatory and immunostimulating potential. [17] Scientific literatures are continuously reporting herbal drugs having anti-ulcer potential. There is need to evaluate the potential of ayurvedic remedies as adjuvants to counteract side effects of modern therapy. [17]

The present investigation is aimed at studying the anti-ulcer activity of the methanolic extract of leaves of *Abutilon indicum* L. in order to justify the traditional claims endowed upon this herbal drug as a rasayana.

MATERIALS AND METHODS

Plant Material

The leaves of *Abutilon indicum* L. were collected at in the month of June, 2008 from local area of Sangli, Maharashtra state, India and authenticated by Dr. U. S. Yadav of the Department of Botany, Willingdon College, Sangli, where a voucher specimen has been preserved for future identification.

Extraction

The leaves were separated from the fresh stems and dried on filter paper sheets under shade at room temperature until with changing of color of filter papers. The shade-dried, coarsely powdered leaves (500 g) were successively extracted with petroleum ether (60-80^{o}C) for 8 hr. to remove fatty matter. The defatted marc was then subjected to soxhlet extraction with 95% methanol to obtain methanolic extract. The methanolic extract were evaporated under reduced pressure at low temperature (30^{o}C) to dryness to yield brownish yellow color extracts of *A. indicum*, stored in an airtight container in refrigerator for further experimental studies.

Preliminary Phytochemical Screening

Methanolic extract of *A. indicum* were subjected to preliminary phytochemical screening for the detection of various plants constituents. [18]

Animals

Inbred colony of albino wistar rat of weighing between 200-250 g was housed in groups 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.

Test Compound Formulations

The aqueous suspension of methanolic extract of leaves of *Abutilon indicum* (MEAI) was prepared in 0.5 % carboxymethylcellulose (CMC) solution in distilled water prior to oral administration to animals. It was used within seven days and stored at 8^{o}C while for further use, freshly prepared solution was used. The vehicle alone served as control.

Chemicals

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

Acute Toxicity Studies

Acute toxicity studies were performed according to organization for economic co-operation and development (OECD) guidelines. [19] Animals were divided in groups (n=5). The animals were fasted for 4 h. with free access to water only. The MEAI extracts was administered orally in doses of 2500 and 5000 mg/kg to different groups of mice and observed over 14 days for mortality and physical/behavioral changes.

Assessment of Anti-Ulcer Activity

Pyloric ligation induced gastric ulceration [20]

Albino rats of either sex were divided into four 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 distilled water. Methanol extract of *A. indicum* at 250 and 500 mg/kg, (p. o.) were given to the animals in the treatment group. Ranitidine (50 mg/kg) was used as a standard. After 1h of drugs treatment, they were anaesthetized with the help of anesthetic ether; the abdomen was opened by a small midline incision below the xiphoid process. Pyloric portion of the stomach was slightly lifted out and ligated according to method of Shay et al. [21], 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 pyloric ligation. The abdomen was opened, cardiac end of the stomach was dissected out and the contents 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, total and free acidity. Each stomach was examined for lesions in the fore stomach portion and indexed according to severity.

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 numbers of ulcers were counted.

Scoring of ulcer will be made as follows:

Normal colored stomach..... (0)
Red coloration..... (0.5)
Spot ulcer.....…. (1)
Hemorrhagic streak... (1.5)
Deep Ulcers.....… (2)
Perforation.....… (3)

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows:

Ulcer index (U_I) was measured by using following formula:

$$U_I = U_N + U_S + U_P \times 10^{-1}$$

Where,

U_I= Ulcer Index; U_N = Average number of ulcers per animal; U_S = Average number of severity score; U_P = Percentage of animals with ulcers

Percentage inhibition of ulceration was calculated as below:

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

Table 1: Effect of methanolic extract of *A. indicum* on gastric content, P^H, total and free acidity in pyloric ligation induced ulceration in rats

Treatment	Dose (mg/kg)	Gastric Content (ml)	pH	Acidity(mEq/l)	
				Total	Free
Control (Distilled water)	10	8.5±0.20	3.21±0.21	120±0.25	95.1±1.5
MEAI	250	4.7±0.15*	3.85±0.22	75±0.48*	70±0.15*
	500	4.1±0.10*	4.87±0.65*	72±0.35*	67±0.13*
Ranitidine	197	4.1±0.10*	5.35±0.15*	3±0.17*	37±0.24*

Values are expressed as (Mean ± S.E.M.), n= 6, *p< 0.05 when compared with control group.

