



Antiulcer activity of Methanolic extract of *Acalypha indica* Linn. (Euphorbiaceae) by Pylorus Ligtire and Swim Stress Induced Ulceration

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ABSTRACT

Medicinal plants are nature's gift to mankind. The plant kingdom holds many species containing substances of medicinal properties; the plant was used in folk medicine against pneumonia asthma and anthelminthic, diuretic, carminative, expectorant and emetic properties. The phytochemical investigation showed the presence of amino acids, proteins, alkaloids, steroids, flavonoids, gums and mucilage. The present study intended to evaluate the anti-ulcer activity was done in pylorus ligation and swim stressed induced ulcer in Wistar rats by using methanolic extract of *Acalypha indica* (MEAI). The MEAI showed significant reduction of gastric volume secretion, acidity and ulceration in pylorus legated and swim stressed rats at $p < 0.001$. From the evaluation the *Acalypha indica* Linn. it could be added to the normal flora of the plant kingdom.

Key words: *Acalypha indica*, Swim stress, Pylorus ligation.

INTRODUCTION

The Plant kingdom remains as a virtual gold mine of drugs yet to be discovered there are several hundreds of medicinal plants that have a history of curative properties against various diseases and ailments. The plant *Acalypha indica* (L.), *Euphorbiaceae* was selected and systematic study was carried out. The Plant has formed the basis for traditional medicinal systems for the ten decades, with the first records dating from about 2600 BC in Mesopotamia. Peoples used oils from cedar, cypress, licorice, myrrh, and poppy juice, among other things, substances that are still in use today for the treatment of a variety of illnesses and infections. Ancient Egyptian, Chinese, and Indian documents showed that medicine in these societies included numerous plant-based remedies (Newman D.J., et al., 2000; Dev S., 1999; Fallarino M., 1994).

The World Health Organization (WHO) defines traditional medicine as the "diverse health practices, approaches, knowledge and beliefs incorporating plant, animal and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly combination to maintain well-being, as well as to treat, diagnose, or prevent illness". It's clear, however, that there is a need to validate the information through an organized infrastructure for it to be used as an effective therapeutic means, either in conjunction with existing therapies, or as a tool in novel drug discovery. Traditional medicine utilizes biological resources and the indigenous knowledge of traditional plant groups, the latter being conveyed verbally from generation to generation. This is closely linked to the conservation of biodiversity and the related intellectual property rights of indigenous people (Timmermans K.R., et al., 2003).

In India medicinal plants have great importance in providing health care to about 80% population research workers therefore take great interest in the pharmacognostic study of herbal drugs and the traditional remedies and recently; there has been an wide upsurge in the scientific investigation in the area. There is a big trade in the herbal drug however due to non-availability or scarce supply of genuine importance crude drugs. Their substitution or adulterants with a similar appearance have entered the market. But these materials do not possess desired medicinal properties. Also at time different herbal drugs have same commercial name and the same drug has different names in different localities and regions in the country. In order to defect spurious crude drug and to avoid confusion in the vernacular names of medicinal plants as the area of

pharmacognosy needs a thrust supported by adequate resources to investigate significantly.

Modern science provides precise techniques for the extraction, isolation, separation and purification of the active phytochemical constituents as well as result of pharmacological testing procedures must of the ancient recipes are gradually regaining popularity and new plants desired drugs usually find their way to novel medicines as purified substance. Out of the total of about 2,25,000 species of plants known about 33,000 are claimed to have medicinal properties (Asolkar et al., 1981). The present study was selected the herb *Acalypha indica*; the plant kingdom holds many species of plants containing substances of medicinal value. Large number of plants was constantly being screened for their pharmacological value such as antiulcer, anthelminthic, expectorant, diuretic, bronchitis, pneumonia and rheumatism.

In present scenario, natural medicine has been gained prominence because they are economically easy to available and relatively free from side effects. The important of systematic phytochemical and pharmacological screening for medicinal plants cannot be therefore under estimated. Systematic investigation has been carried out on a larger number of medicinal plants.

As a result development of modern isolation and chromatographic separation technique; like column, paper, thinlayer, HPLC and Gas chromatography. As well as the sensitive methods of instrumental analysis such as UV, IR, NMR, ESR, ORD, CD and Mass spectroscopy. The increased global demand for polyherbal formulation was a reflective positive impact of consolidated efforts aimed at reviving science of pharmacy.

