

EVALUATION OF ANTI-ULCER ACTIVITY OF *CITRULLUS COLOCYNTHIS* FRUIT AGAINST PYLORUS LIGATION INDUCED ULCERS IN MALE WISTAR RATS

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ABSTRACT

The present study was designed to investigate and compare the antiulcer potential of aqueous and ethanolic extracts of *Citrullus colocynthis*. Preliminary aqueous and ethanol extracts of *Citrullus colocynthis* was subjected to the acute oral toxicity study according to the OECD guideline no. 423. Based on which, dose level i.e. 200 mg/kg and 400 mg/kg were selected for the further study. Antiulcer activity was evaluated by pylorus ligation ulcer model. Effect of concurrent administration of aqueous and ethanolic extracts of fruits *Citrullus colocynthis* at doses of 200 mg/kg and 400 mg/kg was given by oral route. In pylorus ligation ulcer model, various parameters were studied viz. gastric volume, pH, total acidity, free acidity, ulcer index and percentage inhibition of ulceration was determined. Ranitidine at 50 mg/kg was used as the standard drug. Pretreatment of aqueous and ethanolic extracts 400 mg/kg of *Citrullus colocynthis* fruits showed significant ($P < 0.001$) decrease in the gastric volume, total acidity and free acidity. However, pH of the gastric juice was significantly ($P < 0.001$) increased at the dose, 400 mg/kg. It showed also significant ($P < 0.001$) decrease in number of ulcers and ulcer score index in pylorus ligation ulceration model. In conclusion the antiulcer properties of the extracts may be attributed to the presence of phytochemicals like flavanoids, saponins, alkaloids and tannins present in the plant extract with various biological activities.

Keywords: *Citrullus colocynthis*, Pylorus ligation, flavanoids, Ulcer index, Aqueous extract, Ethanolic extract.

INTRODUCTION

Peptic ulcer disease (PUD) is one of the most common gastrointestinal disorders, which causes a high rate of morbidity particularly in the population of non-industrialized countries¹. Peptic ulcer occurs due to an imbalance between the aggressive and the defensive factors². Although a number of antiulcer drugs such as H₂ receptor antagonists, proton pump inhibitors and cytoprotectants are available for ulceration, all these drugs have side effects and limitations. Herbal medicine deals with plants and plant extracts in treating diseases. These medicines are considered safer because of the natural ingredients with no side effects³.

Citrullus colocynthis (L.) Schard belongs to the family Cucurbitaceae and native of Asia, and Africa found in Syria, and Egypt. It was cultivated in Spain and occurs throughout the India, particularly in Tamil Nadu, Gujarat, and Punjab. It was an annual plant resembling the common water melon^{4,5}. *Citrullus colocynthis* was a small scarbid perennial creeping herb with prostrate or climbing stem, bearing smooth spherical fruits which are mottled green when young and some what yellow when ripe⁶. This plant was a well recognized in the traditional medicine and was used by people in rural areas. The fruits are useful in tumors, leucoderma, ulcers, asthma, and bronchitis⁷. Root was given in cases of asthma, inflammation of the breast, ulcers, urinary diseases and rheumatism. Oil from seeds was used for poisonous bites, bowel complaints, epilepsy and also for blackening the hair^{8,9}.

The main chemical constituents of *Citrullus colocynthis* reported in the literature are docosan-1-ol acetate, 0, 13-dimethyl-pentadec-13-en-1-ol, 11, 14-dimethyl hexadecane, 14-ol 2-one, 10, 14- dimethyl hexadecane 14, ol, 2-one, linoleic acid, oleic acid, carbohydrate, amino acid, organic acid, lipid, sterols and phenols^{10, 11, 12, 13}.

MATERIALS AND METHOD

Collection of plant material

Citrullus colocynthis fruits were obtained from the local area of Kadapa district, Andhra Pradesh, India. The plant was authenticated by Dr.Madhava chetty, Taxonomist, S.V. University, Tirupathi, India. The collected plant was washed immediately and dried at room temperature for one month, powdered mechanically, sieved (10/44) and stored in air-tight containers.

