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Research Article

**EVALUATION AND COMPARISON OF VARIOUS
EXTRACTS OF *RHYNCHOSIA MINIMA* (LINN) DC AGAINST
PYLORUS LIGATION INDUCED ULCERS IN RATS****N. Yellasubbaiah^{1*}, B. Nagasudha¹ Sujit Kumar Mohanty¹ and C. Ayyanna²**¹Dept. of Pharmaceutical Chemistry, C.E.S. College of Pharmacy, Chinnatekur, Kurnool,
A.P.,²Dept. of Pharmacology, C.E.S. College of Pharmacy, Chinnatekur, Kurnool, A.P.,**Abstract:**

The plant *R. minima* (Linn) DC (Fabaceae) is an indigenous medicinal plant used traditionally as abortifacient, anthelmintic, used in the treatment of wounds, asthma and piles. In the current study aqueous, ethanol and ethyl acetate extracts of this plant were compared and evaluated for antiulcer activity by pylorus ligation model. All these extracts also subjected for the phytochemical analysis for the investigation chemical constituents and toxic potential. Aqueous extract was found to be presence of flavanoids, tannins and glycosides; alcohol extract revealed the presence of alkaloids, carbohydrates and isoflavanoids; while ethyl acetate extract was found to be the presence of tannins, flavanoids and alkaloids. The aqueous and ethyl acetate extracts were found to be non-toxic up to 4000mg/kg dose level while ethanol extract was found to be toxic at the dose level of 3000mg/kg after single dose administration of the extracts. During the comparison of anti-ulcer activity, treatment with aqueous and ethyl acetate extracts showed significant reduction in ulcer index, free acidity as well as total acidity in pylorus ligated rats. However, ethanol extract showed relatively less reduction in ulcer index, free acidity as well as total acidity. The anti-ulcer activity observed in aqueous extract treatment group was nearly equivalent when compared with standard Ranitidine Hcl.

Keywords: *R. minima* (Linn.) DC, Aqueous, Ethanol and Ethyl acetate extracts, toxicity, anti-ulcer activity, Ranitidine Hcl.

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INTRODUCTION:

The plant *Rhynchosia minima* Synonym(s): *Dolicholus minimus*, *Dolichos minimus*, *R. minima* var. *diminifolia* Family: Fabaceae, locally known as Nela Alumu (Telugu) is an indigenous medicinal plant used traditionally as abortifacient, antihelminthic, used in the treatment of wounds, asthma and piles. The seeds are bitter and poisonous and seed extract shows specific agglutinating action on human RBC [1]. Rangaswamy *et al.*, 1974 [2] Studied the phytochemistry of the seed coat and pericarp and found to contain gallic acid, Hydroquinone diacetate and other phenolics. Elisabeth *et al.*, 1977 studied phenolics and flavonoids in the leaves and reported that all flavonoids of the leaf extract were present in the form of C-glycosylflavones [3]. The hydroquinone present in the seeds of *R. minima* is supposed to be involved in seed germination [1]. Flavonoid profiles of seven species of *Rhynchosia* including *R. minima* were reported by Adinarayana *et al.*, 1985 [4]. New flavonoids were identified in the leaf extract of *R. cyanosperma* [5]. In all these studies the medicinal uses of the phytochemical principles were not discussed. However, Gundidza *et al.*, 2009 [6] demonstrated range of 8 essential oils which showed high antibacterial activity against several bacterial and fungal species. Anthelmintic activity *R. minima* of was reported by Mali RG *et al.*, 2008 [7].

Gastric ulcer, one of the most widespread, is believed to be due to an imbalance between aggressive and protective factors. The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs. These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility (T. Rajkumar *et al.*, 2011) [8]. Keeping in view the frequent folklore use of *R. minima*, the present study was carried out to determine the anti-ulcer activity *R. minima* whole plant using animal models.

