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Anti-diarrhoeal activity of leaves of *Averrhoa carambola* Linn.

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Abstract

Objective: This study screens the aqueous and ethanolic extracts of *Averrhoa carambola* Linn for antidiarrhoeal activity in Castor oil induced diarrhea model and Prostaglandin induced diarrhea model, Gastro-intestinal motility test model in mice.
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 antidiarrhoeal activity by different *in vivo* assay methods. 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.
Results: Acute oral toxicity study indicated that ethanolic extract of *Averrhoa carambola* (EEAC) and aqueous extract of *Averrhoa carambola* (AEAC) was safe up to a dose of 2000 mg/kg body weight of mice. Results of antidiarrhoeal activity study revealed that the extracts of *Averrhoa carambola* Linn leaves showed significant anti-diarrhoeal activity in a dose-dependent manner when compared to control.
Conclusion: Results indicated a possible role of the EEAC & AEAC in the prevention & treatment of diarrhea. The phenolic/flavonoid contents of EEAC & AEAC having antioxidant potential might be responsible for the antidiarrhoeal property of *Averrhoa carambola* leaves.
Keywords: *Averrhoa carambola*, diarrhea, antidiarrhoeal activity; gastrointestinal motility; flavonoids.

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1. Introduction

Diarrhea may be defined in terms of stool frequency, consistency, volume, or weight [1]. Patient's conceptions of diarrhea often focus around stool consistency. Indeed, fecal consistency is determined by the water holding capacity of the stool (that is, the amount of non-bound "free" water) and this perhaps best defines the concept of diarrhea. However, quantification of this in clinical practice may prove difficult and so other criteria, such as the passage of more than three stools per day or stool weight, provide alternative means of definition. A stool weight of 200 g/day is often regarded as the upper limit of normal but this can be misleading as stool weights vary greatly and "normal" stool volumes can exceed this value, particularly when non-Western diets are encountered. Conversely, distal colonic pathology may not increase stool weight above 200 g/day. A pragmatic definition incorporates these elements: diarrhea is the abnormal passage of loose or liquid stools more than three times daily and/or a volume of stool greater than 200 g/day.

Acute, watery diarrhea is usually caused by a virus (viral gastroenteritis) [2]. Medications such as antibiotics and drugs that contain magnesium products are also common offenders. Recent dietary changes can also lead to acute diarrhea. These including intake of coffee, tea, colas, dietetic foods, gums or mints that contain poorly absorbable sugars. Acute bloody diarrhea suggests a bacterial cause like Campylobacter, Salmonella or Shigella [3]. Traveling to developing areas of the world can result in exposure to bacterial pathogens common in certain areas. Eating contaminated foods such as ground beef or fresh fruit can cause diarrhea due to *E. coli*. Chronic bloody diarrhea is

most likely due to Inflammatory Bowel Disease (IBD)[4]. These include ulcerative colitis or Crohn's disease. Pain with defecation suggests rectal inflammation. Other less common causes include ischemia of the gut, infections, radiation therapy and colon cancer or polyps.

The term motility encompasses both motor activity and transit. Motor activity refers to the intestinal contractions and relaxations of the gut and to intestinal tone. These result in the propulsion of digest along the gastro¬intestinal tract, i.e., transit, or in the mixing of the digester. A number of special features of the motor system of the gastrointestinal tract (smooth muscle and enteric nervous system) enable these events to occur, namely: the specific anatomical arrangement of smooth muscle layers and neural plexuses, the spontaneous electrical rhythmicity of the gastrointestinal smooth muscle, the "pace¬maker" sites within the stomach and intestines, and the functional integration of the enteric nervous system with the central nervous system [5].

