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Anti ulcer activity of leaves of *Averrhoa carambola* Linn

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Abstract

Objective: This study screens the aqueous and ethenolic extracts of *Averrhoa carambola* Linn for antiulcer activity in ethanol induced and pylorus ligated models in rats.
Materials and methods: The plant material of *Averrhoa carambola* Linn was extracted by using various solvents like petroleum ether, chloroform, ethanol and water by using soxhlet apparatus. The extracts were screened for antiulcer activity by different *in vivo* assay methods. Oral acute toxicity study of ethanol *extract of Averrhoa carambola* (EEAC) and aqueous *extract of Averrhoa carambola* (AEAC) was done in Wistar albino rats according to OECD guideline. The antiulcer activity of EEAC and AEAC was evaluated in ethanolic and pylorus ligated rats at the doses of 100 & 200 mg/kg body weight. Analysis of biochemical parameters and Histopathological examinations were carried our as per standard protocols.
Results: Acute oral toxicity study indicated that EEAC and AEAC were safe up to a dose of 2000 mg/kg body weight of rats. The results of antiulcer activity study revealed that the extracts of *Averrhoa carambola* Linn leaves showed significant anti-ulcer activity in dose dependent manner when compared to control. Histopathalogical observations of treated stomach tissues further confirmed the antiulcer efficacy of EEAC & AEAC.
Conclusion: Results indicated possible role of the EEAC and AEAC in the prevention and/or treatment of ulcers. The phenolic / flavonoid contents of EEAC & AEAC having antioxidant potential might be responsible for antiulcer property of *Averrhoa carambola* leaves.
Keywords: *Averrhoa carambola*, Ulcer, histopathology, anti ulcer activity; pylorus ligation; flavonoids.

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1. Introduction

The term 'stomach ulcer' is a pretty broad term that includes quite a few different types of ulcers. Mostly, ulcers are named after the location where they are found but, as with most things in life, there is an exception the peptic ulcer. For instance a peptic ulcer can be found anywhere in your esophagus, stomach or duodenum. Peptic ulcers are the areas of degeneration and necrosis of gastrointestinal mucosa exposed to acid peptic secretions. Though they can occur at any level of the alimentary tract that is exposed to hydrochloric acid and pepsin, they occur most commonly in either the duodenum or the stomach in the ratio of 4:1. Acute peptic ulcers or stress ulcers are multiple, small mucosal erosions, seen most commonly in the stomach but occasionally involving the duodenum. Chronic peptic ulcers would mean gastric and duodenum ulcers, the two major forms of ‘’peptic ulcers disease’’ of the upper GI tract in which the acid pepsin secretions are implicated in their pathogenesis. Peptic ulcers are common in the present day life of the industrialized and civilized world [1].

H. pylori infection and the use of non-steroidal anti-inflammatory drugs (NSAIDs) are the predominant causes of peptic ulcer disease. A variety of other infections and comorbidities are associated with a greater risk of peptic ulcer disease (e.g., cytomegalovirus, tuberculosis, Crohn’s disease, hepatic cirrhosis, chronic renal failure, sarcoidosis, myelo proliferative disorder). Critical illness, surgery, or hypovolemia leading to splanchnic

hypoperfusion may result in gastroduodenal erosions or ulcers (stress ulcers); these may be silent or manifest with bleeding or perforation. Smoking increases the risk of ulcer recurrence and slows healing [2].

