



Anti ulcer activity of hydroalcoholic extract of *abutilon indicum* leaves on indomethacin-induced gastric ulcer in albino rats

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ABSTRACT

The leaves extract of *abutilon indicum* leaves herbal preparation that has been suggested as useful in the treatment of various diseases anti microbial, anti wound killing agents and anti oxidant etc. In this study to determine the gastro protective effect of *abutilon indicum* leaves in a model of Indomethacin induced ulcer rat. The extract was given by oral gavages (100 and 200mg/kg) three times at 12 h intervals after administering indomethacin 30mg/kg. Treatment with the extract resulted in a significant decreased of the ulcerated area. The results were comparable with the positive control (ranitidine 50mg/kg). Further the results were confirmed using Histopathological studies of the stomach. Thus, we concluded that hydro-alcoholic extract of *abutilon indicum* leaves possess good preventive and therapeutic action on the gastric ulcers.

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Introduction

Peptic ulcer is a serious gastrointestinal disorder that requires a well targeted therapeutic strategy. A number of drugs including proton pump inhibitors and H₂ receptor antagonists are available for the treatment of peptic ulcer, but clinical evaluation of these drugs has shown incidence of relapses, side effects, and drug interactions. This has been the rationale for the development of new antiulcer drugs and the search for novel molecules has been extended to herbal drugs that offer better protection and decreased relapse. Drugs of plant origin are gaining popularity and are being investigated for a number of disorders, including peptic ulcer¹. Peptic ulcer disease encompassing gastric and duodenal ulcer is the most prevalent gastrointestinal disorder. The pathophysiology of PUD involves an imbalance between offensive (acid, pepsin, and *H. pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors). An estimated 15,000 deaths occur each year as a consequence of PUD. In India, PUD is common. In the Indian Pharmaceutical industry, antacids and antiulcer drugs share 6.2 billion rupees and occupy 4.3% of the market share. Today, there are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric acid and the second with re-enforcing gastric mucosal protection². Recently, there has been a rapid progress in the understanding of the pathogenesis of peptic ulcer. Most of the studies focus on newer and better drug therapy. These have been made possible largely by the availability of the proton pump inhibitors, histamine receptor blockers, drugs affecting the mucosal barrier and prostaglandin analog³. However, the clinical evaluation of these drugs showed development of tolerance and incidence of relapses and side effects that make their efficacy arguable. This has been the rationale for the development of new antiulcer drugs, which includes herbal drugs⁴. Indian Medicinal plants and their derivatives have been an invaluable source of therapeutic agents to treat various disorders including PUD⁵.

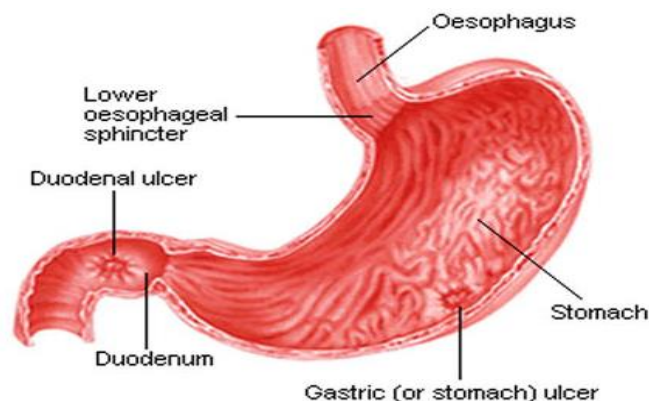


Figure No.1: Ulcer Stomach

Chemical substances derived from plants have been used to treat human diseases since the dawn of medicine. Roughly 50% of new chemical entities introduced during the past two decades are from natural products. Recent technological advances have renewed interest in natural products in drug discovery. Therefore, efforts should be directed towards isolation and characterisation of the active principles and elucidation of the relationship between structure and activity. Furthermore, detailed analysis of the active constituents of natural drugs should be directed towards clinical relevance. Standardisation is indispensable to maintain reproducible quality in biological evaluation. Although the clinical efficacy of these preparations is reported by traditional practices, they have not been scientifically validated.