Preparation of extracts

Lyophilized aqueous extracts

100 g of fresh fruits of *Citrullus colocynthis* were ground with a mixer and added to 500 ml of distilled water. The mixture was allowed to reflux for 30 min, after which the solution was allowed to cool (4 h at 4°C). The mixture was then filtered using filter paper (Whatman no.1) under the vacuum of a water pump. The filtrate obtained was lyophilized, yielding the lyophilized aqueous extract¹⁴ and labeled as AECCT and stored in desiccator.

Preparation of ethanolic extract

About 50 grams of the powder of fruits are homogenized in 100 ml of ethyl alcohol (90%) in homogenizer and operated for 5 minutes. The process was repeated for three to four times with the same quantity of alcohol. The extract was filtered through filter paper under vacuum. The alcohol extract was evaporated through rotary evaporator under reduced pressure at 40°C¹⁵ and labeled as EECCT and stored in desiccator.

Drugs and chemicals

All the drugs, chemicals, and reagents were procured from S.D. Fine Chemicals, (Mumbai, India). All the chemicals used were of analytical grade.

Phytochemical investigation

Phytochemical tests were carried out to find out the presence of phytoconstituents viz flavanoids, saponins, glycosides, carbohydrates, phenols etc and the results are shown in Table: 1

Experimental animals

Male wistar rats (200 – 250g) were used in this experiment. They were housed in standard cages by maintaining a temperature of 22 ± 2°C at 12:12 hours light dark cycle. The animals were fed with standard pellet diet and water ad libitum. Institutional Animals Ethics Committee (IAEC) approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA (NO: 1423/PO/a/11/CPCSEA), Department of Animal Welfare and Government of India.

Acute toxicity studies

Acute toxicity studies for aqueous extract and ethanolic extract of *Citrullus colocynthis* were conducted as per OECD guidelines 423 using wistar rats. Each animal was administered aqueous extract and ethanolic extract by oral route. The animals were observed for any changes continuously for the first 2 h and up to 24 h for mortality and behavioral changes (OECD guidelines).

Evaluation of antiulcer activity

Male wistar rats were divided into six groups, each consisting of six rats. Animals were fasted for 24 h before the study, but had free access to water. One group represented the control group, which received only distilled water. Second & Third Groups received aqueous extract of *Citrullus colocynthis* (AECCT) 200 mg/kg and 400 mg/kg (p. o.) respectively. Fourth & fifth Groups received ethanolic extract of *Citrullus colocynthis* (EECCT) 200 mg/kg and 400 mg/kg (p. o.) respectively. Ranitidine, in the dose of 50 mg/kg were administered orally for sixth group as reference standard drug. After 1h of drugs treatment, they were anaesthetized with the help of anesthetic ether; the abdomen was opened by a small midline incision below the xiphoid process. Pyloric portion of the stomach was slightly lifted out and ligated according to method of Shay et al.,¹⁶ avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall was closed by interrupted sutures. Rats were sacrificed by an over dose of anaesthetic ether after four hours of pyloric ligation. The abdomen was opened, cardiac end of the stomach was dissected out and the contents were drained into a glass tube. The volume of the gastric juice was measured and centrifuged at 2000 rpm for 10 min. From the supernatant, aliquots (1 ml of each) were taken for the determination of pH, total and free acidity. Each stomach was examined for lesions in the fore stomach portion and indexed according to severity.