The carambola tree is slow-growing, short-trunked with a much-branched, bushy, broad, rounded crown and reaches 20 to 30 ft (6-9 m) in height [3]. Its deciduous leaves are spirally arranged and alternate, imparipinnate, 3.8-6.3 by 2-3.2 cm long, with 5 to 11 nearly opposite leaflets, ovate or ovate-oblong; soft, medium-green, and smooth on the upper surface (plate b). Sepals 3.4 mm, petals are more than twice as long as the sepals small cluster of red stalked, lilac, purple-streaked, downy flowers, about 1/4″ (6 mm). Fruits are oblong, longitudinally 5- to 6-angled, 2 1/2 to 6″ (6.35-15 cm) long and up to 3 1/2″ (9 cm) in diameter, have thin, waxy, orange-yellow skin and juicy, crisp yellow flesh when fully ripe. The leaves of *A. carambola* L. are antipruritic, antipyretic, anthelmintic and are also useful in scabies, various types of poisoning, intermittent fevers and intestinal worms. The leaves have been eaten as a substitute for sorrel. In Brazil, the leaves are recommended to treat diabetes [4]. Treatment with hydroalcholic extracts of the leaves resulted in reduction in fasting glycemia, which was not mediated by an inhibition of hepatic gluconeogenesis and / or an increased glucose uptake by muscles. Hydroalcoholic extracts of the leaves also showed anti–ulcer activity, with a different mechanism of action for the anti–ulcerogenic activity [5]. As hydro alcoholic extract contains triterpenes, flavonoids, and mucilage, the partial anti-ulcer activity could be due to their effects.

2. Materials and methods

All chemicals and reagents used in the study were of analytical grade and were procured from Rankem, Mumbai and Himedia Laboratories Ltd., Mumbai. Commercial reagent kits used for determination of biochemical parameters and enzymatic assays were purchased from SPAN Diagnostics Ltd., Surat (India).

2.1. Plant material

The leaves of *Averrhoa carambola* Linn were collected from the Padmapur village of Dharmanagar of North Tripura district in the month of January and then the leaves are dried in a proper manner under shadow around 20-25 days at room temperature. The leaves were subjected to size reduction to a coarse powder with the help of mixer grinder. The plant is authenticated by Dr. B.K.DATTA, Professor of Botany, Plant Taxonomy and Biodiversity Laboratory, Department of Botany, Suryamaninagar, 799022, Tripura, India.

2.2. Preparation of different extracts

2.2.1. Extraction with petroleum ether

At first the finely ground leaves are placed in a ‘thimble’ made by a strong filter paper in a chamber of soxhlet (2000 ml). The powders are extracted at 55℃ using round bottomed flask for 72 hrs. The extracting solvent in round bottomed flask is heated and its vapour condenses in condenser. The condensed extractant drips into the thimble containing the crude drug and extract it by contact. After completion of extraction petroleum ether is filtered and concentrated to dry mass. The extract is air dried to remove all traces of the solvent and the percentage yield was calculated. Petroleum ether extraction was done to defat the powder [6].

2.2.2. Extraction with chloroform

The marc left after petroleum ether extraction, is dried and subsequently extracted with 1200ml of chloroform (61℃) in a soxhlet using round bottomed flask for 72 hrs. Then the extract is concentrated by using rotary evaporator and dried to get a yellowish green colour residue. By using chloroform extraction terpenoid lactones are obtained [7].

2.2.3. Extraction with ethanol

The marc left is again packed in the soxhlet. The solvent is heated using isomentle and began to evaporate. For ethanol extraction the temperature used is 78℃. The extraction had for 18-20 hrs and after completion of extraction solution was evaporated to dryness under reduced pressure and controlled temperature by using rotary evaporator [8].

2.2.4. Extraction with distilled water

The marc left after ethanol extraction is placed in a stoppered container with the distilled water (1176ml) and chloroforms (24ml) and allowed to stand at room temperature for a period of 7 days with frequent agitation until the soluble matter has dissolved. Then the mixture is strained, the marc is pressed and the combined liquids are clarified by filtration. At last the s olution is dried using rotary evaporator [9].

2.2.5. Experimental animals and housing conditions

Healthy Wister albino rats (150-200g) of either sex were maintained under standard environmental conditions (temperature 25 ± 2°C, relative humidity 50 ± 5%) with a 12 h light /dark cycle. They were fed on with normal laboratory chow pellet diet and drinking water was given *ad libitum*. Animals were allowed to acclimatize for 7 days before commencement of the experiment.

