Original Article

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Fruits of *Averrhoa carambola* inn protects Wistar rats against indomethacin and pylorus ligation-induced ulcers

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ABSTRACT

Background: Peptic ulcer disease is one among the major cause of hospitalization in the gastroenterology department. **Objective:** The study was conducted to evaluate the gastroprotective and anti-ulcerogenic activity of *Averrhoa carambola* L. aqueous extract and its ghritha formulation on rat models. **Materials and Methods:** The antiulcer activity of *A. carambola* L. fruit extract was assessed by inducing ulcer using indomethacin and using pylorus ligation-induced ulcer models. The drug activity was compared to standard drugs omeprazole 30 mg/kg and sucralfate at a dose of 100 mg/kg. The parameters such as gastric pH, gastric acid volume, total acidity, free acidity, and ulcer index was assessed. **Results:** The aqueous fruit extract of *A. carambola* L. showed potent ulcer protective activity up to 71.36% and 76.48% in indomethacin and pylorus ligation, respectively. Similarly, ghritha formulation also showed the ulcer protective activity up to 74.78% and 80.22% in both models at a dose of 1160 mg/kg. There was a reduction in the gastric volume, total acidity, free acidity, and ulcer index and a rise in the gastric pH of test animals. **Conclusion:** A significant gastroprotective and anti-ulcerogenic activity are shown by the fruit extract of *A. carambola* L. both of the ulcer model.

Keywords: Gastroprotective activity, antiulcer activity, ghritha formulation, ulcer index, indomethacin, pylorus ligation

INTRODUCTION

Plants are used for their medicinal value long before the advent of modern medicine. As per the WHO data in India, 64% of the population used traditional medicine for primary health care.[1] *Averrhoa carambola* L. is a woody plant and thought to have originated in tropical Southeast Asia, perhaps in Sri Lanka and the Moluccas.[2,3] Commonly known as carambola, the various parts of the plant have been researched for their therapeutic potential. Scholarly articles have been published on antihyperglycemic,[4] hypoglycemic,[5] antioxidant,[6] depressant[7] activity of the plant parts. Carambola fruits are also used to treat throat inflammation and mouth ulcers.[8] Generally, products based on plants, especially those that are edible and used as food supplements, have minimal adverse effects. *A. carambola* L. fruit is edible and proved to have anti-inflammatory activity and can mitigate mouth ulcers.

A peptic ulcer is a chronic disease causing lesions on the stomach or duodenum covering, or both. The microbial cause of peptic ulcer disease (PUD) is said to be *Helicobacter pylori* infection.[9] PUD occurs when there is a poor defensive mechanism, or presence of strong aggravating factors, or both. Gastric acids and excessive use of NSAIDs are the most common aggravating factors, while bicarbonates and prostaglandin are the defensive factors.[10] There are several conventional antiulcer drugs, but they have demonstrated adverse effects, relapses, and drug interactions.

Ghritha is a lipid-based formulation frequently used in Ayurveda in which the plant extract is incorporated with ghee in the presence of water by heating and constant trituration.

During this process, the aqueous phase is evaporated and the plant constituents get transferred to the lipid phase.[11,12] We have hypothesized that the ghritha formulation of the fruit extract will mitigate the mucosal damage in PUD based on the fact that the lipid portion can provide the gastric lumen with a protective coating and make absorption of the phytomolecules across the mucosal barrier easier. Hence, this study was performed to evaluate the antiulcer and gastroprotective property of the star fruit which is formulated in the form of ghritha.

MATERIALS AND METHODS

Plant Material Collection and Authentication

The fruits of *A. carambola* L. were collected from Mangaluru, Karnataka, India. A sample of *A. carambola* L. fruit was kept in the institutional herbarium assigned with the specimen number is 18PY006R.

Materials

The used chemicals, including omeprazole, indomethacin and sucralfate, were of analytical grade and were procured from Aristo pharmaceuticals and Loba Chemie Pvt. Ltd., Mumbai and Fourrts Laboratories. Pvt. Ltd, Chennai, respectively.