Macroscopic evaluation of stomach

The stomachs were opened along the greater curvature, rinsed with saline to remove gastric contents and blood clots and examined by a 10X magnifier lens to assess the formation of ulcers. The numbers of ulcers were counted. Scoring of ulcer will be made as follows:

- Normal colored stomach..... (0)
 Red coloration..... (0.5)
 Spot ulcer..... (1)
 Hemorrhagic streak... (1.5)
 Deep Ulcers..... (2)
 Perforation..... (3)

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows: Ulcer index (UI) was measured by using following formula: $UI = UN + US + UP \times 10^{-1}$

Where,

UI= Ulcer Index; UN = Average number of ulcers per animal; US = Average number of severity score; UP = Percentage of animals with ulcers

Percentage inhibition of ulceration was calculated as below:

$$\% \text{ Inhibition of Ulceration} = \frac{(\text{Ulcer index}_{\text{Control}} - \text{Ulcer index}_{\text{Test}})}{\text{Ulcer index}_{\text{Control}}} \times 100$$

Determination of pH

An aliquot of 1ml gastric juice was diluted with 1ml of distilled water and pH of the solution was measured using pH meter.

Determination of total acidity

An aliquot of 1ml gastric juice diluted with 1ml of distilled water was taken into a 50 ml conical flask and two drops of phenolphthalein indicator was added to it and titrated with 0.01N NaOH until a permanent pink colour was observed. The volume of 0.01N NaOH consumed was noted. The total acidity is expressed as mEq/L by the following formula:

$$\text{Acidity} = \frac{\text{Vol. of NaOH} \times N \times 100 \text{ mEq/L}}{0.1}$$

Determination of free acidity

Instead of phenolphthalein indicator, the Topfer's reagent was used. Aliquot of gastric juice was titrated with 0.01N NaOH until canary yellow colour was observed. The volume of 0.01N NaOH consumed was noted. The free acidity was calculated by the same formula for the determination of total acidity.

Statistical Analysis

The results are expressed as the mean \pm SD for each group. Statistical differences were evaluated using a One-way analysis of variance (ANOVA) followed by Dunnett's t-test. Results were considered to be statistically significant at $P < 0.001$.

RESULTS

Acute oral toxicity study

Acute oral toxicity was carried out by up-down regulation method. It is found that aqueous and ethanolic extract of *Citrullus colocynthis* were safe at limit dose 4000 mg/kg with no mortality in studied subjects. 1/10th of this dose i.e. 400 mg/kg were used in the subsequent study respectively.

Preliminary phytochemical screening

Preliminary Phytochemical results of *Citrullus colocynthis* are shown in table- 1, in the present study, both the aqueous and ethanolic extract of *Citrullus colocynthis* found to contain phytochemicals namely, flavanoids, saponins, alkaloids, glycosides and tannins¹⁷.

Table 1: Qualitative phytochemical analysis of both aqueous extract and ethanolic extract of *Citrullus colocynthis*

Tests	Aqueous extract of <i>Citrullus colocynthis</i> Linn.	Ethanolic extract of <i>Citrullus colocynthis</i> Linn.
Alkaloids	+	+
Flavanoids	+	+
Terpenoids	+	+
Proteins	+	+
Saponins	+	+
Steroids	+	+
Essential oils	+	+
Tannins	+	+
Glycosides	+	+
Carbohydrates	+	+

Symbol '+' = Presence and '-' = Absence

Antiulcer activity

At the end of the study, the stomach was isolated and washed with saline, it was than observed for ulceration and ulcers were scored, Ulcer index and percentage protection against ulcers was calculated.

Pylorus ligation induced gastric ulceration

Pylorus ligation showed superficial, deep ulcers and perforations in the control animals (fig 1). However, animals treated with AECCT at 200 mg/kg and 400mg/kg, EECCT at 200 mg/kg and 400mg/kg

doses showed significant reduction in the number of ulcer and ulcer index (Table 2), showed 27.98, 57.29 and 34.62, 60.76% ulceration inhibition respectively whereas ranitidine showed 73.45%

ulceration inhibition. Anti-ulcerogenic effect of *Citrullus colocynthis* in Pylorus ligation induced ulcers was comparable to that of ranitidine, 50 mg/kg.

