

STUDY OF THE ANTI-ULCEROGENIC ACTIVITY OF THE ETHANOLIC EXTRACTS OF RHIZOME OF *Curcuma caesia* (EECC) AGAINST GASTIC ULCERS IN EXPERIMENTAL ANIMALS

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ABSTRACT

The aim of the study is to study the anti-ulcer activity of the ethanolic extract of the rhizome of *Curcuma caesia* on experimental animal models. Four groups of albino rats weighing 150-200 grams were taken for the study (n=5). Group A : control (3% gum acacia 5ml/kg/day orally for 7 days). Group B : Experimental control (Aspirin 400mg/kg orally as single dose on 7th day). Group C : Test (Curcuma caesia extract 500mg/kg/day orally for 7 days plus Aspirin 400mg/kg orally on 7th day) and Group D: Standard (Ranitidine 150mg/kg orally for 7 days and Aspirin 400mg/kg orally on 7th day). The stomachs of the sacrificed rats were removed and (1) volume of gastric juice (2) ulcer index (3) pepsin activity (4) free acidity (5) total acidity (6) gastric mucus secretion were studied. The ulcer index, pepsin activity, free and total acidity and volume of gastric juice in group III and IV showed significant decrease in comparison to group II whereas there was increase in gastric mucus secretion ($p < .01$).

Keywords: aspirin, gastric ulcer, ranitidine**INTRODUCTION**

An ulcer is defined as disruption of the mucosal integrity of the stomach and/or duodenum leading to a local defect or excavation due to active inflammation. Ulcers occur within the stomach and/or duodenum and are often chronic in nature¹. The etiology of peptic ulcer is not clearly known. It results probably imbalance due to an imbalance between the aggressive and defensive factors. Reactive oxygen metabolites, free radicals, nitric oxide and genetic and environmental factors are also thought to play a role in the pathogenesis of ulcer².

In the last few years efforts have been taken to identify new antiulcer drugs from natural sources. Plants are the source of certain known anti-ulcer drugs³.

Black Turmeric (*Curcuma caesia*) is a perennial herb with bluishblack rhizome, native to North-East and Central India. It is also sparsely found in Papi Hills of East Godavari, the root hills of the Himalayas and North Hill forest of Sikkim. The rhizomes of Black Turmeric have a high economical importance owing to its putative medicinal properties. The rhizomes are used in the treatment of hemorrhoids, leprosy, asthma, cancer, epilepsy, fever, wound, vomiting menstrual disorder, smooth muscle relaxant activity, anthelmintic, aphrodisiac, inflammation, gonorrhoeal discharges, etc. Preliminary phytochemical screening of crude methanol extract of *Curcuma caesia* demonstrated strong positive test for flavonoids and tannin, additionally, alkaloids and saponins were also present⁴.

Literature reviews indicated that the ulcer protective activity of this plant has not been evaluated so far. Thus, the aim of the study was to evaluate the protective role of *C. caesia* on ulceration induced by aspirin in albino rats.

MATERIALS AND METHODS**Collection, identification and extraction of plant materials**

Fresh rhizomes of *Curcuma caesia*, approximately one kilogram collected in the month of April- May, 2011 were used for the study. The plant was authenticated by Dr. M. Islam, Professor of Life Science, Dibrugarh University, Assam, India. The plant material was air dried at room temperature. The dried rhizomes were grounded to fine powder and stored in air tight container. Preparation of extract- The powder obtained was soaked in ethanol for 24 hours in percolator. After 24 hours it was allowed to percolate slowly and extract was collected in Petri dishes⁵. The extract was concentrated in vacuum using rotary flash evaporator.

Animals

The experiments were carried out in albino rats of the species *Rattus norvegicus* of either sex weighing 150-200 gms. The animals were procured from Central Animal House, Assam Medical College &

Hospital. The study was conducted in accordance with CPCSEA (Committee for the Purpose of control and supervision of Experiment on Animals) guidelines and the study was approved by the Institutional Animal Ethical Committee (Registration no.- 634/02/a/CPCSEA). Before starting the experiment the animals were acclimatized for one week under laboratory conditions. They were fed with standard diet and water *ad libitum* was provided.

