



ANTIULCER ACTIVITY OF *AMMANIA BACCIFERA* LINN

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ABSTRACT

The petroleum ether, methanol, chloroform and aqueous fractions of the ethanolic extract of *Ammania baccifera* Linn whole plant were evaluated for their antiulcer potential at a dose of 400 mg/kg b.w. p.o. against pyloric ligation and indomethacin induced gastric ulcer models in Albino rats. Ranitidine was used as a reference standard for both the models. The antiulcer effect was assessed by parameters such as gastric volume, pH, free acidity, total acidity, ulcer index and percent inhibition. The methanolic fraction exhibited highly significant antiulcer effect against both pyloric ligation and indomethacin induced gastric ulcers in Albino rats. The phytochemical studies revealed the presence of alkaloids, carbohydrates, steroids, tannins, triterpenes, and flavanoids in the ethanolic extract; steroids in the pet. ether fraction; alkaloids, steroids, triterpenes and flavanoids in the methanolic fraction; alkaloids in the chloroform fraction; flavanoids in the aqueous fraction.

Key words: *Ammania baccifera*, Antiulcer, Pylorus ligation, Indomethacin, Ulcer index, Gastric acidity, pH, Mucosal defense, Flavanoids, Triterpenes.

INTRODUCTION

Peptic ulcer is a conglomerate of heterogeneous disorders, which manifest itself as a break in the lining of the gastrointestinal mucosa bathed by acid/pepsin¹. Although a number of synthetic antiulcer drugs are available for ulcer treatment, all these drugs possess one or other adverse effect and limitations². A retrospection of healing power of plants, a return to natural remedies is absolute need of our time. Medicine of plant origin is based upon the premise that plants contain natural substances that can promote health and alleviate illness with less adverse effects^{3,4}.

A. baccifera Linn, an indigenous medicinal plant belonging to the family Lythraceae

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is an erect, branched, smooth, slender, annual herb usually found in open, damp, marshy rice fields⁵. It has been used traditionally for treating leucorrhoea, snake-bite poisoning, abscess, intermittent fever, ulcers, polyuria, ringworm infestations, swellings, depression, hypertension, weakness, flatulence, seminal weakness, tuberculosis, bacterial and parasitic infections^{6,7}. Some of the traditional uses of *A. baccifera* such as antisteriodogenic⁸, antifertility⁹, antiurolithic¹⁰, analgesic¹¹, anti-inflammatory and antiarthritic¹² potentials have already been proven experimentally. An extensive literature survey revealed no pharmacological validation on antiulcer activity of this plant. Hence the objective of the present study was to investigate the antiulcer activity of various fractions of ethanolic extract *Ammania baccifera* whole plant using experimental models.

EXPERIMENTAL

Plant material

The whole plant of *Ammania baccifera* (L.) was collected from in and around the region of Shimoga, Karnataka, India in the month of June. The plant was identified and authenticated by Prof. Rudrappa, Botanist SRNM College, Shimoga, Karnataka. A voucher specimen (NCP/04/2010-11) was preserved in the Herbarium of Pharmacognosy Department, National College of Pharmacy, Shimoga for future reference. The whole plant was dried under shade, powdered by a mechanical grinder to obtain coarse powder.

Preparation of extract and fractions

The powdered plant material (1400 g) was extracted using 90% ethanol (5.2l) in a Soxhlet extractor (hot extraction). The ethanolic extract was evaporated using Rota flash evaporator under reduced pressure and low temperature and then on a water bath. The ethanolic extract was then fractionated with petroleum ether, methanol, chloroform and water^{13,14}.

Preliminary phytochemical screening

The ethanolic extract of *Ammania baccifera* and the various fractions of ethanolic extract of *Ammania baccifera* were subjected to preliminary phytochemical screening according to standard procedures¹⁵.

Animals

The animals used in acute toxicity study (Swiss Albino mice of either sex weighing 25-30 g) and for the evaluation of antiulcer activity (Wistar Albino rats of either sex weighing 150-200 g) were procured from the Department of Pharmacology, National

College of Pharmacy, Shimoga. The study was approved by Animal Ethical Committee (Ethical clearance No. NCP/IAEC/CL/24/02/2009-10). The study was carried out in accordance with CPCSEA guidelines¹⁶.

Acute toxicity study¹⁷

The acute toxicity study was performed according to the OPPTS (Health effect test guideline 2004, Office of Prevention, Pesticide and Toxic Substance) by Up and Down procedure using Swiss albino mice of either sex. The fractions were suspended in Tween 80 (0.1%) and administered orally upto a dose of 4000 mg/kg b.w. p.o.

Antiulcer studies

Pyloric ligation model (shay rat model)^{18,19}

Wistar Albino rats of either sex weighing 200-250 g were housed in individual cages. Rats were divided into six groups of each consisting of six animals and placed in spacious cages with grating floor to avoid coprophagy and fasted for 24 hours prior to pyloric ligation. The animals were maintained under standard conditions proposed by (CPCSEA). The test fractions were suspended in Tween 80 (0.1%) so as to obtain a final concentration of 1gm in 10 mL. The vehicle, standard drug Ranitidine and all fractions were administered orally.