Table 2: Results of antiulcer activity of both aqueous and ethanolic extracts of *Citrullus colocynthis* against pylorus ligation method

Group	Treatment	Ulcer index	% Protection	P ^H of gastric juice	Vol. of Gastric juice (ml)	Free acidity (meq/ltr)	Total acidity (meq/ltr)
I	Control (1ml/kg)	17.33±0.03	---	2.9±0.10	8.6±0.28	6.56±0.07	7.77±0.25
II	AECCT (200 mg/kg)	12.48±0.24	27.98**	3.3±0.02**	6.9±0.01**	6.1±0.12**	7.28±0.45**
III	AECCT (400 mg/kg)	7.4±0.12	57.29***	4.5±0.25***	5.8±0.25***	4.82±0.06***	6.8±0.12***
IV	EECCT (200 mg/kg)	11.33±0.71	34.62**	3.8±0.05**	6.3±0.06**	5.65±0.19**	7.15±0.08**
V	EECCT (400 mg/kg)	6.8±0.18	60.76***	4.9±0.17***	5.2±0.18***	4.24±0.29***	6.5±0.05***
VI	Standard (50mg/kg)	4.6±0.57	73.45***	5.6±0.09***	4.5±0.29***	3.43±0.08***	5.4±0.14***

Results are expressed as the mean ± SEM; n=6 in each group, statistical comparisons as follows * Significance at P < 0.05, ** significance at P < 0.01, *** significance at P < 0.001 for Dennett's test vs. control AECCT = Aqueous extract of CCT; EECCT = Ethanolic extract of CCT