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:

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

Determination of free acidity

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.

Ethanol induced ulcer model [22]

The ulcer was induced by administering ethanol. All the animals were fasted for 36 hours before administration of ethanol. The Albino rats of either sex were divided into five groups, each consisting of six rats. One group represented the control group, which received ethanol Second & Third Groups received methanolic extract of *A. Indicum* 250 and 500 mg/kg and, Ranitidine, in the dose of 50 mg/kg were administered orally for Four group as reference standard drug. The gastric ulcers were induced in rats by administrating absolute ethanol (90%) (0.5 ml/100g) orally, after 45 min of methanolic extract and ranitidine treatment. They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1h latter with anaesthetic ether and stomach was incised along the greater curvature and ulceration will be scored. A score for the ulcer was study similar to pyloric ligation induced ulcer model.

Statistical analysis

The results are expressed as the mean ± SD for each group. 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 $P<0.05$.

RESULTS

Acute Oral Toxicity Study

Acute oral toxicity was carried out by up-down regulation method. It is found that MEAI were safe at limit dose 2500 mg/kg and 5000 mg/kg with no mortality in studied subjects. 1/10th of these doses i.e. 250 mg/kg and 500 mg/kg were used in the subsequent study respectively.

Preliminary Phytochemical Screening

The MEAI were found to contain carbohydrates, proteins, amino acids, saponin glycosides, flavonoids, alkaloids, tannins and phenolic compounds. TLC analysis showed the presence of flavonoids. [23]

Pyloric Ligation Induced Gastric Ulceration

Effect of methanol extract of *A. indicum* on pyloric ligation induced ulceration is shown in Table 1. The pyloric ligation has caused the accumulation of gastric secretions of 8.5±0.20 ml with pH 3.21±0.21 in a control group. The total acidity and free acidity of the gastric secretions were found to be 120±0.25 and 95.1±1.5 mEq/l respectively. Pretreatment with the *A. indicum* extract, significantly ($P<0.05$) reduced the volume of gastric secretions 4.7±0.15 and 4.1±0.10ml at the doses of 250 and 500 mg/kg respectively. P^H of the gastric fluid was significantly ($P<0.05$) elevated up to 4.87±0.65 only at higher dose of the extract. In addition, total acidity and free acidity were also reduced significantly ($P<0.05$) in a dose dependant manner. Further it is observed that pyloric ligation has caused gastric ulcerations and pretreatment with *A. indicum* extract has reduced them significantly ($P<0.05$) in a dose dependent manner. In this model, percentage inhibition of ulceration was found to be 48.76 and 75.45 at 250 and 500 mg/kg respectively. The gastroprotection offered by the test extract was comparable to that of the standard drug, ranitidine (50 mg/kg).

Ethanol Induced Gastric Ulceration

Ethanol at dose of 0.5 ml/kg showed superficial, deep ulcers and perforations in the control animals (Table 2). However, animals treated with methanol extract of *A. indicum* at 250 and 500 mg/kg doses showed significant ($P<0.05$) reduction in the number of ulcer and ulcer index (Table 2). It showed 41.17 and 56.48% ulceration inhibition at the dose of 250 and 500 mg/kg respectively whereas ranitidine showed 72.49% ulceration inhibition. Anti-ulcerogenic effect of *A. indicum* in ethanol induced ulcers was comparable to that of ranitidine, 50 mg/kg.