Abutilon indicum is a small shrub in the Malvaceae family⁶, native to tropic and subtropical regions and sometimes cultivated as an ornamental⁷. This plant is often used as a medicinal plant and is considered invasive on certain tropical lands. In traditional medicine, *A. indicum* various parts of the plant are used as a demulcent, aphrodisiac, laxative, diuretic, sedative, astringent, expectorant, tonic, anti-inflammatory, anthelmintic, and analgesic and to treat leprosy, ulcers, headaches, gonorrhoea, and bladder infection⁸. The whole plant

is uprooted, dried and is powdered. In ancient days, maidens were made to consume a spoonful of this powder with a spoonful of honey, once in a day, for 6 months until the day of marriage, for safe and quick pregnancy⁹. The plant is very much used in Siddha medicines. The root, bark, flowers, leaves and seeds are all used for medicinal purposes by Tamils¹⁰. The leaves are used as adjunct to medicines used for pile complaints. The flowers are used to increase semen in men.

Materials and Methods

Collection of *Abutilon indicum*

The Leaves of *Abutilon indicum* L. (Family: Malvaceae). The leaves were separated from the fresh stems and dried on filter paper sheets under shade at room temperature until with changing of colour of filter papers. The shade-dried, coarsely powdered leaves (500 g) were successively extracted with petroleum ether (60-80°C) for 8 hr. to remove fatty matter. The defatted marc was then subjected to soxhlet extraction with 75% ethanol and 25% water to obtain hydroalcoholic extract. The hydroalcoholic extract were evaporated under reduced pressure at low temperature (30°C) to dryness to yield brownish yellow colour extracts of *A. indicum*, stored in an airtight container in refrigerator for further experimental studies.

Phytochemical Evaluation¹¹

Identification of Constituents by Phytochemical Test

The extracts were subjected to qualitative tests for identification of phytoconstituents present in it viz. alkaloids, carbohydrates, glycosides, phytosterols, fixed oils & fats, phenolic compounds & tannins, proteins and free amino acids, gums & mucilage's, flavonoids, lignins and saponins.

Test of Alkaloids

A small portion of the solvent free petroleum ether, hexane, alcohol and aqueous extracts were stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate may be tested carefully with various alkaloidal reagents such as,

- Mayer's reagent - Cream precipitate
- Dragendroff's reagent - Orange brown precipitate
- Hager's reagent - Yellow precipitate
- Wagner's reagent - Reddish brown precipitate

Test for Carbohydrates & Glycosides

The minimum amount of extracts were dissolved in 5ml of distilled water and filtered. The filtrate was subjected to test for carbohydrates and glycosides.

a. Molisch's Test

The filtrate was treated with 2-3 drops of 1% alcoholic alpha naphthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube.

b. Fehling's Test

The filtrate was treated with 1ml of Fehling's solution and heated. Orange precipitate was obtained shows the presence of carbohydrates.

Another portion of the extracts was hydrolysed with hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to Legals, Borntrager's test to detect the presence of different glycosides.

c. Legal's Test

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Purple colour in ammoniacal layer was observed.

Test for Phytosterol (Liebermann Burchard Test)¹²

One gram of the extract was dissolved in few drops of dry acetic acid, 3 ml of acetic anhydride was added followed by few drops of conc sulphuric acid. Appearance of bluish green colour showed the presence of phytosterol.

Test for Fixed oils and Fats

A small quantity of the extracts was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil. Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hrs. Formation of soap or partial neutralization of alkali indicates the presence of fixed oil and fats.

Test for Tannins and Phenolic Compounds¹³

Small quantities of extracts were dissolved separately in water and tested for the presence of phenolic compounds and tannins with

- Dilute Ferric chloride solution 5% - Violet colour
- 1% solution of gelatin containing 10% NaCl - White precipitate
- 10% Lead acetate solution - White precipitate

Test for Proteins and Free Amino Acids

Small quantities of extracts were dissolved separately in a few ml of water and treated with:

- Million's reagent** - Appearance of red colour shows the presence of proteins and free amino acids.
- Ninhydrin reagent** - Appearance of purple colour shows the presence of proteins and free amino acids.
- Biuret test** - Equal volume of 5% solution of sodium hydroxide and 1% solution of copper sulphate were added. Appearance of pink colour shows the presence of proteins and free amino acids.

Test for Gums and Mucilages

About 10ml of extract were added separately to 25ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates.

Test for Flavonoids

- With aqueous sodium hydroxide solution, blue to violet colour (Antho cyanins), yellow colour (Flavones), yellow to orange (Flavonones).
- With concentrated sulphuric acid, yellowish orange colour (anthocyanins), yellow to orange colour (Flavones), orange to crimson (Flavonones).
- Shinoda's Test

The various extracts were dissolved separately in alcohol, to this a piece of magnesium followed by conc. hydrochloric acid drop wise were added & heated. Appearance of magenta colour shows the presence of flavonoids.

Test for lignin

With alcoholic solution, phloroglucinol and conc. hydrochloric acid, appearance of red colour shows the presence of lignin. The results of chemical tests of whole plant powder and extracts.