2.2.6. Phytochemical screening

Preliminary phytochemical screening was carried out for detection of the presence of various phytoconstitutents such as alkaloids, glycosides, flavonoids, phenolic compounds, tannins, saponins, proteins, amino acids and steroids [10-15].

2.3. Acute oral toxicity study

The acute toxicity of *Averrhoa carambola* Linn was determined in albino mice of either sex weighing from 20 - 25g according to the Organization for Economic Co-operation and Development (OECD) guidelines for Testing of Chemicals number 420 (OECD, 2001). The study was initiated with a sighting study aimed to determine the dose for the acute toxicity study. The sighting study comprised of albino mice dosed in a stepwise procedure using the fixed doses of 5, 50, 300 and 2000 mg/kg. The test substance is administered in a single dose. Animals should be fasted prior to dosing for 3-4 hrs. Following the period of fasting, the animals should be weighed and the test substance administered. After the substance has been administered, food may be withheld for a further 1-2 hrs [16-17].

2.4. Anti ulcer activity

2.4.1. Ethanol induced ulcer model

Animals are fasted for 24 h before the study, but had free access to water. Different groups had given different treatment as discussed earlier. After 1 hr of distilled water, lansoprazole and different extract treatment, each rat received 99.80% ethanol 1ml/200g. After 1 hour of ethanol treatment animals are sacrificed by cervical dislocation and stomach are excised along with greater curvature. Then washed with 0.9% saline, stomach is then observed for ulceration and ulcers are scored. Ulcer index and percentage protection against ulcers was calculated [18]. Mean ulcer score is expressed as ulcer index. Ulcer index are noted according to the scores (0: No visible ulcers, 0.5: Red coloration, 1: Spot ulcers, 1.5: Hemorrhagic streaks, 2: Ulcer > 3 mm but < 5 mm, 3: Ulcers > 5 mm)

Ulcer inhibition (%) = (Ulcer index Control -Ulcer index Test)/ Ulcer index Control × 100

2.4.2. Pylorus ligation method

Animals are fasted for 24 hrs before the study, but had free access to water. Different groups had given different treatment as discussed earlier. After 30min of distilled water, Lansoprazole and extract treatment, pylorus ligation had done. Under light anaesthesia the abdomen is opened by a small midline incision below the process, pyloric portion of the stomach is slightly lifted out and ligated avoiding traction to the pylorus or damage to its blood supply. The stomach is replaced carefully and the abdominal wall is closed by interrupted sutures. The animals are deprived of both food and water during the post operative period and are sacrificed at the end of 4hrs of pyloric. The abdomen is opened and a ligature is placed around the esophagus close to the diaphragm. The stomach is removed and the contents are drained in a centrifuge tube. Then the contents are centrifuges at 1000 rpm for 10min. The stomachs are washed under running tap water and then focused under magnifying lens to note the ulcers [19-20]. Mean ulcer score is expressed as ulcer index. Ulcer index are noted according to the scores (0: No visible ulcers, 0.5: Red coloration, 1: Spot ulcers, 1.5: Hemorrhagic streaks, 2: Ulcer > 3 mm but < 5 mm, 3: Ulcers > 5 mm).

Ulcer inhibition (%) = (Ulcer index Control -Ulcer index Test)/ Ulcer index Control × 100

2.4.3. Determination of pH

An aliquot of 1ml gastric juice was taken in a test tube and pH of the solution was measured using pH meter.

2.4.4. Determination of free acidity and total acidity

1 ml of gastric juice was pipette into a 100 ml conical flask, 2 or 3 drops of Topfer's reagent was added and titrated with 0.01N Sodium hydroxide until all traces of red colour disappears and the colour of the solution turns to yellowish orange. The volume of alkali added was noted. This volume corresponds to free acidity. Then 2 or 3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge appears. Again the total volume of alkali added was noted now this volume corresponds to total acidity.