Preparation of Fruit Extract

A. carambola L fruit was sliced, dried in the shade, powdered and the aqueous extract was prepared by extracting 100 g of the dried powder with 500 ml of water by heating on a water bath till the total volume was reduced to half. The decoction was filtered and the filtrate was further condensed to get the extract of semisolid consistency. The ethanolic extract was prepared by the continuous extraction process using the Soxhlet extractor. 100 g of the fruit powder was extracted with 95% ethanol. The extract was concentrated using a rotary evaporator under reduced pressure and was stored in a desiccator.[13]

Preparation of Ghritha Formulation of *A. carambola* L. Fruit Extract

A. carambola L. fruit extracts weighing 5 g was added to 20 ml of ghee followed by the addition of 80ml water, which served as a medium for the incorporation of extracts into the oil (ghritha) phase. These contents were boiled and stirred continuously till all the aqueous part was evaporated. The drug incorporated ghritha was filtered and stored in an airtight container for further use.[14] The ratio of the basic ingredients taken was as per Ayurveda texts. One part of the extract, four parts of cow ghee, and 16 parts of water was taken while preparing the ghritha formulation.[11]

Preliminary Qualitative Phytochemical Investigation

The extracts were subjected to preliminary qualitative phytochemical analysis to check the presence of alkaloids, carbohydrates, flavonoids, saponins, tannins, steroids, and proteins by standard methods.

Experimental Animals

The animal experimental protocol was approved by the Institutional Animal Ethics Committee, bearing the no NGSMIPS/IAEC/MARCH-2019/133. Wistar rats weighing between 180 and 200 g were procured from the Nitte Gulabi Institute of Pharmaceutical Sciences (Karnataka, India). They were kept under standard conditions maintaining the temperature between 23 and 27°C with a 12 h day/light cycle. Free access to food and water *ad libitum* was provided throughout the experiment. All the experimental procedures were done according to CPCSEA guidelines, New Delhi.

Acute Toxicity Study

The acute toxicity studies were performed as per OECD 425 guidelines using five female albino Wistar rats.[15] A limit test was performed by single oral administration of the test drug to one animal at the dose of 2000 mg/kg body weight. The animal was observed continuously once in ½ h for the next 4 h for general behavioral, neurological, and autonomic profile and finally for death after 24 h. The animals were observed for central nervous system toxicity symptoms for 24 h.[6] As no mortality was observed, the same dose was administered sequentially to four more animals and the results were recorded.

Animal Grouping and Treatment for Indomethacin-induced and Pylorus Ligation Ulcer Models

Forty-two Wistar rats were randomly divided into seven groups. Group 1 was taken as normal control and administered the normal saline. Group 2 (ulcer control) animals received a single dose of 30 mg/kg of indomethacin. Group 3 received the standard treatment of sucralfate 100 mg/kg *p.o.* Group 4 and 5 received 400 mg/kg *p.o.* of aqueous extract and ethanolic extract of *A. carambola,* L., respectively. Group 6, 7 received 1160 mg/kg *p.o.* of ghritha of aqueous extract of *A. carambola* L. and Ghee, respectively. The test drugs were administered 10 min before the administration of indomethacin. A single dose of 30mg/kg of indomethacin was administered orally to induce the gastric ulcer.[16-18]

Isolation of the Stomach

Six hours after indomethacin administration, animals were anesthetized and sacrificed humanely by cervical dislocation. The abdomen was cut open, and the stomach was excised and opened from the greater curvature. The opened stomach was cleaned with warm water and examined under a 3X magnifier. The mean count of lesions was calculated after adding the dimensions of lesions of all animals in a group divided by the number of animals.[18]

Pylorus Ligation-induced Ulcer Model

This model also used forty-two Wistar rats randomly divided into seven groups i.e, six animals in each group. Group 1 was normal control. Group 2 (ulcer control) animals received a single dose of 0.25%w/v Critical micelle concentration, 5 ml/kg *p.o.* Group 3 received the standard treatment of omeprazole 30 mg/kg *p.o* for the pyloric ligation model. Group 4 and 5

received 400 mg/kg *p.o.* of aqueous extract and ethanolic extract and groups 6, 7 received 1160 mg/kg *p.o.* of ghritha of aqueous extract of *A. carambola* L. and Ghee, respectively. This procedure was performed under anesthesia on 14–16 h fasted rats. The rats were anesthetized, the abdomen was opened by a small incision below the xiphoid process. Pylorus ligation was done without injuring peripheral tissue and blood vessels, the stomach was replaced inside and the incision was sutured. The rats were housed in single cages such that to avoid coprophagy and cannibalism. Post-surgery drinking water to the animals was withheld. The rats were sacrificed (after 6 h), stomachs were removed and opened and the ulcers were observed.[19]