Acute toxicity study as per OECD guideline 425¹⁴

In the assessment and evaluation of the toxic characters of the substance, determination of acute oral toxicity is usually an initial step. It provides information of health hazards likely to arise from a short-term exposure by the oral route. Acute oral toxicity is the adverse effects occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24h. Data from an acute study may serve as a basis for classification and labelling. LD (medium lethal 50 doses), oral, is a statistically derived single dose of a substance that can be expected to cause death in 50% of animals when administered by the oral route. The LD₅₀ value expressed in terms of test substance per unit weight of test animal (mg/kg). It is initial step in establishing a dosage regimen in sub chronic

and other studies and may provide initial information on the mode of toxic action of a substance.

The concept of the up and down (UDP, stair case method) was first designed by Dixon and Mood. In this method animals of a single sex, usually females, with the first animal receiving a dose just below the best estimate of the LD₅₀. Depending on the outcome for the previous animal, the dose for the next is increased or decreased, usually by the factor of 3.2. This sequence continues until there is a reversal of the initial outcome (the point where an increasing dose results in death rather than survival or decreasing dose result in survival rather than death) then, additional animals are dosed following the up-down principle until a stopping criterion is met. If there is no reversal before reaching the selected upper (2000 or 5000 mg/kg) limit dose, then a specific number of animals are dosed at the limit dose. The option to use an upper limit dose of 5000 mg/kg should be taken only when justified by a specific regulatory need.

Healthy Wistar rats weighing between 180-220 g were used to carry out acute oral toxicity studies by the 'staircase' method. The hydroalcoholic extracts of *Abutilon indicum* leaves in was administered orally by gavages in graduated dose to several groups of experimental animals, one dose being used per group. Subsequently, observations of effects were made at 0,1,2,4 and 24 h for any mortality.

Pharmacological Evaluation

Experimental Design

Indomethacin induced gastric ulcer in rats

Albino rats (80-100g) were divided in to 5 groups, each group consisting 6 rats. All groups were treated for 6 days.

Group A was treated with normal food diet daily.

Group B was treated with Gum acacia (5mg/kg) daily + Indomethacin (30 mg/kg) on 6th day. **Group C** was treated with Ranitidine (50mg/kg) daily + Indomethacin (30 mg/kg) on 6th day

Group D was treated with hydro-alcoholic extract (100mg/kg) daily + Indomethacin (30 mg/kg) on 6th day.

Group E was treated with Hydro-alcoholic extract (200mg/kg) daily + Indomethacin (30 mg/kg) on 6th day. The Indomethacin was given in groups 2, 3, 4 and 5 on 6th day to over night fastened rats. Tested drugs treated before 45 min. of ulcerogen. After 4 hrs of ulcerogen drug the animals were sacrificed and stomach and gastric fluid was collected for determining ulcer index parameters.

Statistical analysis

Statistical analysis of the results was done using the statistical functions of the Graph pad Prism 5.0 software. The results were expressed in terms of mean \pm SD. The significance of difference between mean values for the various treatments were tested using one way analysis of variance test (ANOVA test) followed by Dunnett Multiple Comparisons Test and the p values less than 0.05 were considered significant.

Result and Discussion

The preliminary phytochemical screening like Saponins, Tannins, Amino acids, Proteins, Glycosides, Cardiac glycosides, Alkaloids, Carbohydrates and Flavonoids was done with the hydro-alcoholic extract of *Abutilon indicum* leaves according to the procedure. In the above chemical test the hydro-alcoholic extract of *Abutilon indicum* leaves gives positive results for Saponins, Tannins, Amino acids, Proteins, Cardiac glycosides, Alkaloids, Carbohydrates and Flavonoids except glycosides. The results of preliminary test of hydro-alcoholic extract extract of *Abutilon indicum* leaves were shown in Table No.1. The Hydro-alcoholic extract *Abutilon indicum* at the dose of 100 and

200 mg/kg and ranitidine at 50mg/kg produced a significant reduction in the ulcer index and increases in % protection. The ulcer index of aspirin treated group was found to be 20.5 \pm 4.8, whereas it was reduced to 8.8 \pm 2.8 ($P < 0.001$), 11.4 \pm 0.2 ($P < 0.01$) and 09.8 \pm 2.2 ($P < 0.001$) in ranitidine, hydro-alcoholic extract 100 and 200 mg/kg treated groups, with protection index of 52.5, 30.89 and 42.62 % respectively as shown in Table No.2 and Figure No.2.