2.5. Statistical analysis

Values are expressed as mean ± SEM; n = 6; *P <0.05, **P<0.01 and ***P<0.001 when compared to control. Statistical analysis was performed using the IBM SPSS 19.0 statistical software package, for Windows. Statistical differences at 5% level of probability ($p < 0.05$) between the groups were analyzed by one-way ANOVA followed by Student's *t*-test. Values are expressed as mean ± SEM; n = 6; *P <0.05, **P<0.01 and ***P<0.001 when compared to control.

3. Results

3.1. Phytochemical screening

The percentage yield of crude dried extract was found to be 23.6%, w/w per dry weight of powdered leaves. The results of preliminary phytochemical screening revealed the presence of Tannins, Flavonoids, Phenols, Terpenoids, Sterols, Fats, Fixed oils etc.

3.2. Ethanol induced ulcer model

The extracts of *Averrhoa carambola* Linn leaves showed significant anti-ulcer activity in dose dependent

manner when compared to control which is evident by decrease in the mean ulcer index in different groups.

The mean ulcer index of ethanolic extract group at a dose of 100mg/kg & 200mg/kg is found to be 2.333 ± 0.441 & 1.75 ± 0.461 respectively. The mean ulcer index of aqueous extract group at a dose of 100mg/kg & 200mg/kg is found to be 2.167 ± 0.307 & 1.33 ± 0.307 respectively. For standard (atropine sulphate) it is found to be 0.75 ± 0.171. Here it is found that the aqueous extract is more potent than ethanol extract. The result complied in table no 1 and histopathology showed in figure 1.

3.3. Pylorus ligation model

The extracts of *Averrhoa carambola* Linn leaves showed significant anti-ulcer activity in dose dependent manner when compared to control. The mean ulcer index of ethanolic extract group at a dose of 100mg/kg & 200mg/kg is found to be 2.417 ± 0.473 & 1.917 ± 0.352 respectively. The mean ulcer index of aqueous extract group at a dose of 100mg/kg & 200mg/kg is found to be 2.08 ± 0.49 & 1.5 ± 0.258 respectively. For standard (atropine sulphate) it is found to be 0.833 ± 0.247. The result showed in in table no 2 and figure 2.

Table 1: Effect of ethanolic and aqueous extract of dried leaves of *Averrhoa carambola* L. on ethanol induced ulcer on rats

S. No	Treatment	Dose	Avg. mean ulcer index	% ulcer protection
1	Control	Distilled water	3.833±0.358	0%
2	Standard	Lansoprazole (8mg/kg) P.O.	0.75±0.171***	80.43%
3	EEAC1	100 mg/kg	2.333±0.441	39.13%
4	EEAC2	200 mg/kg	1.75±0.461**	54.34%
5	AEAC1	100 mg/kg	2.167±0.307*	43.47%
6	AEAC2	200 mg/kg	1.33±0.307***	65.22%

Values are expressed as mean ± SEM; n = 6; *P <0.05, **P<0.01 and ***P<0.001 when compared to control.

Table 2: Effect of ethanolic and aqueous extract of *Avorrhoa carambola* L. leaves on pylorus ligated rat

Groups	Dose (p.o.)	Gastric content (ml)	pH	Free acidity (meq/l/ 100g)	Total acidity (meq/l/ 100g)	Avg. mean ulcer index.	% ulcer protection
Control	Distilled water	6.933±0.299	1.662±0.072	33.303±2.475	77.115±1.657	3.917±0.352	0%
Standard	Lansoprazole (8mg/kg)	3.383±0.491**	3.448±0.348***	16.167±1.475**	35.728±2.287***	0.833±0.247***	78.73%
EEAC1	100 mg/kg	5.467±0.699	2.08±0.115	28.468±3.067	70.487±3.243	2.417±0.473	38.29%
EEAC2	200 mg/kg	4.333±0.612*	2.818±0.279*	20.693±3.165*	49.632±4.483***	1.917±0.352**	51.06%
AEAC1	100 mg/kg	5.167±0.605	2.113±0.309	26.998±2.782	64.505±4.989	2.08±0.49*	46.89%
AEAC2	200 mg/kg	3.95±0.769*	3.108±0.342**	19.723±3.115*	42.68±4.895***	1.5±0.258**	61.71%

Values are expressed as mean ± SEM; n = 6; *P <0.05, **P<0.01 and ***P<0.001 when compared to control.

