

Assessment of *Coccinia Indica* root extract for antiulcer activity in experimental animals

Ashish Dixit^{1,*}, Vibhu Sahani², Anil Kumar khareya³ and Vivek Tiwari⁴

¹ Spectrum Hi Pharmacy College, Sultanpur, Uttar Pradesh, India.

² LLRM Medical College, Meerut, Uttar Pradesh, India.

³ Kunwar Harvansh Singh College of Pharmacy, Jaunpur, Uttar Pradesh, India.

⁴ Ramdham Mahavidhyalaya (Pharmacy), Mau Uttar Pradesh, India.

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Abstract

The objective is to evaluate the anti-ulcer activity of ethanolic extract of root of *Coccinia grandis* (Linn.), Anti-ulcer activity of the three extract was studied in rats by using pylorus ligated ulcer model and it was subjected to preliminary phytochemical studies for the identification of phytoconstituents and also studied for color, consistency and percentage yield of various extracts. Ranitidine was used as the standard drug for comparison. The animals were sacrificed after 06 hrs after the ligation. Stomach was dissected out and contents were drained into tubes and were centrifuged and volume was noted. The PH of gastric juice was recorded using a PH meter. The contents were subjected for analysis of free and total acidity and Na⁺, k⁺ ion concentration. The numbers of ulcers per stomach was noted and severity of ulcers scored. Then the blood samples were collected and subjected to estimation of serum calcium and alkaline phosphatase level. The expected result is to get an anti-ulcer activity of the leaf extracts of *Coccinia grandis* should owing to the presence of one or more phytoconstituents, which may reduce the acidity of the gastric juice and also prevents the mucosal damage and ulcer formation. The Ethanolic Extract 400mg/kg expected to showed comparable anti ulcer-activity as that of standard Ranitidine.

Keywords: Gastric ulcer; *Coccinia grandis*; Ranitidine; Experimental animals

1. Introduction

Peptic ulcer disease is one of the most common gastrointestinal disorders, which causes a high rate of morbidity impaired bicarbonate neutralization, impaired mucus secretion and precipitate lesions on the mucosal layer [2, 3]. In recent years, a powerful association between peptic ulcers and infection of *Helicobacter pylori* has been adopted. At least 70-90% of patients with gastric ulcers and 80-95% with duodenal ulcers are infected by *H. pylori* and eradication of this Microorganism seems to be curative for the disease [4]. There is a balance between the aggressive (i.e. acid, pepsin, active oxidants, *H. pylori*) and the mucosal protective (i.e. mucus, bicarbonate, prostaglandin's) factors in stomach. Thus, drug therapy of peptic ulcer has been commonly targeted at either counteracting the aggressive factors or stimulating defensive one [5]. Despite the progress in conventional chemistry and pharmacology in producing highly effective drugs, some of them are expensive and have different adverse effects [6, 7]. However, screening plants for active drugs is still important and might provide a useful source of new anti-ulcer compounds for developing pharmaceutical drugs or alternatively as simple dietary adjuncts to existing therapies [8]. *Coccinia grandis* is one of the traditional indigenous plants of India and is widely distributed. It is commonly used for respected therapeutic uses (E.g. anti inflammatory, aphrodisiac, uterine discharges, astringent, Anti pyretic, galactagogues, expectorant, leprosy, skin diseases, intermittent fever, agalactia, asthma, cough, bronchitis, jaundice, antispasmodic) on the contrary, the flavonoids that have potentially anti-ulcerative properties [9].

* Corresponding author: Ashish Dixit

2. Material and methods

2.1. Plant material

The plant roots of *Coccinia Indica* were identified and collected from Siwal khas, Meerut, U.P., INDIA during the month of October 2021 2017 then roots of *Coccinia Indica* was authenticated from the CSIR-National Institute of Science Communication and Information Resources, Raw Material Herbarium and Museum, Delhi (RMHD). The plant roots were cleaned, reduced to small fragments and air dried under shade at room temperature then coarsely powdered in grinding mill and passed via sieve no. 40. The sieved powder was stored in airtight container and kept at room temperature. The powdered (500 gm) plant material was successively extracted with ethanol using Soxhlet extractor [10].