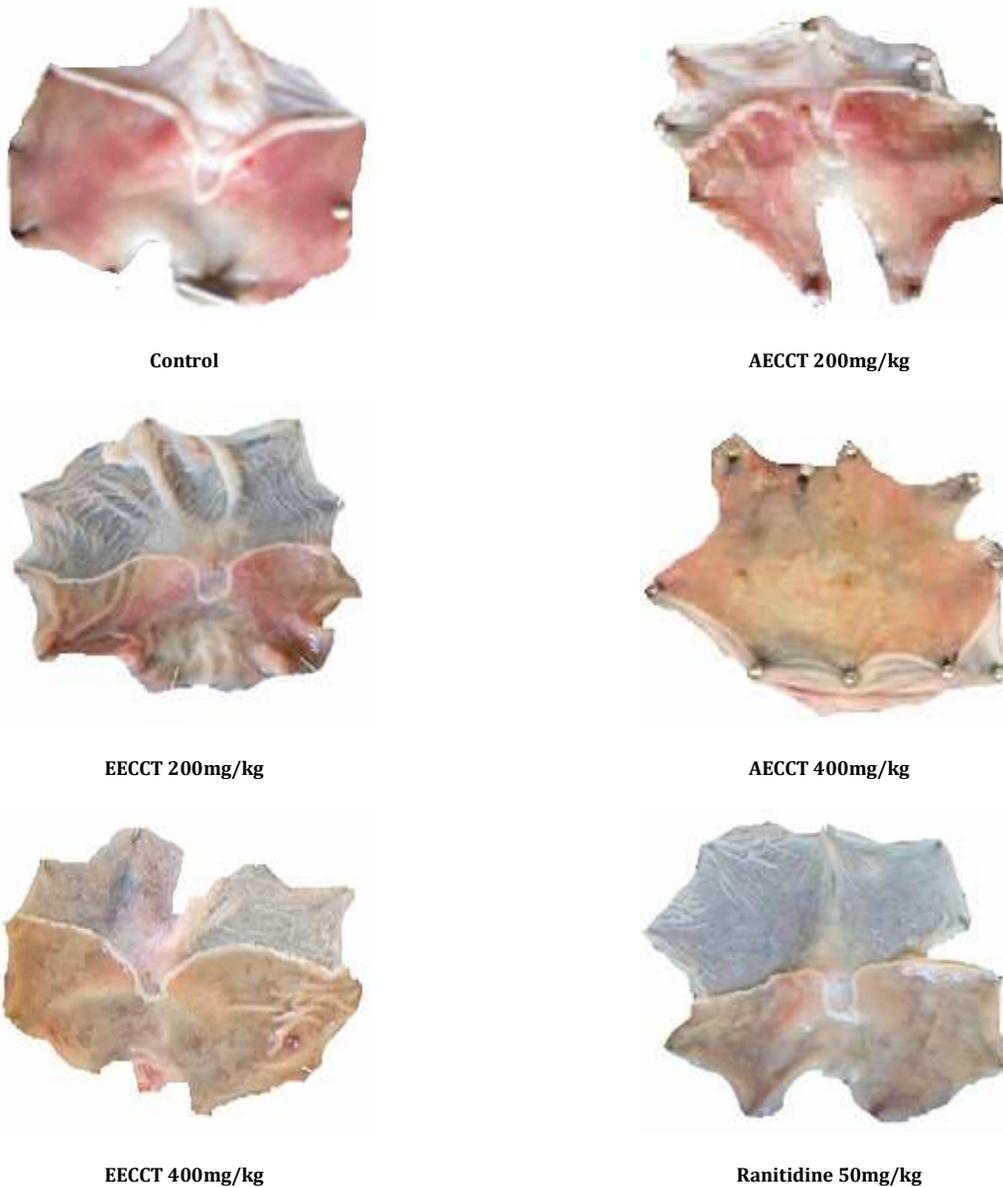


Fig 1: Effect of AECCT and EECCT on pylorus ligation induced ulcer model in male wistar rats

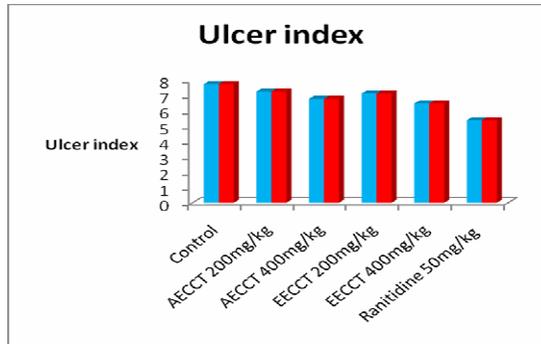


Fig. 2: Antiulcer effect of AECCT and EECCT on ulcer index in pylorus ligation induced ulcer model in rats.

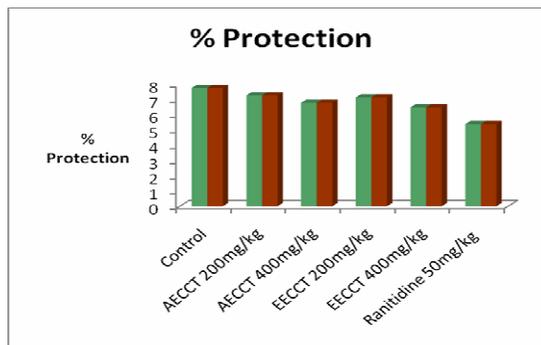


Fig. 3: Antiulcer effect (% inhibition) of AECCT and EECCT in pylorus ligation induced ulcer model in male wistar rats.

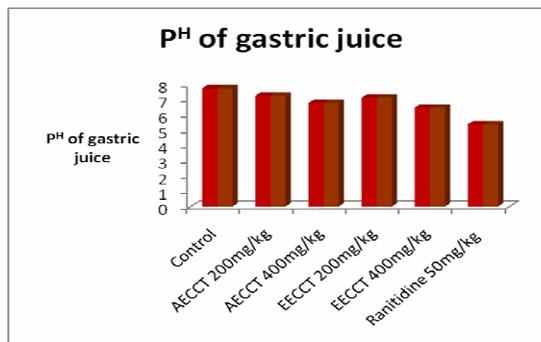


Fig. 4: Antiulcer effect of AECCT and EECCT on PH of gastric juice in pylorus ligation induced ulcer model in male wistar rats.