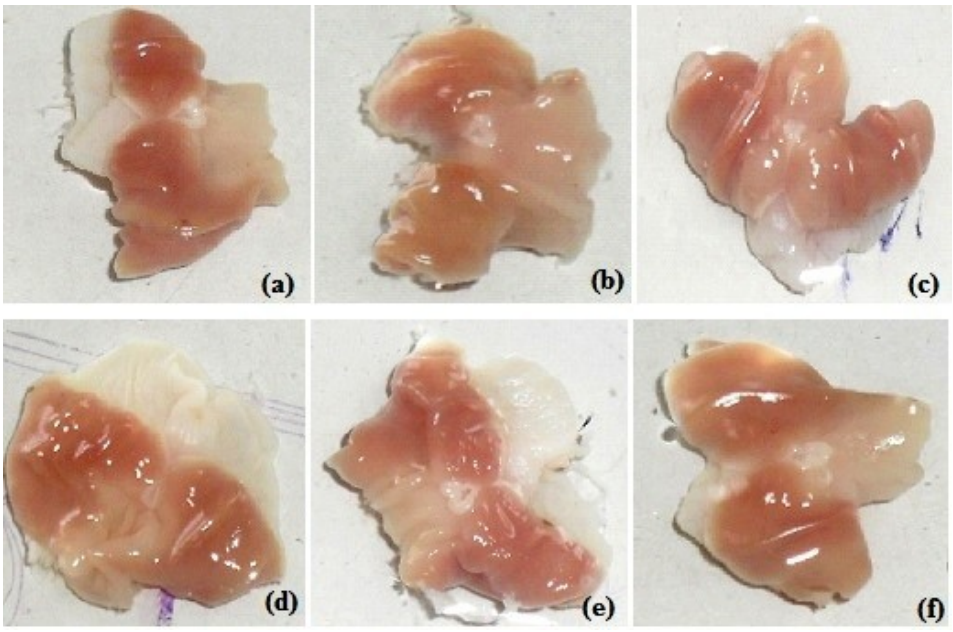

Figure 1: **Histopathology of isolated stomach of ethanol induced ulcer in rats, a) Distilled water b) Lansoprazole c) EEAC1, d) EEAC2, e) AEAC1, f) AEAC1**