2.2. Animals

Male Wistar rats 150-220 gm were selected for the study. The animals were obtained from animal's house facility of Spectrum Hi Pharmacy College, Sultanpur (Uttar Pradesh) which were housed in polypropylene cages at 24 ± 2 °C in the college animal house and fed with commercial pellet diet supplied by Kamadhenu Agencies, Bangalore and water ad libitum. The food was withdrawn 18 hours before the experiment but allowed free access of water. To avoid Coprophagy and fighting, the rats were fasted in wire-bottomed cages. All animal experiments were carried out in accordance with the guidelines of CPCSEA.

2.3. Acute toxicity testing

Acute oral toxicity test was performed as per OECD-423 guidelines. All the animals were randomly distributed into one control group and three treated groups, containing six animals per group. Groups 1, 2 and 3 were orally administered 100, 500 and 1000 mg/kg body weight ethanolic extract following the method of Lorke (Lorke *et al.*, 1983). The control group received vehicle alone. The animals were observed continuously for first 72 hours and 7 days for any symbols of behavioral changes, toxicity, mortality and body weight [11].

2.4. Pylorus ligation induced ulcer

An identification mark was given to the rats of each group using picric acid as dye following standard operating procedure. Every one rat of a group was marked at a particular position viz.- Head; Tail; Back; and one was left Blank i.e. unmarked. Every rat was weighed and the dose was calculated accordingly.

Male Wistar rats 150-220 gm were selected and randomly divided into four groups with six animals in each group and orally administered with following treatments:

- Group 1 – Control group received normal saline 2 ml/kg.
- Group 2 – Standard group received Ranitidine 50 mg/kg by oral route.
- Group 3 – EECI (Ethanolic extract of *Coccinia Indica* at a dose of 200 mg/kg, b.w., p.o.).
- Group 4 - EECI (Ethanolic extract of *Coccinia Indica* at a dose of 400 mg/kg, b.w., p.o.).

2.5. Experimental Procedure

The assay was performed using method of Shay *et al.* (N. L. Dashputre *et al.*, 2011) Rats were fasted for 36 hr prior to experimentation. The drug/extract/vehicle was administered orally one hour before ligation. After one hour rats were anesthetized using a mild dose of anesthetic ether. A midline abdominal incision was made. The pylorus was ligated, care being exercised that neither damage to the blood supply nor traction on the pylorus occurs. The abdominal wall was closed by sutures. Animals were permitted to recover and stabilize in individual cages. After 6 hr of surgery, rats were sacrificed, abdomen was opened and a ligature was placed around the oesophagus close to the diaphragm. The stomach was removed, along the greater curvature the stomach was opened and the contents were drained in a centrifuge tube. Stomach was washed with tap water. Following parameters were recorded in this model: Volume of gastric secretion, Free Acidity, Total Acidity, pH, Ulcer Index, % protection.

2.6. Statistical analysis

The result were express as Mean \pm SEM. Statistical analysis was carried out using student-t test. $P < 0.01$ was considered statistically significant.

3. Results

3.1. Acute toxicity testing

Acute toxicity studies revealed that the ethanolic extract of *Coccinia Indica* was safe upto 1000 mg/kg body weight and approximately LD₅₀ is more than 1000 mg/kg. No lethality or any toxic reactions was observed up to the end of the study period.

3.2. Effect of ethanolic extract of *Coccinia Indica* in pyloric ligation induced peptic ulcer -

Table 1 Effect of EECI against Pylorus ligation induced ulcers in rats

S.No	Group	Gastric volume	pH	Total acidity	Free acidity
01.	Control	4.42±0.18	1.56±0.27	90.00±2.10	70.64±6.35
02.	Standard	2.21±0.16**	3.2±0.29**	53.45±2.67**	36.72±1.67**
03.	EECI 200 mg/kg	3.06±0.11**	1.69±0.05**	44.17±1.82**	37.56±1.89**
04.	AECCI 400 mg/kg	2.44±0.22**	2.13±0.73**	40.03±3.35**	33.81±1.92**

All the values were expressed as Mean ± standard error of mean (SEM), n=6/groups, P<0.01 When compared with control group (ANOVA) followed by student-t test.