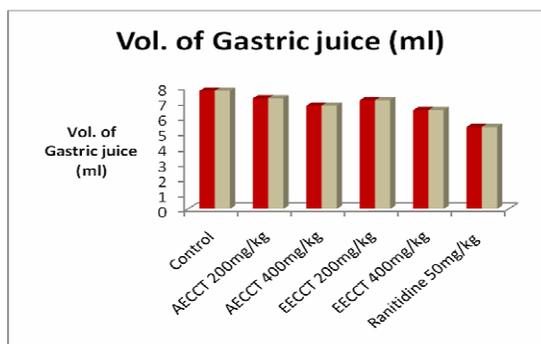


Fig. 5: Antiulcer effect of AECCT and EECCT on volume of gastric juice in pylorus ligation induced ulcer model in male wistar rats.

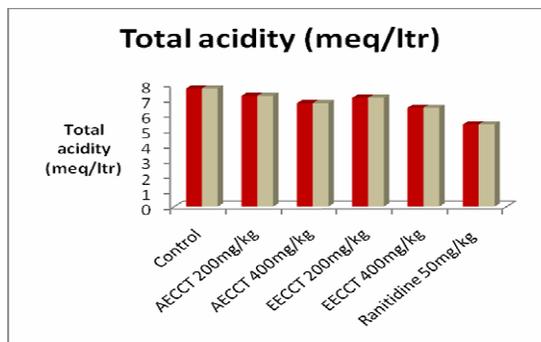


Fig. 6: Antiulcer effect of AECCT and EECCT on total acidity in pylorus ligation induced ulcer model in male wistar rats.

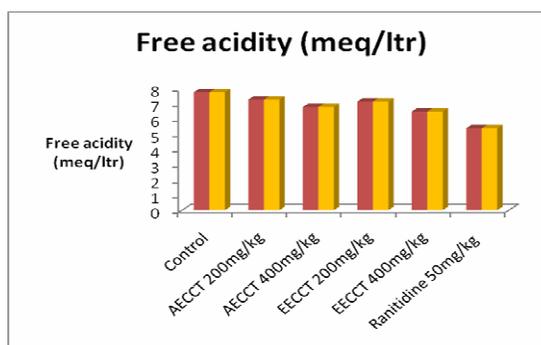


Fig. 7: Antiulcer effect of AECCT and EECCT on free acidity in pylorus ligation induced ulcer model in male wistar rats.

DISCUSSION

The fruit extracts (aqueous and alcoholic extracts) of *Citrullus colocynthis* were subjected for phytochemical investigation and LD₅₀ studies. It was found that both the aqueous and ethanolic extract contained flavanoids, saponins, sterols, and alkaloids. Both the extracts were tested for their lethal effect up to the dose level of 4000 mg/kg (up and down method). None of them have produced abnormal behavior or mortality in rats.

Similarly both the extracts were evaluated for their anti-ulcer activity in Pylorus ligation induced ulcer model in male wistar rats. Both the extracts produced a significant ($p < 0.001$) anti-ulcer activity but similar to the above experiment a relatively better anti-ulcer activity was recorded with ethanolic extract. The significant increase in the antiulcer activity of *Citrullus colocynthis* could be attributed to the presence of flavanoids, alkaloids, tannins, saponins glycosides and phenolic compounds. Flavanoids are among the cytoprotective materials for which antiulcerogenic efficacy has been extensively confirmed. It is suggested that, these active compounds would be able to stimulate mucus, bicarbonate and the prostaglandin secretion and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen¹⁸. So the antiulcer activity of *Citrullus colocynthis* may be attributed to its flavanoids content.

CONCLUSION

Fruit extracts of *Citrullus colocynthis* (CCT) exhibited a significant anti-ulcer activity in experimental male wistar rats. EECCT exhibited relatively better anti-ulcer activity than AECCT. The difference in the evaluated activity could be due to the number /quantity of phytoconstituents present in these extracts.

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