Estimation of Gastric pH, Total Acidity, and Free Acidity

The stomach was cut open and the gastric content was centrifuged for 10 min at a speed of 3000 rpm. pH, total acidity, and free acidity were checked from the supernatant layer. The supernatant was diluted with 10 ml distilled water and to that 2–3 drops of Topfer's reagent was added and the solution was titrated against 0.01N sodium hydroxide until the solution turns orange as an endpoint. The quantity of alkali added was recorded to calculate the free acidity. The total volume of sodium hydroxide was used to titrate the solution till the endpoint corresponded the total acidity.[19]

Quantification of Ulcer

The degree of ulceration was represented as the ulcer index. The abdomen was cut open along the greater curvature and pinned on a soft board. The severity of the ulcer sores was observed using a hand lens (10×). Ulcer sore was graded on a scale between 0 and 3. Ulcer index was calculated using the following formula.[20]

$$\text{Ulcer index} = (\text{ulcerated area/total stomach area}) \times 100$$

$$\text{Protection \%} = (\text{Ulcer index in control} - \text{Ulcer index of test})/\text{Ulcer index in control} \times 100.$$

Statistical Analysis

All the data exhibited as Mean ± SEM and were subjected to the one-way analysis of variance test (ANOVA) this was followed by the post hoc Scheffe's test. The statistical analysis was performed using SPSS 20.0. The $P < 0.05$ was considered as statistically significant.

RESULTS

Percentage Yield

The percentage yield was found to be 19% and 19.18% in the aqueous and ethanolic extract, respectively.

Preliminary Qualitative Phytochemical Investigation

The preliminary phytochemical analysis of aqueous and ethanolic extracts of *A. carambola* L. fruit revealed the presence of various phytoconstituents like alkaloids, carbohydrates, steroids, etc., as represented in Table 1.

Table 1: Phyto-constituents of Averrhoa carambola L. fruit extracts

Sl. No	Test	Aqueous extract	Ethanolic extract
1	Alkaloids		
	a. Dragendroff's test	+	+
	b. Hager's test	+	+
	c. Wagner's test	+	+
	d. Mayer's test	+	+
2	Carbohydrates:		
	a. Molish's test	+	+
	b. Benedict test	+	+
	c. Fehlings test	+	+
3	Flavonoids:	+	+
	a. Shinoda test		
4	Steroids:		
	a. Liberman Burchard's test	+	-
	b. Salwoski test	+	-
5	Triterpenoids:		
	a. Libermann Buchard's test	+	-
6	Proteins:		
	a. Biurette test	+	+
	b. Millons test	+	+
7	Saponins	+	+
8	Tannins	+	+
9	Glycosides		
	a. Keller-killiani test	+	+
10	Phenols:		
	a. Ferric chloride test	+	+

Present(+)/Absent (-)

Physicochemical Parameter of Ghritha of Aqueous Extract of *A. carambola* L (GACaq)

Ghritha was analyzed for physicochemical properties. Specific gravity, rancidity, acid value, etc., were assessed, which are represented in Table 2.

Acute Toxicity Study

The fruit extract of *A. carambola* L. was checked for toxicity. Toxicity studies were performed as per OECD 425 guidelines. The extract was found to be nontoxic up to 2000mg/kg body weight given orally. No morbidity, mortality, or organ-specific toxicity was observed, confirming the safety of the aqueous extracts. The toxicity study was not conducted using the ethanolic extract because the ethanolic extract was not used in the preparation of ghritha formulation.

Effect of GACaq on Indomethacin-induced Ulcer Model

Treatment with 400 mg/kg of aqueous extract and ethanolic extract of *A. carambola* L. showed significant improvement

against ulceration. Ghritha formulation of the aqueous extract has a synergetic effect, as shown in Table 3. The aqueous extract, ethanolic extract, and ghritha formulation produced ulcer index 3.607, 4.49, and 3.17 when compared with the ulcer control group (12.57). The percentage protection produced by the extracts is 71.36%, 64.74%, and 74%, respectively, and standard sucralfate (100 mg/kg) produced 85.1% protection against indomethacin-induced ulcer model in rat.

Effect of GACaq on Pylorus Ligation-induced Ulcer Model

Treatment with 400 mg/kg of the aqueous and ethanolic extract showed a significant decrease in total and free acidity and an increase in gastric pH. A synergetic reduction in total and free acidity and an increase in gastric pH was observed when treated with GACaq. The details are referenced in Table 4.

Morphological Study of the Stomach

In the normal control group, stomach integrity was observed to be normal. Severe bleeding, spot ulcers were observed in the disease control group. The animals in the standard and the extract group animals showed less degree of ulcerations, and stomach integrity was maintained.