The plant *Acalypha indica* (L.) was selected for the present investigation. Ayurvedic knowledge supported by modern science was necessary to isolate characterize and standardize the active constituents from herbal sources for anti ulcer activity. Since, all parts of investigated plant were being used for various human ailments and very less scientific work has been performed. The present investigation was carried out to exposure the chemical and therapeutically potential by evaluating phytochemical and antiulcer of the whole plant of *Acalypha indica* (L.) is presented in the article.

PLANT PROFILE

Acalypha indica (L.)

Botanical name : *Acalypha indica* (L.)
Family : Euphorbiaceae

Vernacular names

Tamil : kuppameni
Malayalam : kuppaimeni
Telugu : kuppacheetu
Hindi : kohkla

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MATERIALS AND METHODS

Description

A small erect herb upto 60 cm tall or a little more, with a few ascending branches, these angled and pubescent; leaves broadly ovate, subteloid, rather coarsely toothed, on petioles as long as or longer than the 3-5cm long blades; nerves 3-5 from base, thereafter pinnately arranged; stipules minute; flowers sessile on erect axillary spikes longer than the leaf; male flowers, crowded distally; stamens 8, female flowers scattered along the inflorescence axis, each subtended by a conspicuous semicupular foliaceous toothed green bract nearly 7mm long; capsule hispid, 1mm broad, 3-locular (Stone.,1970).

Distribution

It is distributed widely in Tamilnadu, west Bengal, Andhra Pradesh and Kerala.

Phytochemical and pharmacological aspects

The plant *Acalypha indica* (L.) is used in folk medicine against pneumonia, asthma and as an anthelmintic (Blaschek et al., 1976) and it has also been described in homeopathic pharmacopoeias as active towards hemoptysis (The homeopathic pharmacopoeia of united states, 1941). The herb is also said to possess diuretic, carminative, expectorant, emetic properties, laxative, cure tooth problems and ear ache. A paste of the leaves applied to the burns with lime juice it is early cases of ring worm. Fresh juice of leaves is applied with oil, salt or lime used in rheumatoid arthritis, to cure scabies and other skin infections. The powdered leaves are used for bed sores maggot infested wounds (The wealth of india, 1985).

EXPERIMENTAL PROCEDURE

Plant material

The whole plant of *Acalypha indica* (L.) was collected from Tirunelveli district of Tamilnadu, India, during October 2005. The sample was identified and the voucher specimen number was deposited. The powdered whole portion of plants

of known quantity was taken in a Soxhlet apparatus and extracted with methanol; the material was extracted continuously for 72 hours. The excess solvent present in the crude extracts were removed by distillation and concentrated under vacuum and then dried. The yield of prepared extract was around 21.00% w/w (Trease & Evans 1989).

Phytochemical screening

The preliminary phytochemical group test of the methanol extracts of whole plants of *Acalypha indica* (L.) (MEAI) was performed by the standard methods (Tyler et al., 1993; Pollock and Stevens, 1965; Trease and Evans, 1996 and Plummer, 1985)

Preliminary phytochemical tests for crude MEAI

Test for Carbohydrates and Glycosides

A small quantity of various extracts were dissolved separately in 4ml of distilled water and filtered. The filtrate was subjected to the following tests to detect the presence of 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. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

(b) Fehling's test

The filtrate was treated with each 1ml of Fehling's solution A and B and heated on a water bath. A reddish precipitate was obtained shows the presence of carbohydrates. Another portion of extracts were hydrolyzed with dilute hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to the following tests to detect the presence of glycosides.

(c) Legal's test

To the hydrolysate 1ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red color shows the presence of glycosides.

(d) Borntrager's test

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal volume of dilute ammonia solution was added. Ammonia layer acquires pink colour shows the presence of glycosides.

Detection of Fixed Oils and Fats

(a) Filter paper test

Small quantities of various extracts were pressed separately between the filter papers. Appearance of oil stain on the paper indicated the presence of fixed oils.

(b) Saponification test

Few drops of 0.5M alcoholic potassium hydroxide was added to small quantities of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap indicates the presence of fixed oils and fats. 3.3

2.3.3. Detection of Proteins and Free Aminoacids

Small quantities of various extracts were dissolved in few ml of water and then they were subjected to the following tests.

(a) Million's test

The above-prepared extracts were treated with Million's reagent. Red colour formation shows the presence of proteins and free amino acids.

(b) Biuret test

To the above prepared extracts equal volume of 5% sodium hydroxide and 1% copper sulphate solution were added. Violet color produced shows the presence of proteins and free amino acids.

(c) Ninhydrine test

The extracts were treated with Ninhydrine reagent. Purple color produced shows the presence of proteins and free amino acids.