MATERIALS AND METHODS:

Plant Material

Rhynchosia minima (Linn) DC plant was procured in the spring season, from Medicinal garden of CES College of Pharmacy, a Chinntekur locality in Kurnool. The Leaves were identified and authenticated by botanist Dr. M. Palanisamy, Scientist 'C' Botanical survey of India, Southern regional center, Coimbatore. A specimen voucher of the plant has been deposited in the Department of Pharmacognosy, CES College of Pharmacy, Chinntekur, Kurnool.

Preparation of extracts

The whole plant of *R. minima* (Linn) DC was shade dried under room temperature for one week and the whole plant was powdered mechanically. The finely powdered plant was kept separately in an airtight container until the time of use. About 200 grams of finely powdered plant were successively extracted in a Soxhlet extractor using absolute ethanol for 24h [9]. The aqueous extract was prepared by maceration of finely powdered plant material with water for 48h. These extracts were screened for phytochemical screening and antiulcer activities.

Qualitative phytochemical screening

Aqueous, ethanol and ethyl acetate extracts of *R. minima* (Linn) DC were screened for their preliminary phytochemical investigation for the presence of various phytochemical constituents [10,11].

Acute Toxicity Studies

The acute toxicity of aqueous, alcoholic and ethyl acetate extracts of *R. minima* (Linn) DC plant were determined in Wistar rats fasted for 3 h (which examined that/DEL). The highest oral dose administered was 4 g/kg body weight (which was equivalent to powder crude drug 28.95 g/kg of body weight). Up to 4 g/kg dose levels no signs of toxicity appeared. The LD50 of the test extracts were calculated using AOT 425 software¹². Oral toxicity: not considered as toxic (DL50 oral/rats >40 g/kg body weight).

Preparation of drugs and chemicals

Standard ranitidine injection (Aciloc Injection, Cadila, Health Care Ltd., Mumbai, India), Tween 80 (laboratory grade) were used. Other chemicals used were: ethyl acetate, absolute alcohol, anesthetic ether, formalin and EDTA ethylene diamino tetra acetic acid (EDTA).

Experimental animals

Adult Wistar rats (150–180 g) were used to study anti-ulcer activity. All these animals were maintained under standard husbandry protocol and conditions (light/dark period of 12 h light/dark and temperature 25 ± 3 °C) with free access to food and water all experiments were carried out between 10 am to 5 pm daily [11].

In-vivo anti-ulcer activity

Pylorus ligation method

In this method Wistar rats of both sexes weighing between 150-250 gm having free access to drinking water were placed in separate single-single cages with raised bottom in order to avoid cannibalism and coprophagy¹⁵. The rats were randomly allotted to five groups containing six animals each [11] as follows.

Group 1: Group 1st received aqueous extract (500 mg/kg body weight), Group 2nd received alcoholic extract (500 mg/kg), Group 3rd received ethyl

acetate extract (500 mg/kg), Group 4th received standard (ranitidine 20 mg/kg) and Group 5th received (Tween 80) all the drugs are given by oral route (Table 1).

Under ether anesthesia a midline abdominal incision was made. The pylorus was ligated representing that neither blood supply was damaged nor traction occurred on the pylorus. The test compounds were given orally by gavage. The animal was kept for 6 h under experimental conditions [12]. At the end of treatment, the mucosa of animals in each group was examined under microscope. The number of ulcers and their severity [10] were recorded using arbitrary scale as follows.

0 = no ulcer, 0.5 = spot ulcer, 1.0 = superficial ulcers, 2.0 = deep ulcers and 3.0 = perforation.

Mean of ulcer score for each animal was expressed by the formula given below:

Percentage protection=

$$100 \frac{\text{Ulcer index of treated group}}{\text{Ulcer index of controlled group}} \times 100$$

The volume of the gastric content was measured after centrifugation, while acidity was determined by titration with 0.01 N NaOH using Toppfer's reagent and phenolphthalein as indicators [15].

Statistical analysis

The results are expressed as mean \pm SEM. Statistical difference between means were determined by one-way ANOVA followed by Dunnett's post hoc test (Del/was) were used to analyze and compared data with $P > 0.05$ as the limit of significance (SEM= standard of error of mean).