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 [6,7]. 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 clusters of red stalked, lilac, purple-streaked, downy flowers, about 1/4″ (6 mm) wide (Plate c). 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. Treatment with hydroalcoholic extracts of the leaves resulted in a 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 antiulcer activity, with a different mechanism of action for the antiulcerogenic activity. As hydro-alcoholic extract contains triterpenes, flavonoids, and mucilage, the partial anti-diarrhoeal 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. Then 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 (2000ml). The powders are extracted at 55℃ using round-bottomed flask for 72 hrs. The extracting solvent in a round-bottomed flask is heated and its vapor condenses in the condenser. The condensed extractant drips into the thimble containing the crude drug and extracts 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 remove the fat material from the powder [8].

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 color residue. By using chloroform extraction terpenoid lactones are obtained [9].

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, the solution was evaporated to dryness under reduced pressure and controlled temperature by using rotary evaporator[10]

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 chloroform (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 clarified by filtration. At last, the solution is dried using rotary evaporator [11].

2.2.5. Experimental animals and housing conditions

Healthy albino mice 20-25g (weight interval within ± 20%) 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.

2.3 Phytochemical screening

Preliminary phytochemical screening was carried out for detection of the presence of various phytoconstituents such as alkaloids, glycosides, flavonoids, phenolic compounds, tannins, saponins, proteins, amino acids, and steroids [12–17].

2.4 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 fasted prior to dosing for 3-4 hrs. Following the period of fasting, the animals were weighed and the test substance administered. After the substance has been administered, food was withheld for a further 1-2 hrs [18–19].

2.5. Anti-diarrhoeal activity

2.5.1. Castor oil induced diarrhea model

Animals are kept for overnight fasting with free access to water before the experiment. Different groups had given different treatment as discussed earlier. After 30 minutes of distilled water, loperamide and different extract treatment, each mouse received 0.5 ml of castor-oil orally. Each mouse was then housed separately in the perforated cage over a clean filter paper. Then diarrhoeal episodes were observed for a period of 4 hrs, during that period frequency of defecation, number of fecal drops and mean weight of stool were noted [20-21]. The anti-diarrhoeal activity was determined in terms of percentage of protection, which was calculated by the following formula-

$$\% \text{ protection} = \frac{\text{Mean weight of stool of Control animals} - \text{Mean weight of stool of drug/extract treated animals}}{\text{Mean weight of stool of control animals}} \times 100$$

2.5.2. Prostaglandin-induced diarrhea model

Animals were kept for overnight fasting with free access to water before the experiment. Different groups had given different treatment as discussed earlier. After 30 minutes of distilled water, loperamide and different extract treatment, each mice received misoprostol 1mg/kg p.o. Each mice was then housed separately in a perforated cage over a clean filter paper. Then diarrhoeal episodes were observed for a period of 4 hrs, during that period frequency of defecation, number of fecal drops and mean weight of stool were noted. The anti-diarrhoeal activity was determined in terms of percentage of protection, which was calculated by the previous formula.

2.5.3. Gastrointestinal motility test model

Animals have fasted for 24 hrs with free access to water before the experiment. Different groups had given different treatment as discussed earlier. After 30 minutes of distilled water, atropine sulfate and different extract treatment, each mice received 5% 0.5ml of charcoal meal orally. Each animal was sacrificed thirty minutes after administration of charcoal meal. The distance covered by the charcoal meal in the intestine was expressed as a percentage of the total distance traveled from the pylorus to the caecum. The mean percentage movement of charcoal meal in ratio to the intestinal length and percentage of inhibition was calculated by following the formula-

$$\% \text{ travelled} = \frac{\text{Distance traveled by the charcoal meal}}{\text{The total length of the small intestine}} \times 100$$

$$\% \text{ of inhibition} = \frac{\text{Mean length of the Small intestine} - \text{Distance traveled by the charcoal meal}}{\text{Mean length of the small intestine}} \times 100$$

2.6. 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. Castor oil induced diarrhea model