Detection of Saponins

The extracts were diluted with 20ml of distilled water and it was agitated in a measuring cylinder for 15 minutes. There is no formation of foams indicates the absence of saponins.

Detection of Tannins and Phenolic Compounds

Small quantities of the various extracts were taken separately in water and test for the presence of phenol compounds and tannins was carried out with the following reagents.

- 1) 5% Ferric chloride solution - No violet color
 - 2) 1% solution of gelatin containing 10% sodium chloride - No precipitate
 - 3) 10% lead acetate solution - No precipitate
- Above findings shows the absence of phenolic compounds and tannins.

Detection of Phytosterols

Small quantities of various extracts were dissolved in 5ml of chloroform separately. Then chloroform solution was subjected to the following tests to detect the presence of phytosterols.

a) Salkowski test

To 1ml of above prepared chloroform solution, few drops of concentrated sulphuric acid was added; brown color produced and showed the presence of phytosterols.

b) Libermann Burchard test

The above prepared chloroform solution was treated with a few drops of concentrated sulphuric acid followed by few drops of diluted acetic acid, 3ml of acetic anhydride. A bluish green color appeared indicates the presence of phytosterols.

Detection of Alkaloids

Small quantities of various extracts were separately treated with few drops of dilute hydrochloric acid and filtered. The filtrates were used for the following tests.

- 1) Mayer's reagent - cream precipitate
- 2) Dragendroff's reagent - orange brown precipitate
- 3) Hager's reagent - yellow precipitate
- 4) Wagner's reagent - reddish brown precipitate

Detection of Gums and Mucilages

A small quantity of various extracts 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. No swelling was observed indicates the absence of gums and mucilage.

Detection of Flavonoids

Small quantities of various extracts were dissolved separately in aqueous sodium hydroxide. There is no appearance of yellow color indicates the absence of flavonoids.

Pharmacological screening on anti-ulcer activity

Group separation

Albino rats either sex weighing about (100-130g) was taken in a polypropylene cages and provided food and water. Famotidine 20 mg/kg as a reference drug and vehicle (5%W/v of acacia) 5ml/kg. Doses of 100 and 200 mg/kg of *Acalypha indica* (L.) extract were prepared as aqueous suspensions.

Pylorus ligation Methods

Albino rats either sex weighing about 100-130g (pregnancy was excluded) were taken in a individual animal cages and fasted (water allowed) for 48 hours prior to pyloric ligation, care being taken to avoid coprophagy. Under light ether anesthesia the abdomen was opened by a small midline incision below the xiphoid process; pyloric portion of the stomach is slightly lifted out and ligated avoiding traction to the pylorus or damage to its blood supply. The stomach is replaced carefully, and the abdominal wall was closed interrupted sutures. The drugs are administered orally two hours prior to pyloric ligation. They are deprived of both food and water during the postoperative period, and are sacrificed at the end of 6th hours after operation. Stomach is dissected out and the contents are drained into the tube and this is subjected to analysis for pH and for free and total acidity. The stomach is then open along the greater curvature and is examined for any ulceration. The degree of ulceration is graded from zero to five depending on the size and severity of ulcers (Shay J.P., et al., 1945).

Test drug	Name of the drug	Concentration of drug
Group 1	Vehicle (Control)	5 ml/kg (5%w/v Acacia)
Group 2	Famotidine	20 mg/kg
Group 3	Extract of <i>Acalypha indica</i>	100 mg/kg
Group 4	Extract of <i>Acalypha indica</i>	200 mg/kg

Total acidity

A volume of 2 ml of diluted gastric juice was titrated with 0.01N sodium hydroxide run from a micro pipette using phenolphthalein as an indicator. The acidity was expressed as mg. HCl/100g. body weight of rat.

Free acidity

It is determined in similar manner using toppers reagent as indicator and sodium hydroxide was run until canary yellow color was observed.

Ulcer index

The method of (Anderson and Soman., 1965) was used for scoring the ulcer index. Gastric mucosal damage, induced using stress model was not affected by different doses of the MEAI; Famotidine was used as the reference drug, significantly reduced the ulcerative index in all two models of gastric mucosal lesion.

Swim stress induced ulceration

In this method the ulcer evaluation was carried out in a similar way of pylorus ligation model. Instead of pylorus ligation the rats were forced to swim for 5 hours in a stainless steel water tank (60x90cm) with a 60 cm water level at constant temperature of 33±1°C. Dose of the extract 100 and 200 mg/kg and Famotidine were given through orally 2 hours before forcing the rats to swim.