RESULTS AND DISCUSSION:

Physical characteristics of extracts:

The Aqueous extract of *R. minima* (Linn) DC was thick dark brown color, sticky in nature and the percentage yield of the extract was found to be 32% w/w.

Ethanol extract of *R. minima* (Linn) DC was slightly black green in color, sticky in nature and the percentage yield of the extract was found to be 22.42% w/w.

Ethyl Acetate extract of *R. minima* (Linn) DC was green in color, sticky in nature and the percentage yield of the extract was found to be 21.76% w/w.

Table 1: Experimental groups and treatment given

Groups	Treatment (dose/kg, p.o.)
Group 1	Aqueous extract 500 mg/kg
Group 2	Alcoholic extract 500 mg/kg
Group 3	Ethyl acetate extracts 500 mg/kg
Group 4	Ranitidine 20mg/kg (standard)
Group 5	Tween 80

Preliminary phytochemical screening of extracts:

Qualitative phytochemical screening was carried out using several tests of *R. minima* (Linn) DC Aqueous extract was found to be presence of flavanoids, tannins and glycosides; alcohol extract revealed the presence of alkaloids, carbohydrates and isoflavanoids; while ethyl acetate extract was found to be the presence of tannins, flavanoids and alkaloids.

Acute toxicity study

The aqueous and ethyl acetate extracts were found to be non-toxic up to 4000mg/kg dose level while ethanol extract was found to be toxic at the dose level of 3000mg/kg after single dose administration of the extracts.

Effect of *R. minima* (Linn) DC extracts in Pylorus ligation induced gastric ulcers.

Pretreatment with all extracts significantly decreased ulcer index $P < 0.001$, with aqueous while $P < 0.01$ with ethyl acetate extract and $P < 0.05$ with ethanol extract. There was significantly rise in pH with reduction in volume of gastric contents, free acidity, total acidity with/in extract treated group as compared to extract untreated rats. Significant with aqueous and ethyl acetate extract and percentage protection was comparable with that of standard (ranitidine). The order of percentage inhibitions showed by the extracts is Aqueous > Ethyl acetate > Ethanol the results were represented in Table 2.

All the extracts of *R. minima* (Linn) DC in pylorus ligation induced ulcer model reduced ulcer index, gastric volume, free acidity and total acidity. Thus, they exhibit the antisecretory mechanism involved in the extracts for their antiulcerogenic activity. Ulcer index parameter was used for the evaluation of anti-ulcer activity since factors such as gastric volume, free acidity and total acidity is directly related to ulcer formation.

The results indicated that *R. minima* (Linn) DC extracts produced antiulcerogenic effects possessing antisecretory, cytoprotective and H₂ blocking/proton pump inhibition mechanism. The present study demonstrated the potential of *R. minima* (Linn) DC to exert anti-ulcer activity especially the aqueous extract.

Table 2: Effect of *R. minima* (Linn) DC extract on ulcer index, pH, volume of gastric juice, free acidity, total acidity and percentage protection in pylorus ligated rats.

Treatment group's	aqueous extract	Ethanolic extract	Ethyl acetate extracts	Standard (ranitidine)
Control				
Dose mg/kg	500	500	500	20
Ulcer Index	0.81±0.29***	1.34±0.35*	1.02±0.35**	0.76±0.61***
pH	4.12±0.71*	3.24±0.12	5.16±0.14**	4.98±0.127**
Volume of gastric juice	2.74±0.48**	3.42±0.56	4.27±0.14	2.31±0.52**
Free acidity	38.62±12.14*	54.46±16.13	47.51±14.52	35.39±11.34*
Total acidity	54.67±10.54**	112.7±14.58	82.58±18.15*	49.35±19.54*
% protection	81.42	72.58	79.82	88.48

Results are expressed as mean ± SEM, significance at *P<0.05, **P<0.01, ***P<0.001 compare to control.