The results clearly elucidate the anti-ulcerative property of *Abutilon indicum* leaves since a significant inhibition in the formation of gastric lesions¹⁵ with histological evident is presented in aspirin induced gastric ulcer in rats. Even though there is no significant difference observed between the ranitidine and *Abutilon indicum* leaves treated animals for the anti-ulcerative index, the *Abutilon indicum* leaves found to have better protective effect by both macroscopic and microscopic examinations¹⁶.

In the C-reactive protein estimation, a prominent agglutination was appeared in the serum of aspirin treated animals where no such agglutination appearance was observed in the other groups of experimental animals. The C-reactive protein concentration is a marker for systemic inflammation.

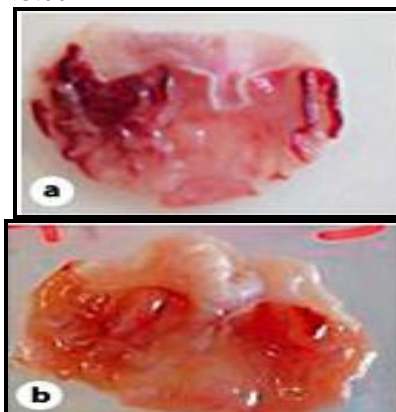
Table No.1: Phytochemical screening results of *Abutilon indicum* leaves

S.No	Phytoconstituent	Result
1	Saponins	Present
2	Tannins	Present
3	Amino acids	Present
4	Proteins	Present
5	Glycosides	Absent
6	Cardiac glycosides	Present
7	Alkaloids	Present
8	Carbohydrates	Present
9	Flavonoids	Present

Table No.2: Anti ulcer activity of hydroalcoholic extract of *Abutilon indicum* leaves

S.No	Treatment	Dose in mg/kg	Ulcer index (mm ² /rat)	% protection
1	Control	-	-	-
1	Indomethacin	30	20.5 \pm 4.8	-
2	Ranitidine	50	8.8 \pm 2.8**	52.5
3	<i>Abutilon indicum</i> extract	100	11.4 \pm 0.2*	30.89
4	<i>Abutilon indicum</i> extract	200	09.8 \pm 2.2**	42.62

Values are mean \pm SEM, n=6. Statistically, significance *p<0.01, **P<0.001



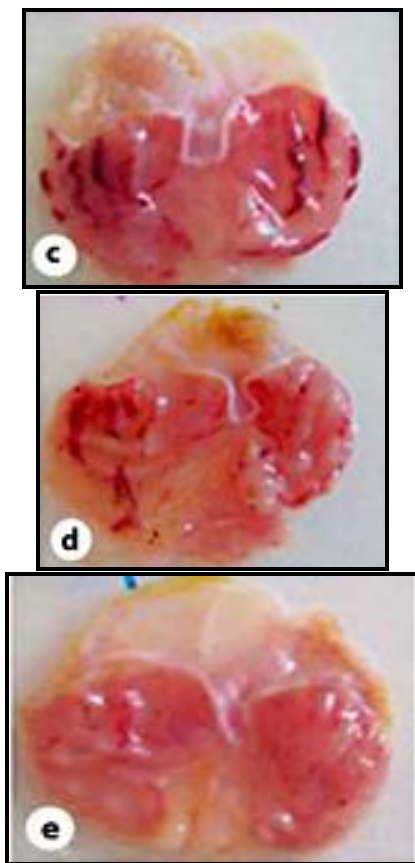


Figure No.1: Macroscopic Assessment of the Healing of Acute Gastric Mucosal Injury Induced by Indomethacin in Rats

This revealed that compared to the vehicle treated rats, oral administration of indomethacin (30 mg/kg) produced acute lesions in the gastric mucosa within 4 h with number of blood clots in the ulcer spots and perforations

Fig. a and b. The six day ulcerated (untreated experimental control) stomach showed lesser spots but the tissues were hyaline in nature

Fig. c. In comparison, stomachs of the rats were healthy almost without any ulcer spots

Fig. d, e. The stomachs of the rats treated with extract were equivalent to those of the normal control rats. In comparison, stomachs of the rats treated with misoprostol showed less ulcer spots but the tissues remained hyaline in nature.

Conclusion

The Chemical substances derived from plant have got a very long history in treatment of human diseases. Nearly 50% of new chemical entities introduced during the past two decades were from natural products. Further research is required to isolate the active phyto constituents present in the extract and experimentation on the healing action of drug on chronic ulcer as well as on the possible side effects. The investigation on mode of action may pave way for establishment of new anti-ulcer therapy regimen.

Conflict of Interest

The author does not have a direct financial conflict of interest with any of the commercial identities mentioned in this paper

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