Acute toxicity studies

Acute oral toxicity test for the ethanolic extract of the tubers of *Curcuma caesia* was carried out as per OECD Guidelines 425⁶. Mortality in the acute oral toxicity test was not seen in the limit test up to dose 2000mg/kg. One-fourth of the upper bound dose of the extract from the limit test was decided to be considered for the experiments⁷.

Materials required for the study

- ethanolic extract of the rhizome of *Curcuma caesia* (EECC).
- 3 % gum acacia as vehicle for all preparations
- Aspirin (obtained from BD Pharmaceutical Works)
- Ranitidine (obtained from Ranbaxy Laboratories)

Experimental design-

Four groups of albino rats of either sex of species *Rattus norvegicus* weighing 150-200gms were taken for the study taking five animals in each group.

Group A - Normal control (3% gum acacia 5ml/kg/day orally for 7 days).

Group B - Experimental control received Aspirin (400mg/kg) orally as a single dose on the 7th day.

Group C - Extract treated received ethanolic extract of the rhizome of *Curcuma caesia* (EECC) (500mg/kg/day orally for 7 days) plus Aspirin (400mg/kg orally on the 7th day) and

Group D - Standard group received Ranitidine (150mg/kg orally for 7 days) plus Aspirin (400mg/kg orally on the 7th day).

On the 7th day, the groups B, C and D were given aspirin (400mg/kg) as single dose. After 24 hrs pyloric ligation was done in all the animals and kept for 4hrs. Thereafter, the rats were sacrificed and stomachs were removed and the following biochemical estimation were done- 1) Pepsin activity 2) free acidity 3) total acidity 4) ulcer index 5) gastric mucus secretion 6) Volume of gastric juice.

Biochemical assessment

1) Pepsin activity - By the method of Debnath PK (1974)⁸ and Lowry OH (1951)⁹. One ml of diluted gastric juice mixed with 2% haemoglobin solution in 0.06M HCl and incubated for 20 mins. Then

0.6 M ice cold Trichloroacetic acid was added. Later solution centrifuged and supernatant mixed with Reagent C (alkaline copper sulphate solution), Reagent E (diluted Folin reagent) and optical density was measured at 610 nm against a blank distilled water.

2)Free acidity and Total acidity-By the method of Kulkarni SK (1999)^[10]. Briefly 2 drops of Toppers reagent was added and diluted supernatant of gastric juice in a conical flask. 0.01 N NaOH was taken in a burette and allowed to titrate till flask change to yellow colour. Then 2 drops of phenolphthalein added and titration continued till orange colour is reached.

$$\text{Acidity(mEq/L)} = \frac{\text{Volume of NaOH} \times \text{Normality} \times 100}{0.1}$$

3) Ulcer index -By the method of Goyal RK (2002)^[11]. The dissected stomachs of the sacrificed rats were opened along the greater curvature and the ulcer index calculated from the glandular portion of the stomach. The ulcer index was calculated as,

$$\text{Ulcer index} = 10/x$$

where x = Total mucosal surface/Total ulcerated area

Each lesion was measured along the greatest length. In case of petechiae, five of these were considered to be equivalent to 1mm² of ulcer area. The total area of the glandular portion of the stomach and that of ulcerated mucosa were measured for determination of the ulcer index.

4)Gastric mucus - As described by Crone SJ (1974)^[12]. Briefly excised glandular portion of stomach was soaked in 0.1% alcian blue solution buffered with 0.05M sodium acetate and HCl. Uncomplex dye adhered to tissue washed with 0.025M sucrose again soaked in MgCl₂ and resulting blue solution mixed with ether and optical density measured against 605nm.

5)Volume of Gastric Juice: By the method of Deshpande SS (2003)^[13]. Contents of the resected stomachs of the rats were taken in graduated test tubes and allowed to centrifuge at 2000 rpm for 10 mins. The supernatant fluid was measured for volume of gastric juice and expressed as ml/4 hrs. Then the juice was subjected to the biochemical tests.