Group 1 (n=6): Control group treated with saline (10 mL/kg b.w. p.o.)

Group 2 (n=6): Received reference standard ranitidine at a dose (20 mg/kg b.w. p.o.)

Group 3 (n=6): Received Pet. ether fraction of *A. baccifera* (400 mg/kg b.w. p.o.)

Group 4 (n=6): Received methanol fraction of *A. baccifera* (400 mg/kg b.w. p.o.)

Group 5 (n=6): Received chloroform fraction of *A. baccifera* (400 mg/kg b.w. p.o.)

Group 6 (n=6): Received aqueous fraction of *A. baccifera* (400 mg/kg b.w. p.o.)

The pyloric ligation was performed 30 minutes after drug administration. Under light ether anesthesia the abdomen was cut opened by a small incision below the xiphoid process; pyloric portion of the stomach was slightly lifted out and ligated avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall closed by interrupted sutures. The animals were deprived of both food and water during the postoperative period and sacrificed at the end of 4 hours post operation. Stomachs were dissected out; contents drained into tubes and centrifuged at 2000 rpm for 10 min. The supernatant was collected and used for further study. The stomach was then cut

open along the greater curvature and the inner surface was examined for ulceration by giving score number.

The parameters studied include gastric volume, pH, free and total acidity and ulcer index. The severity of the ulcer was scored with the following scores.

0 = No ulcer

1 = Superficial ulcers

2 = Deep ulcers

3 = perforation

Mean ulcer score for each animal was expressed as Ulcer Index (UI). The percentage protection was calculated using the formula.

$$\text{Percentage of ulcer protection} = \frac{\text{UI of control} - \text{UI of test}}{\text{UI of control}} \times 100$$

Measurement of pH: The pH of gastric juice was measured by using a pH meter.

Estimation of total and free acidity

1 mL of gastric juice was pipetted into a 100 mL conical flask, 2 to 3 drops of Topfer's reagent was added and titrated against 0.01 N NaOH until all traces of the red colour disappears and the colour of solution becomes yellowish orange. The volume of alkali consumed was noted which corresponds to free acidity. Then 2 to 3 drops of phenolphthalein solution was added and titration continued until a definite red tinge reappears. Again the total volume of alkali added was noted which corresponds to total acidity.

Acidity was calculated by using the formula

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{0.1} \times 100 \text{ meq/L/100 g}$$

Indomethacin induced gastric ulcer¹⁹

The rats were grouped and treated as under pyloric ligation model. The vehicle, standard reference (Ranitidine at a dose of 20 mg/kg b.w) and test fractions were administered orally 30 minutes prior to the oral administration of indomethacin in a dose of 20 mg kg⁻¹ b.w. The animals were sacrificed 4 hrs after Indomethacin administration. The

stomachs were removed, opened along the greater curvature. The parameters studied include ulcer index and percentage of ulcer protection.

Statistical analysis

Results were expressed as mean \pm SD (n=6). Statistical analysis was performed with one way analysis of variance (ANNOVA) followed by Turkey-Kramer Multiple Comparison Test using Graph Pad Insat Software.

RESULTS AND DISCUSSION

The percent yield of ethanolic extract of *Ammania baccifera* (EEAB) was 12.56% w/w. The percent yield of petroleum ether (PFEEAB), methanol (MFEEAB), chloroform (CFEEAB) and aqueous fractions (AFEEAB) of ethanolic extract of *Ammania baccifera* were 13.7%, 32.35%, 3.9% and 50.05% w/w respectively.

The preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrates, steroids, tannins, triterpenes, and flavanoids in the ethanolic extract; steroids in the Pet. ether fraction; alkaloids, steroids triterpenes and flavanoids in the methanolic fraction; alkaloids in the chloroform fraction; and flavanoids in the aqueous fraction (Table 1).

Table 1: Phytochemical analysis of ethanolic extract and fractions of *Ammania baccifera* whole plant

Phytochemical	EEAB	PFEEAB	CFEEAB	MFEEAB	AFEEAB
Alkaloids	+	-	+	+	-
Glycosides	-	-	-	-	-
Carbohydrates	+	-	-	-	-
Proteins	-	-	-	-	-
Steroids	+	+	-	+	-
Tannins	+	-	-	-	-
Triterpenes	+	-	-	+	-
Flavanoids	+	-	-	+	+

+ indicates positive, - indicates negative result

Acute toxicity studies showed no mortality up to the dose of 4000 mg/kg b. w. with all fractions, thus indicating the plant as a broad nontoxic one. 1/10th of the maximum tolerated dose was used for the further study.

The experimental findings revealed that the methanol fraction of ethanolic extract of *Ammania baccifera* exhibited more significant ($p < 0.001$) antiulcer effect in both pylorus ligated and indomethacin induced gastric ulcer models in Albino rats. The gastric volume, pH of gastric juice, free acidity, total acidity, ulcer index and ulcer protection of animal treated with methanolic fraction were found to be 5.13 ± 0.05 mL, 6.43 ± 0.05 , 53.07 ± 1.09 mEq/L/100 g, 64.77 ± 0.99 mEq/L/100 g, 4.98 ± 0.08 and 50.34% respectively (**Table 2**). The ulcer index and percentage protection of ulcers in indomethacin induced gastric ulcer model were 5.12 ± 0.12 and 71.68% respectively (**Table 3**). The antiulcer effect of methanolic fraction was comparable to that of standard drug ranitidine. In both the models, the control group (treated with vehicle alone) failed to exhibit any ulcer protective activity.