DISCUSSION

Indomethacin's inhibitory role in prostaglandin synthesis and the development of free radicals are biochemical incidents of gastric ulceration pathogenesis. The inhibition of both cyclo-oxygenase (COX) 1 and COX2 enzymes strongly damages PG syntheses**.** NSAIDs have been implicated in the etiology of ulcers and have been employed in several animal studies for the induction of gastric ulcers.[21,22] In the study, it was confirmed that the administration of 30 mg/kg of indomethacin in rats caused a significant increase ($P < 0.05$) in the degree of ulceration. The administration of fruit extract as well as the formulation showed substantial improvement against ulceration.

The indomethacin-induced ulcer models and pylorus ligation models have some basic mechanism-based differences, such as the inhibition of prostaglandin synthesis by inhibiting COX enzymes and reducing the amount of free radicals. The antiulcer activity shown by using this model probably suggests the inhibition of PG synthesis or trapping in oxygen-free radicals. The Pylorus ligation model of ulcer is also acceptable and extensively used to assess the antiulcer property. The promising antiulcer results in the pylorus model may indicate a mechanism like neutralizing the free and total acid contents in the study, thus elevating the gastric pH. Elevation in the gastric pH indicates the prevention from the self-destruction of the mucosal defense barrier leading to antiulcer activity.

Pylorus ligation leads to the accumulation of a large quantity of gastric acid, which results in an increase in free acidity and total acidity and a reduction in gastric pH. The accumulation of a large volume of gastric juice leads to the self-digestion of the gastric mucosa and leads to the breakdown of the mucosal barrier-forming gastric ulcers.[23] The result in the study showed a significant increase in ($P < 0.05$) in the degree of ulceration. The treatment of aqueous, ethanolic extract and ghritha formulation of *A. carambola* L. showed substantial improvement against ulcerations Tabel 4 corresponding to gastric protection the treatment and formulation of *A. carambola* L. showed a significant increase in total and free acidity and a significant decrease in gastric pH. These extracts produced a good antiulcer activity by reducing free acidity, total acidity, ulcer index, and an increase in gastric pH. These extracts showed ulcer index 3.40, 4.01, and 2.86, when compared to the ulcer control group (14.46) ulcer index in omeprazole, treated group was 1.87 Table 4. The percentage protection of these extracts is 76.48%, 72.26%, and 80.22%, respectively, whereas omeprazole produced percentage protection of 87.06%. With 80.22% protection, ghritha formulation was found to be better than just extracts and also, it was better than only ghritha (Ulcer index: 3.98), indicating synergism between the extracts and ghritha. It may be because ghritha protects the lumen of the stomach more effectively. The antiulcer activity maybe because of the flavonoid, steroid, or tannins present in the drug.[24]

Table 2: Physicochemical properties of GACaq

Sl. No	Parameters	Results
1	Specific gravity	0.86 g/cc
2	Rancidity	Fat not oxidized
3	Acid value	5.7783
4	Saponification value	246.84
5	Iodine value	12.9
6	Melting point	42°c
7	Loss on drying	0.29 g