Statistical analysis-For all the above methods, the results were expressed as mean ±SEM. Statistical analysis was done using one way ANOVA followed by Dunnett's multiple comparison test using the Graph pad prism software.

Results

The LD₅₀ value of the extract was found to be more than 2000mg/kg. It is evident from the Table... and Figure.. that the ethanolic extract of the plant exhibited anti-ulcer activity. The treatment of rats with ethanolic extract of Curcuma caesia(EECC-500mg/kg) produce significant reduction of ulcer index, gastric acid volume, pepsin, free and total acidity alongwith increased production of gastric mucus as it is evident from Table...The protection was statistically significant.

Table 1. Effects of extract (EECC) against aspirin induced gastric ulcer(ulcer index and volume of gastric juice) in rats.

Design of treatment	Ulcer index	Volume of Gastric juice (ml/4 hr)
Group-A	0±00	1.92±0.05
Group-B	12.29±0.39 ^a	4.86±0.13 ^a
Group-C	4.18±0.60 ^b	1.14±0.10 ^b
Group-D	1.6±0.26 ^b	0.08±0.08 ^b

N=5 in each group. a- p<.05 when compared to the normal control, b- p<.05 when compared to experimental control. One way ANOVA followed by Dunnett's multiple comparison test.

Table 2: Effects of extract (EECC) against aspirin induced gastric ulcer(free and total acidity) in rats.

Design of treatment	Free acidity (mEq/l)	Total acidity(mEq/l)
Group-A	81.40±1.77	98.40±5.38
Group-B	115.60±2.31 ^a	125.60±1.93 ^a
Group-C	46.40±2.13 ^b	66.80±1.35 ^b
Group-D	59.20±1.85 ^b	70.00±1.63 ^b

N=5 in each group. a- p<.05 when compared to the normal control, b- p<.05 when compared to experimental control. One way ANOVA followed by Dunnett's multiple comparison test.

Table 3: Effects of extract (EECC) against aspirin induced gastric ulcer(pepsin and gastric mucus) in rats.

Design of treatment	Pepsin Activity (µmol tyrosine/ml)	Gastric Mucus (mg of alcian blue/gm glandular tissue)
Group-A	41.20±1.56	13.61±0.51
Group-B	146.00±3.21 ^a	7.62±0.47 ^a
Group-C	49.60±1.77 ^b	17.45±0.71 ^b
Group-D	32.60±1.53 ^b	18.42±1.28 ^b

N=5 in each group. a- p<.05 when compared to the normal control, b- p<.05 when compared to experimental control. One way ANOVA followed by Dunnett's multiple comparison test.

*(GroupA-Normal control, Group B-Experimental control, C-Extract treated, D-Standard drug in all the tables)

Figure 1(a, b, c): Graphical presentation showing decreased production of ulcer index, gastric acid volume, free & total acidity,pepsin alongwith increased production of mucus in extract treated and standard group.(A-normal control, B-experimental control, C-extract treated, D-Standard).

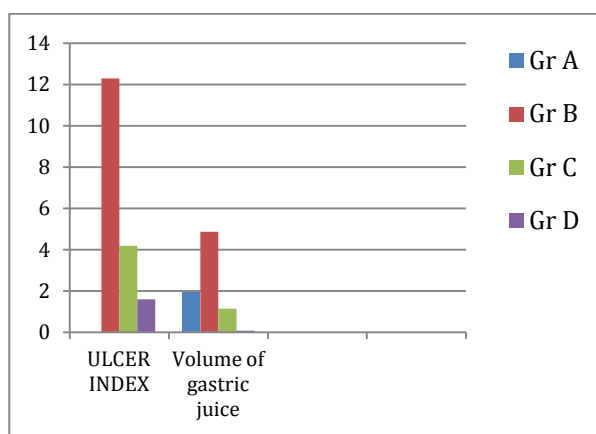


Figure 1(a)