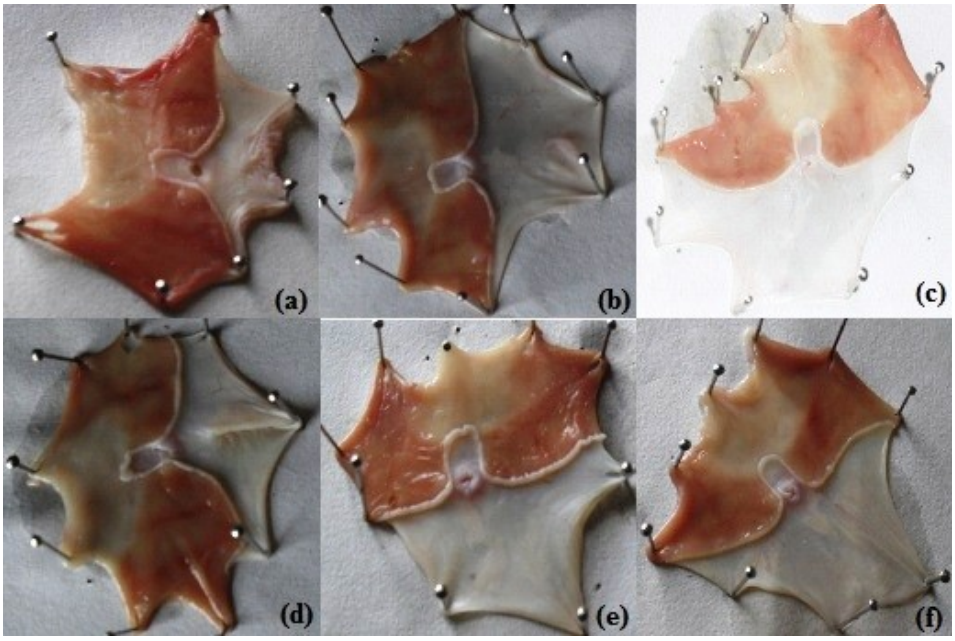

Figure 2: Histopathology of isolated stomach of pylorus ligated ulcer in rats, a) Distilled water b) Lansoprazole c) EEAC1, d) EEAC2, e) AEAC1, f) AEAC1

4. Discussion

Ulcers are caused due to an imbalance between mucosal integrity (defensive) and aggressive factors. For maintenance of the mucosal integrity different therapeutic agents including plant extracts are used to inhibit gastric acid secretion or to stimulate the mucosal defense mechanism by increasing the mucosal production of the surface epithelial cells or by interfering with mediator's synthesis [15-17].

From phytochemical studies it is found that the plants with antiulcer properties contain triterpenes, flavonoids, and mucilage [18]. As the phytochemical study of the ethanolic and aqueous extract of *Averrhoa carambola* Linn leaves contains these constituents, these may have partial anti-ulcer activity due their effects. The present study is carried out to evaluate the anti-ulcer activity of ethanolic and aqueous extract of *Averrhoa carambola* Linn leaves against different animal models of ulcers like ethanol induced ulcer model, Pylorus ligation method.

In the present study ethanol is used to induce gastric ulceration where ethanol produces necrotic lesions by direct necrotizing action which in turn reduces defensive factors like the secretion of bicarbonate and production of mucus. In this study ethanolic and aqueous extract of *Averrhoa carambola* Linn leaves prevented acute gastric mucosa injury induced by ethanol. The protective action was more for higher dose of the extract. As the plant shows anti-oxidant activity, the anti-ulcer property may be due to its anti-oxidant property [19].

It has been proposed that in pyloric ligation ulcers are developed due to accumulation of gastric acid and pepsin, which leads to autodigestion of gastric mucosa. The Anti-ulcer property of *Averrhoa carambola* in pylorus ligation model is evident from its significant reduction in free acidity, total acidity and ulcer index. Because ethanolic and aqueous extract of *Averrhoa carambola* treated animals significantly inhibited the formation of ulcers in the pylorus ligated rats and also decreased volume of gastric contents and increased the pH, it is suggested that *Averrhoa carambola* can suppress gastric damage induced by aggressive factors. Further studies are requiring to find out the active constituents responsible for the pharmacological activity.

5. Conclusion

The preliminary phytochemical studies show that the leaves of *Averrhoa carambola* Linn contain tannins, flavonoids, terpenoids, phenols, sterols, alkaloids, glycosides, carbohydrates etc in different solvent extract. Ethanolic and aqueous extract showed the presence of tannins, flavonoids, terpenoids, alkaloids, glycosides, carbohydrates. In the biological system oxidation plays an important role in energy generation. The oxidation damages vital organs in our body like stomach, kidney and liver etc. From the review of literature it is found that the ethanolic and aqueous extract of the selected plant part having anti-oxidant, antimicrobial activity which may be due to the presence of above chemicals. As these having antioxidant activity these may have organoprotective activity like anti-ulcer, hepatoprotective etc. The experiment showed that the ethanolic and aqueous extract of dried leaves of Averrhoa *carambola* Linn may also have anti-ulcer, activity.

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Conflict of interest: The authors declare that there are no conflicts of interest.

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