Table 2: Effect of methanolic extract of *A. indicum* on gastric ulcer induced by pylorus ligation in rats

Treatment	Dose (mg/kg)	Ulcer Index	% Ulcer Inhibition
Control (Distilled water)	10	3.6±0.45	-
MEAI	250	2.43±0.20*	48.76
	500	2.81±0.27*	75.45
Ranitidine	50	1.65±0.49*	86.39

Values are expressed as (Mean ± S.E.M.), n= 6, *p< 0.05 when compared with control group. (Statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's t-test.)

Table 3: Effect of methanolic extract of *A. indicum* on ethanol induced ulcers

Treatment	Dose (mg/kg)	Ulcer Index	% Ulcer Inhibition
90% absolute ethanol	0.5 ml/kg	4.55±0.52	-
MEAI	250	2.56±0.25*	41.17
	500	2.12±0.35*	56.48
Ranitidine	50	1.50±0.20*	72.49

Values are expressed as (Mean ± S.E.M.), n= 6, *p< 0.05 when compared with control group. (Statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's t-test.)

DISCUSSION

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanisms. To regain the balance, different therapeutic agents including plant extracts may be used. [24] *A. indicum* extract is one such herbal drug used in the present study primarily to evaluate the anti-ulcerogenic in pylorus ligation and ethanol induced ulcers in rats.

The causes of gastric ulcer pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid. [24]

Pylorus ligation induced ulcers are due to auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier. These factors are associated with the development of upper gastrointestinal damage including lesions, ulcers and

life threatening perforation and hemorrhage. Aspirin, phenylbutazone, indomethacin and some non-steroidal anti-inflammatory drugs are also known to cause duodenal and gastric ulceration. Prostaglandin E2 and I2 are predominantly synthesized by the gastric mucosa and are known to inhibit the secretion of gastric acid and stimulate the secretion of mucus and bicarbonate. Hydrophobic surfactant-like phospholipids secretion in the gastric epithelial cells is also stimulated by the prostaglandin. It is also showed development of gastric ulcers in pyloric ligation model. Volume of gastric secretion is an important factor in the production of ulcer due to exposure of unprotected lumen of the stomach to the accumulating acid. [25]

Ethanol is also has been reported to cause disturbances in gastric secretion, damage to the mucosa, alterations in the permeability, gastric mucus depletion and free radical production. This is attributed to the release of superoxide anion and hydroperoxy free radicals during metabolism of ethanol as oxygen derived free radicals has been found to be involved in the mechanism of acute and chronic ulceration in the gastric mucosa. [26]

Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and necrotic aspects of tissue injury. Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intra cellular membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium. [24] It was observed in this study that the extract reduced significantly ethanol- induced ulcer. This may be due to cytoprotective effect of the extract via antioxidant effects. The extract shows protection against characteristic lesions produced by ethanol administration this antiulcer effect of MEAI may be due to both reductions in gastric acid secretion and gastric cytoprotection.

The antiulcer property of *A. indicum* in pylorus ligation model is evident from its significant reduction in free acidity, total acidity, number of ulcers and ulcer index. *A. indicum* treated animals significantly inhibited the formation of ulcers in the pylorus ligated rats and also decreased both the concentration and increased the pH, it is suggested that *A. indicum* can suppress gastric damage induced by aggressive factors.

The preliminary phytochemical analysis of *A. indicum* extract showed the presence of alkaloids, flavonoids, triterpenoids, carbohydrates and glycosides. The significant increase in the antiulcer activity of *A. indicum* could be attributed to the presence of flavonoids (quercetin), 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. [25] So the antiulcer activity of *A. indicum* may be attributed to its flavonoids content. The results of the present study suggest that the methanol extract of *A. indicum* leaves 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.

ACKNOWLEDGEMENT

The authors would like to thank Dr. C. S. Magdum, Principal and Head, Department of Pharmaceutical chemistry, Rajarambapu College of Pharmacy, Kasegaon, Sangli, Maharashtra for providing general support and encouraging our work.

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