RESULTS

1. Phytochemical screening of MEAI

S.No.	Phytoconstituents	MEAI
1.	Alkaloids	+
2.	Proteins Amino Acids	+
3.	Flavonoids	+
4.	Phytosterol	-
5.	Reducing sugars	+
6.	Gums and mucilage	+
7.	Tannins	-
8.	Saponins	+

Pharmacological screening on Pylorus ligation method



Figure.1. Control group. Showed that the heavy ulcerative condition of the stomach sections was observed.



Figure.2. Famotidine 20 mg/kg. There is no any sign of inflammation and mild redness of the ulcer index was observed.



Figure.3. MEAI 100 mg/kg. The stomach sections was showed that normal stomach architecture and without any ulceration with mild redness.



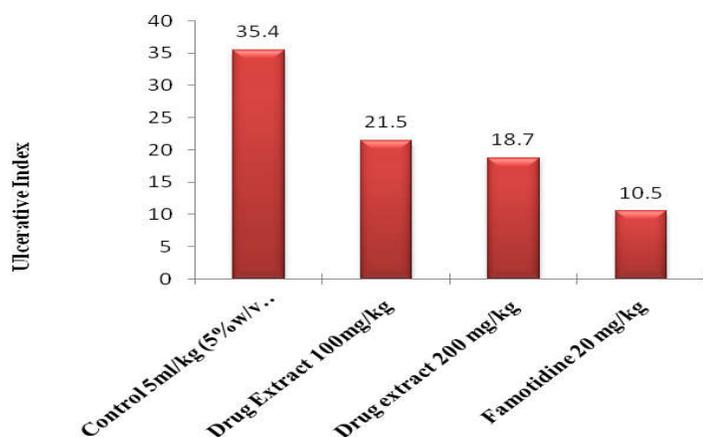
Figure.4. MEAI 200 mg/kg. The stomach was observed that no any sign of redness and inflammation.

Table.2.Parameters assessment treatment upon *Acalypha indica* on pylorus ligated Albino rats

S.No.	Groups	Volume of gastric juice	pH	Total acidity	Free acidity	Ulcer index (In mm)
1.	Control	2.8±0.04	1.3±0.07	98± 7.3	77±6.3	35.4±3.2
2.	Famotidine	0.50±0.02**	4.10±0.18	29.3±1.0	16.2±0.9	10.5±0.8**
3.	MEAI 100 mg/kg	0.78±0.03*	3.8±0.19**	47.2±2.9*	33.2±2.1	21.5±1.2*
4.	MEAI 200 mg/kg	0.64±0.04**	4.0±0.11**	30.1±2.4**	28.3±0.8**	18.7±0.8**

*p<0.01 Vs control, **p<0.001 Vs control by students 't' test. Ulcer index obtained with control, drug extract of plant in a dose 100 and 200 mg/kg, (Control 5ml/kg (5%w/v acacia) and Famotidine 20mg/kg induced by pylorus ligation model.

Figure.5.Parameters assessment treatment upon *Acalypha indica* on pylorus ligated Albino rats



Parameters assessment treatment upon *Acalypha indica* on Swim Stress Induced Ulceration



Figure.6. Control group. The stomach section of the control group showed that the normal stomach veins and without any ulcer index.



Figure.7. Famotidine 20 mg/kg. The stomach sections also described that the standard has not been shown any sign of inflammation and ulcer index.



Figure.8. MEAI 100 mg/kg. The dissected the stomach section was observed that the clear stomach and with mild redness.



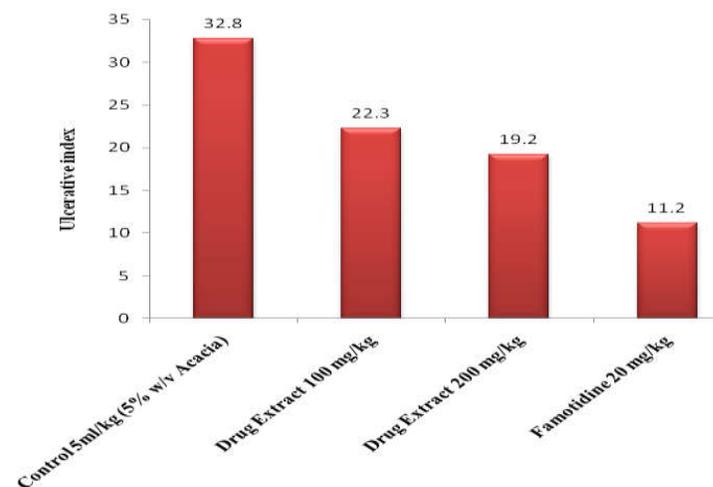
Figure.9. MEAI 200 mg/kg. The stomach section was observed that no sign of redness and inflammation.