Table 2 Ulcer index and % protection of EECI in pylorus ligation induced peptic ulcers in Rats

S. No.	Groups	Ulcer index	% Protection
01.	Control	13.51	-
02.	Std (Ranitidine 50mg/kg)	4.51	81.22**
03.	EECI (200 mg/kg)	14.32	39.34**
04.	EECI (400 mg/kg)	10.20	64.09**

All the values were expressed as Mean ± standard error of mean (SEM), n=6/groups, P<0.01 When compared with control group (ANOVA) followed by student-t test.

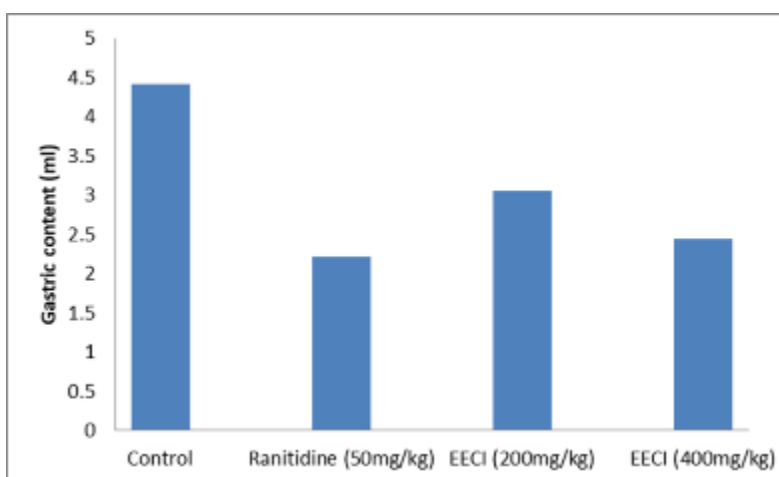


Figure 1 Antiulcer Effect of EECI against Pylorus ligation induced ulcers in Male Wistar rats

The ulcer index and percent of protection against ulcers in the pylorus ligated model in table 2. Treatment with ethanolic extract of *Coccinia Indica* showed significant protection against ulcers (39.34%) at a dose of 200 mg/kg, while the treatment of ethanolic extract of *Coccinia Indica* showed significant protection against ulcers (64.09%) at a dose of 400 mg/kg ($p < 0.01$) when compared with the control animals respectively.

The standard drug, Ranitidine showed significant protective effect against ulcers (81.22 %) at a dose of 50 mg/kg when compared with the control groups in both treatments.

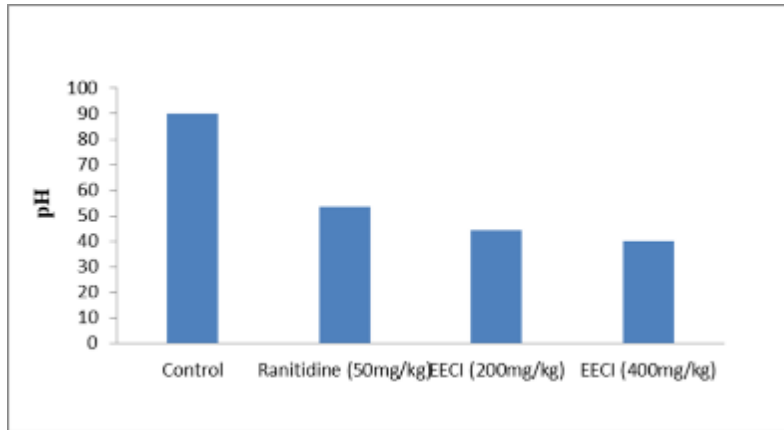


Figure 2 Antiulcer Effect of EECl against Pylorus ligation induced ulcers in Male Wistar rats