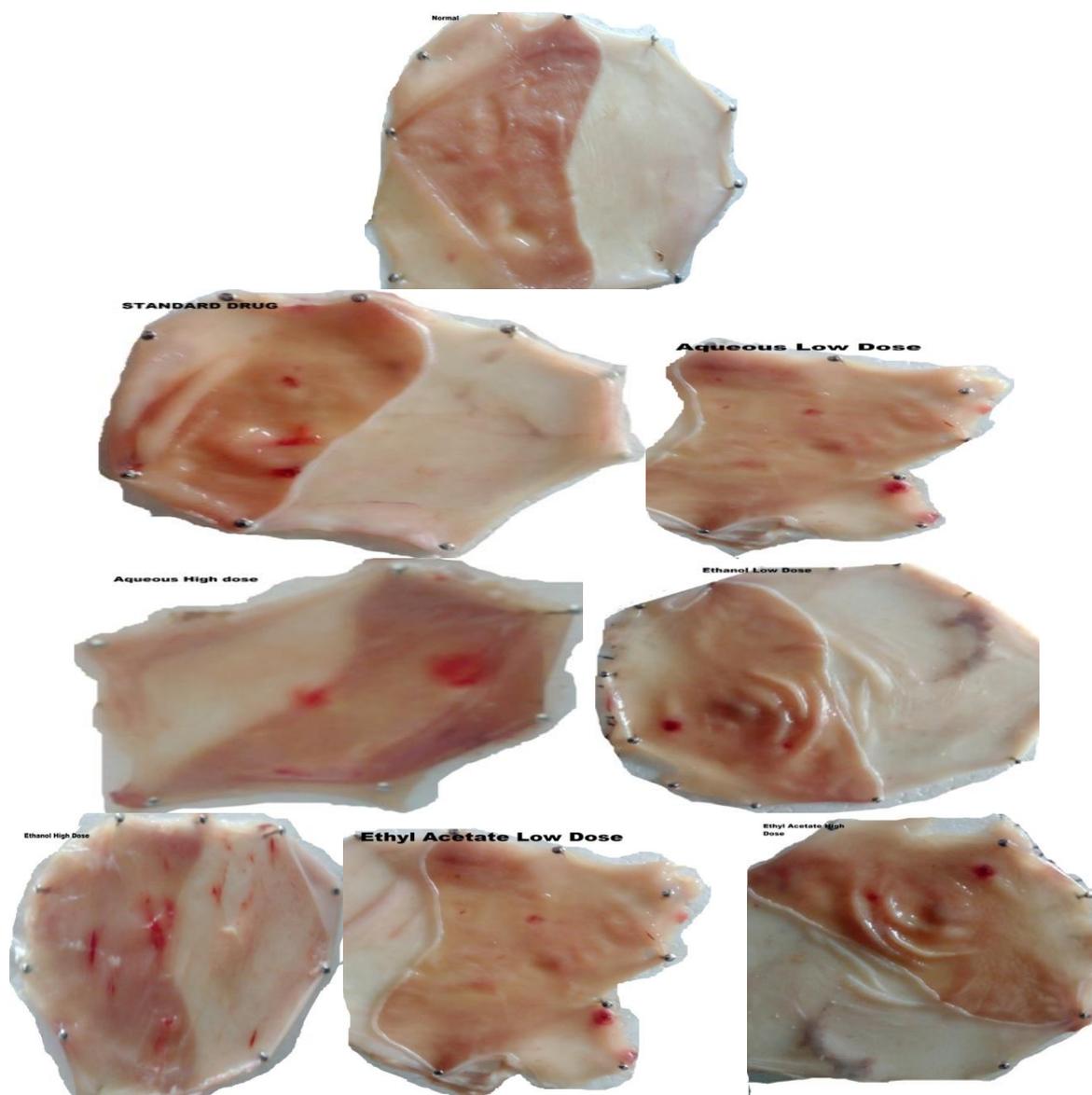


Fig 1: Diagrammatic representation of antiulcer activity *R. minima* DC linn. by pylorus ligation method.

CONCLUSION:

The present work revealed that the extracts of *R. minima* (Linn) DC to exert antiulcer activity by pylorus ligation method especially aqueous and ethyl acetate extracts. The results justified the use of plant extracts in antiulcer diseases traditionally. We suggest that the plant can be viewed as the potential sources of natural antiulcer afford precious functional components.

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