Table. 3. Effect of Methanolic Extract of *Acalypha indica* (L.) (MEAI) on Swim stress induced gastric ulcer in rats

Test drugs	Name of drug	Dose (mg/kg)	Ulcer index (in mm)
Group 1	Vehicle	-	32.8±2.3
Group 2	MEAI	100	22.3±1.3
Group 3	MEAI	200	19.2±0.6*
Group 4	Famotidine	20	11.2±0.8*

* p<0.01 Vs control by students 't' test drug extract of plant in a dose 100 and 200 mg/kg, (control 5ml/kg (5%w/v acacia) and famotidine 20mg/kg induced by swim stress induced ulcer.

Figure.10. Parameters assessment treatment upon *Acalypha indica* on Swim Stress Induced Ulceration



Statistical analysis

The results were expressed as mean±S.E and the significance were evaluated by student's t-test compared with control (Woodson., 1987).

DISCUSSIONS

Various standard phytochemical tests were performed for the MEAI and the results were reported in (Table.1.). The phytoconstituents present in the plant MEAI was alkaloids, glycosides, gums and mucilage, reducing sugars, starch and steroids. The phytoconstituents such as glycosides, steroids present in the MEAI which was reported to possess various therapeutic properties. The presence of wide range of phytochemical constituents indicates that plant could serve as lead for the development of novel agents for various pathological disorders. The results obtained from the phytochemical screening given a basic foundation to elucidate the structure of phytoconstituents present in the selected plant.

Results of present study was established a cytoprotective action of stomach tissue cells treatment upon MEAI on pylorus ligation and swim stress induced ulceration in Wistar rats by conducting various parameters with histopathological sectioning. The plant MEAI was used; two different doses at (100 & 200 mg/kg) and protected the stomach from the cytodestructive damage in the gastric mucosa of rats and the cytoprotection given by the herbal drugs has been considered to be due to the generation of gastric mucosa by anti-ulcer drugs when used in their non-anti-secretory drugs (Robert A et al., 1979). The cytoprotection was significantly observed on MEAI by dose dependently reduced the extent of gastric ulceration in pylorus ligated rats and swim stress induced ulcer without affecting the gastric secretion or pepsin activity. On the

other, the standard drug Famotidine (20 mg/kg) produces anti-ulcer activity by inhibiting gastric secretion and reducing pepsin activity. The protective effect of MEAI against pylorus ligation and swim stress induced duodenal ulcers may be due to the strength of the duodenal mucosa (Garner A., 1988) or by the other mechanisms like increased gastric and duodenal alkaline secretion (Rees WDW, *et al.*, 1982). In the pylorus ligation induced gastric ulcer the MEAI showed (Table.2) that the significance at $p < 0.01$ reduction in gastric volume, free acidity and ulcer index (21.5 & 18.7 mm) on 100 & 200 mg/kg respectively. Simultaneously, the control group showed maximum of ulcer index at (35.4 mm) and the standard drug Famotidine (20 mg/kg) showed that evidenced anti-ulcer activity at (10.5 mm). The histopathological sections also showed in (Figure.1 to 4); significant evidenced anti-ulcer activity by reducing ulcer redness in pylorus ligation induced ulceration by Wistar rats. In particularly the MEAI (200 mg/kg) showed that maximum inhibiting action against the ulceration on pylorus ligation method and by the dose dependent manner. In Swim stress induced ulcer method showed in (Table.3.); MEAI showed that significant activity on ulcer index on both the dose levels (19.2 & 11.2 mm) on 100 & 200 mg/kg respectively., compare to the pylorus ligation induced method; swim stress method having high significance. The histopathological sections (Figure. 6 to 9) were observed that MEAI having significant activity on Swim stress induced method by reducing the ulcer index. The comparative drug evaluation of the treated groups was exposed in graphical peak observation on (Figure. 5 & 10). The results significantly indicated that methanolic extract *Acalypha indica* produced anti-ulcerogenic effects possessing antisecretory and cytoprotective mechanism.

CONCLUSION

From the phytochemical screening major constituents present in the compounds are steroids and alkaloids. The phytochemical screening has given the basic foundation in which the class of compounds possesses the antiulcerogenic activity. From the results of pharmacological screening the phytoconstituents showed significant action against pylorus ligated and swim stressed rats. From the evaluation the *Acalypha indica* Linn. it could be added to the normal flora of the plant kingdom.

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