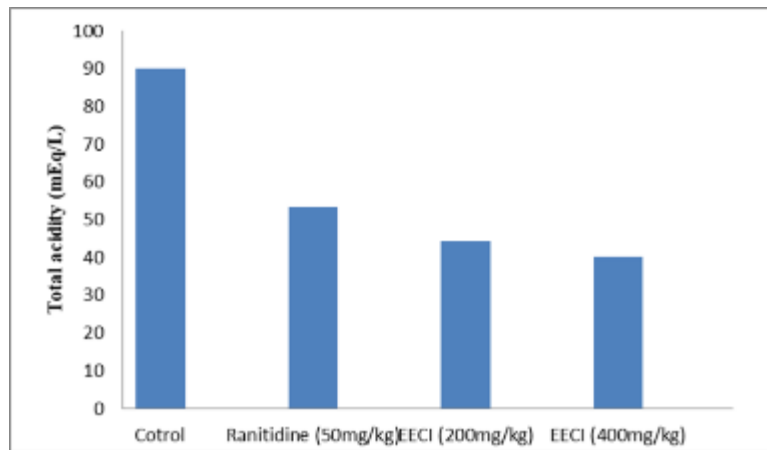


Figure 3 Antiulcer effect of EECl against Pylorus ligation induced ulcers in Male Wistar rats

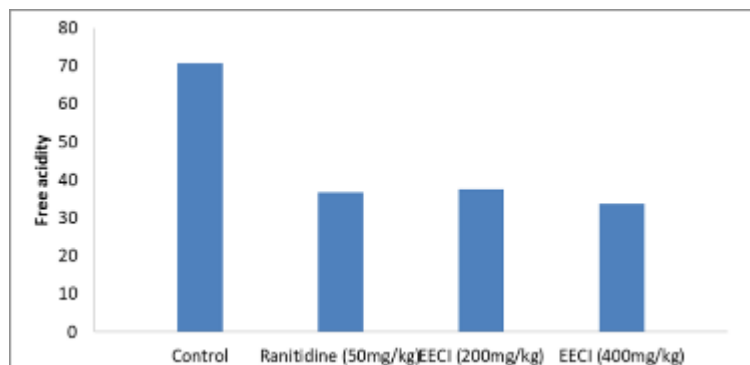


Figure 4 Antiulcer Effect of EECl against Pylorus ligation induced ulcers in Male Wistar rats

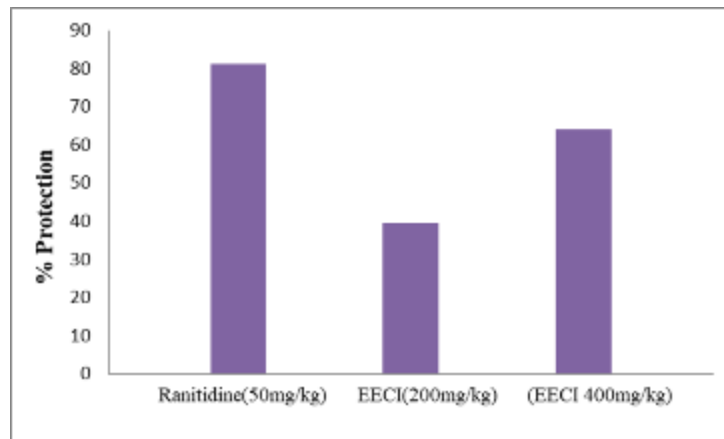


Figure 5 Antiulcer effect % inhibition of EECl in pylorus ligation induced peptic ulcers in Male Wistar rats