Table 2: Effect of different fractions of ethanolic extract of whole plant of *Ammania baccifera* on ulcer index, gastric volume, pH, free acidity, total acidity and percentage of ulcer protection in pylorus ligated model

Groups	Dose (mg/kg b.w.)	Ulcer index	% of inhibition	Vol. of gastric juice (mL)	Free acidity (mEq/L/100g)	Total acidity (mEq/L/100g)	Gastric pH
Control (saline)	-----	10.03 ± 0.15	-----	7.22 ± 0.04	86.03 ± 0.24	98.68 ± 0.90	2.57 ± 0.08
Standard (Ranitidine)	20 mg/kg	$2.23 \pm 0.05^*$	77.76	$3.45 \pm 0.05^*$	$23.32 \pm 0.84^*$	$37.33 \pm 0.60^*$	$7.03 \pm 0.05^*$
PFEEAB	400 mg/kg	$5.25 \pm 0.05^*$	47.65	$5.80 \pm 0.01^*$	$59.27 \pm 0.54^*$	$64.37 \pm 0.49^*$	$6.25 \pm 0.05^*$
MFEEAB	400 mg/kg	$4.98 \pm 0.08^*$	50.34	$5.13 \pm 0.05^*$	$53.07 \pm 1.09^*$	$64.77 \pm 0.99^*$	$6.43 \pm 0.05^*$
CFEEAB	400 mg/kg	$7.13 \pm 0.05^*$	21.04	$6.37 \pm 0.05^*$	$64.15 \pm 0.36^*$	$78.38 \pm 0.79^*$	$4.43 \pm 0.05^*$
AFEEAB	400 mg/kg	$5.24 \pm 0.04^*$	47.75	$5.77 \pm 0.05^*$	$61.85 \pm 0.92^*$	$76.83 \pm 0.81^*$	$6.32 \pm 0.04^*$

Each value represents the mean \pm SD (n=6), Significant * $P < 0.001$ compared to respective control group

Table 3: Effect of different fractions of ethanolic extract of *Ammania baccifera* whole plant on ulcer index and percentage of ulcer protection in Indomethacin induced gastric ulcer in rats

Groups	Treatment and dose (mg/kg b.w.)	Ulcer index	% of inhibition
Control (distilled water)	-----	18.08 ± 0.18	-----
Standard (Ranitidine)	20 mg/kg	3.33 ± 0.14*	81.58
Pet. Ether fraction	400 mg/kg	7.92 ± 0.10*	56.19
Methanol fraction	400 mg/kg	5.12 ± 0.12*	71.68
Chloroform fraction	400 mg/kg	10.15 ± 0.18*	43.86
Aqueous fraction	400 mg/kg	6.97 ± 0.10*	61.44

Each value represents the mean ± SD for six rats; Significant *P<0.001 compared to respective control group

Pyloric ligation model is a simple and reliable method for production of gastric ulceration in rats. It has been proposed that in pylorus-ligation, the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for induction of ulceration^{20,21}. Increased synthesis of nucleic acid and metabolism of carbohydrates and other compensatory mechanisms could also be responsible for the ulceration due to pylorus-ligation²². Gastric acid and pepsin are important factors for the formation of ulcers in pylorus ligated rats²³.

NSAID'S like Indomethacin act by inhibiting COX1, thereby inhibiting prostaglandin synthesis, consequently lipooxygenase pathway is enhanced liberating leukotrienes and these leukotrienes are reported to have a role in ulcerogenesis. In addition there is some evidence that NSAID'S may induce ulcer by causing the back diffusion of H⁺ ion into mucosal cells²⁴⁻²⁸. Therefore, in the present study the gastro protective effect of the test fractions may be due to their ability to inhibit the synthesis of leukotrienes.

Earlier researchers have suggested that, the antiulcerogenic activity may be due to the presence of various phytochemicals such as saponins, tannins and flavanoids in plant extracts or fractions²⁹⁻³³. Flavanoids have been reported to protect the gastric mucosa from damage by increasing the mucosal prostaglandin content and by inhibiting histamine secretion from mast cells by inhibition of histidine decarboxylase³⁴⁻³⁵. Free radical

scavenging ability of flavanoids has been reported to protect the GIT from ulcerative and erosion lesion³⁶.

The present study showed methanolic fraction of ethanolic extract of *Ammania baccifera* whole plant exhibit significant antiulcer effect in both pylorus ligation and indomethacin induced gastric ulcer in Albino rats. The antiulcer activity of *A. baccifera* may be attributed to the presence of flavanoids, triterpenes and steroids in the methanolic fraction. The present study therefore supports the claims of traditional medicinal practitioners as an antiulcer remedy.

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