Table 3: Effect of GACaq on indomethacin-induced ulcer model

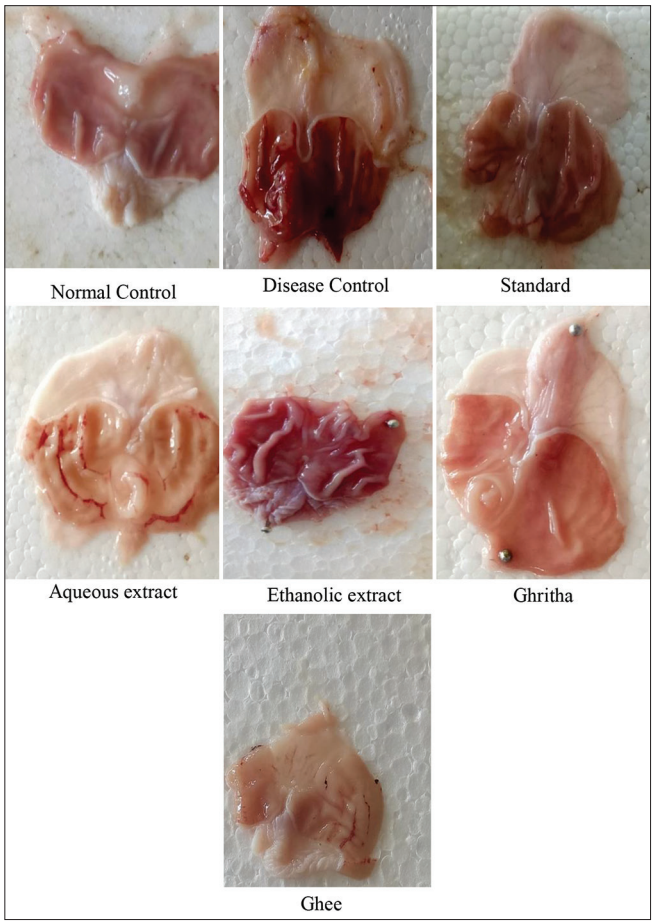
Sl. No	Groups	Treatment	Doses (mg/kg)	Ulcer index	% Protection
1	Group I	Normal control	-	0	.
2	Group II	Ulcer control (Indomethacin)	30mg/kg	12.57±0.113	.
3	Group III	Standard (sucralfate)	100mg/kg	1.72±0.21*	85.1%
4	Group IV	Aqueous extract of *Averrhoa carambola* L.	400mg/kg	3.607±0.061*	71.36%
5	Group V	Ethanolic extract of *Averrhoa carambola* L.	400mg/kg	4.494±0.173*	64.24%
6	Group VI	Ghritha formulation of Averrhoa carambola L.	(1161mg/kg)	3.17±0.123*	74.78%
7	Group VII	Ghee	(1161mg/kg)	4.49±0.088*	64.28%

Data were analyzed using ANOVA and post hoc Scheffe's test and the obtained values are expressed in terms of mean±SEM. * $P<0.05$ when compared to ulcer control

Table 4: Effect of GACaq on various gastric parameters on pylorus ligation induced ulcer model

Sl.No	Treatment	Doses	Gastric volume (ml)	Gastric pH	Free acidity (meq/l)	Total acidity (meq/l)	Ulcer Index	Protection %
1	Normal	-	1.67±0.175	3.16±0.006	42.5±0.22	71.33±0.33	0	.
2	Ulcer control	-	2.85±0.125	2.19±0.06	80.6±0.21	85±0.36	14.46±0.20[b]	.
3	Standard (Omeprazole)	30 mg/kg	2.86±0.12	5.45±0.11*	25.16±0.16[#]	41.66±0.33[a]	1.87±0.30[b]	87.06%
4	Aqueous extract	400 mg/kg	2.4±0.36	4.71±0.04*	35.5±0.22[#]	51.66±0.33[a]	3.40±0.07[b]	76.48%
5	Ethanolic extract	400 mg/kg	1.81±2.47	4.05±0.09*	44.83±0.30[#]	75.83±0.30[a]	4.01±0.07[b]	72.26%
6	Ghritha formulation	1161 mg/kg	2.47±0.34	5.56±0.02*	28.5±0.22[#]	48.3±0.33[a]	2.86±0.020[b]	80.22%
7	Ghee	1161 mg/kg	3±0.420	4.22±0.04*	41.16±0.30[#]	63±0.258[a]	3.98±0.04[b]	72.47%

Data were analyzed using ANOVA and post hoc Scheffe's test and the obtained values are expressed in terms of mean±SEM. *,#, a, b, $P<0.05$ when compared to ulcer control



AQ2 **Figure 1:** Morphological features of the stomach in pylorus ligation induced ulcer

The findings of the study suggested the antiulcer effect of the fruits of *A. carambola* L. in both indomethacin-induced ulcer model and pyloric ligation models. Still, the study was limited by the preparation of ghritha formulation only from aqueous extract, as the ghritha from the ethanolic extract is not used in the Indian traditional system of medicine. The investigators were unable to determine the blood chemical values like prostaglandin and COX as this research was part of an academic curriculum and was limited by time and resources. Similarly, the acute toxicity study was conducted only using aqueous extract of the plant.

CONCLUSION

The results of the study demonstrated the promising findings on the gastroprotective and antiulcer property of aqueous and ethanolic extract of *A. carambola* L. The results also revealed a synergetic effect when the aqueous extract was formulated as ghritha. There was also a marked decrease in free and total acidity and increasing the gastric pH. The gastroprotection activity could be due to the phytoconstituents like alkaloids, flavonoids, proteins, etc. But further research is required to establish the mechanism for the antiulcer property of the plant.

CONFLICTS OF INTEREST STATEMENT

The authors declare that there is no conflict of interest with individuals or organizations that would unacceptably bias the content of this paper.

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