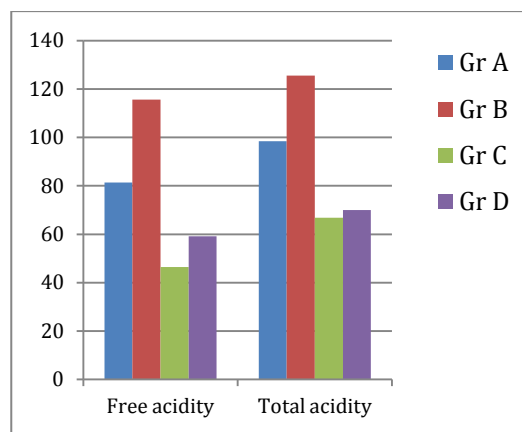


Figure 1(b)

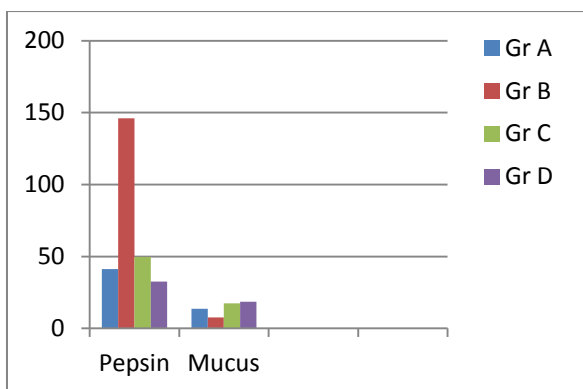


Figure 1(c)

DISCUSSION

It is evident from the results of the study that *Curcuma caesia* (EECC) possesses antiulcer activity in aspirin induced gastric ulcer model. Aspirin causes mucosal damage by interfering with Prostaglandin synthesis, increasing acid secretion and back diffusion of H^+ . In stomach, Prostaglandin play a vital role by stimulating secretion of HCO_3^- and mucus maintaining mucosal blood flow and regulating mucosal cell turnover and repair. Thus the suppression of prostaglandin synthesis by NSAID results in increased susceptibility to mucosal injury and gastro-duodenal ulceration¹⁴.

Aspirin treatment caused a significant increase in the ulcer index, pepsin activity, free and total acidity, volume of gastric juice and decreased mucus production. It has been proposed that in pyloric ligation, the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for induction of ulceration¹⁵. In our study, *Curcuma caesia* extract decreased the gastric volume and gastric acid secretion significantly by pretreatment with aspirin. Prostaglandins are known to have an antisecretory effect on gastric acid production¹⁶. Hence it is assumed that the antiulcer and acid secretion inhibitory effect of EECC may be mediated through prostaglandins.

The mucus membrane of gastrointestinal tract contains rich amount of mucin, the protein involved in mucosal protection and the role of mucin in the pathological of gastrointestinal diseases has been reported¹⁷. Prostaglandins are also known to stimulate the synthesis of mucus¹⁸. In our study the extract showed significant increase in mucus secretion. This indicates that the acid inhibiting and mucus production effect of prostaglandins may be the major mechanism by which *Curcuma caesia* extract promotes ulcer healing.

Human ulcers have been shown to be associated with excess acid secretion due to upregulation of the pepsin enzyme. Proteolytic activity of pepsin as the primary aggressor in gastric mucosal ulceration has been reported¹⁷. In our study pepsin activity was significantly reduced in extract treated rats.

The phytoconstituents like flavonoids, tannins, and saponin have been reported in several antiulcer literatures as possible gastroprotective agents. Flavonoids, tannins are among the cytoprotective active materials for which antiulcerogenic efficacy has been extensively confirmed. Tannins may prevent ulcer development due to their protein precipitating and vasoconstriction effects. Their astringent action can help precipitating microproteins on the ulcer site, thereby forming an impervious layer over the lining that hinders gut secretions and protect the underlying mucosa from toxins and other irritants. Alkaloids prevent ulcers induced by stress¹⁹. This may be also a possible mechanism.

The elevation in mucus and decreased in acidity, pepsin activity and volume of gastric fluid collected from the rats received test drug indicates that the EECC contains some active components. However

further studies are required to establish and elaborate the molecular mechanism of the antiulcer activity of *Curcuma caesia*.

CONCLUSION

The present study suggests that the ethanolic extracts of *Curcuma caesia* has significant ulceroprotective effect against gastric ulcer, which is comparable to standard drug ranitidine.

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