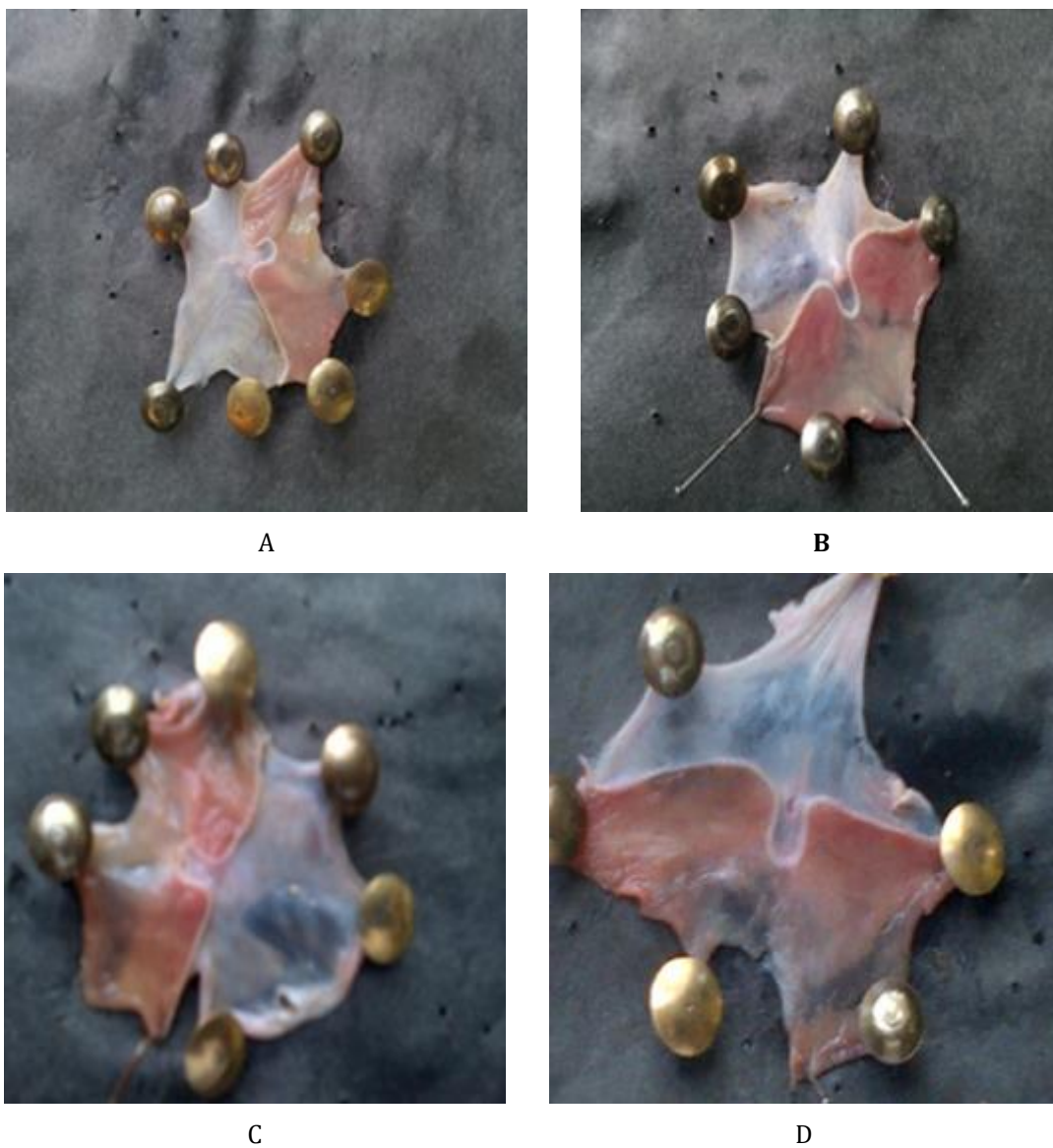


Figure 6 Photograph Showing Pylorus ligation induced model A) Control, B) Standard, C) EECl 200 mg/kg, D) EECl 400 mg/kg

4. Discussion

In present study, the antiulcer activity of *Coccinia Indica roots* extract in model including antiulcer activity by pylorus ligation where ulcer is either due to the effect of acid secretion (Sayed Ahmed Husain *et al.*, 2011). The ethanolic extract (200 mg/kg and 400 mg/kg) has less effective against the various parameters like ulcer index, pH, total and free acidity in animal models when compared with control and standard (Ranitidine 50 mg/kg). While ethanolic extract with a high dose i.e. 400 mg/kg shows significant inhibition of the all above parameters in both the animals' models. The results of the present study suggest that the ethanolic extract of *Coccinia Indica roots* may be beneficial in the treatment of peptic ulcer. Further studies to identify the active moieties and elucidation of the mechanism of action are recommended.

5. Conclusion

The plant *Coccinia Indica* is a perennial or some time annual plant belonging to family *Cucurbitaceae*. The present study reports the antiulcer activity of ethanolic roots extract of *Coccinia Indica* in model pylorus ligation model.

Compliance with ethical standards

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Disclosure of conflict of interest

The manuscript has no conflict of interest.

Statement of ethical approval

The animals were obtained from animal's house facility of Spectrum Hi Pharmacy College, Sultanpur (Uttar Pradesh). All animal experiments were carried out in accordance with the guidelines of CPCSEA.

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