The ethanolic and aqueous extract of *Averrhoa carambola* Linn leaves showed significant anti-diarrhoeal activity in a dose-dependent manner when compared to control which is evident by a decrease in number and weight of fecal matter. The avg. weight of wet faeces of ethanolic extract group at a dose of 100mg/kg & 200mg/kg is found to be 0.242 ± 0.039 & 0.15 ± 0.023 respectively. The avg. weight of wet faeces of aqueous extract group at a dose of 100 mg/kg & 200 mg/kg is found to be 0.302 ± 0.032 & 0.178 ± 0.035 respectively. For standard (loperamide) it is found to be 0.137 ± 0.025. Here it is found that the ethanolic extract is more potent than aqueous extract. The result complied in table no 1 and graphically presented in figure 1, 2 & 3.

3.3. Prostaglandin-induced diarrhea model

The ethanolic and aqueous extract of *Averrhoa carambola* Linn leaves showed significant anti-diarrhoeal activity in a dose-dependent manner when compared to control which is evident by a decrease in number and weight of fecal matter. The avg. weight of wet feces of ethanolic extract group at a dose of 100 mg/kg & 200mg/kg is found to be 0.267 ± 0.027 and 0.187 ± 0.035 respectively. The avg. weight of wet faeces of aqueous extract group at a dose of 100mg/kg & 200mg/kg is found to be 0.307 ± 0.041 and 0.192 ± 0.045 respectively. For standard (loperamide) it is found to be 0.158 ± 0.028. Here it is found that the ethanolic extract is more potent than aqueous extract. The result complied in table no 2 and graphically presented in figure 4, 5 & 6.

3.4. Gastrointestinal motility test model

The ethanolic and aqueous extract of *Averrhoa carambola* Linn leaves showed significant anti-diarrhoeal activity in a dose-dependent manner when compared to control which is evident by a decrease in the distance traveled by charcoal meal. The avg. % distance travelled by charcoal meal of ethanolic extract group at a dose of 100mg/kg & 200mg/kg is found to be 55.50% & 43.92% respectively. The avg. % distance travelled by charcoal meal of aqueous extract group at a dose of 100mg/kg & 200mg/kg is found to be 58.82% & 48.79% respectively. For standard (atropine sulfate) it is found to be 38.82%. Here it is found that the ethanolic extract is more potent than aqueous extract. The result complied in table no 3 and graphically presented in figure 7.

Table 1: Effect of ethanolic and aqueous extract of *Averrhoa carambola* Linn leaves on castor oil induced diarrhoea

S. No.	Treatment	Dose (mg/kg)	Avg. onset time of diarrhoea in min	Avg. total no. of faeces	Avg. of No. of wet faeces	Avg. wt. of wet faeces (g)
1	Control	Distilled water	69.167 ± 13.49	9.167 ± 0.703	6.833 ± 0.601	0.39 ± 0.041
2	Standard	Loperamide (3mg/kg)	200.17 ± 21.651**	4.333 ± 0.882 **	3.0 ± 0.577**	0.137 ± 0.025***
3	EEAC1	100 mg/kg	132.67 ± 25.899	6.667 ± 1.085	4.833 ± 0.792	0.242 ± 0.039*
4	EEAC2	200 mg/kg	189.83 ± 23.796**	5.167 ± 0.872*	3.667 ± 0.494*	0.15 ± 0.023***
5	AEAC1	100 mg/kg	118.17 ± 23.655	6.5 ± 0.671	5.167 ± 0.654	0.302 ± 0.032
6	AEAC2	200 mg/kg	168.67 ± 26.690*	5.333 ± 1.022*	4.0 ± 0.894	0.178 ± 0.035**

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 *Averrhoa carambola* Linn leaves on prostaglandin induced diarrhoea

S. No.	Treatment	Dose (mg/kg)	Avg. onset time of diarrhea in min.	Avg. total no. of faeces	Avg. of no. of wet faeces	Avg. wt. of wet faeces (g)
1	Control	Distilled water	77.167 ± 11.898	8.5 ± 0.671	6.5 ± 0.619	0.343 ± 0.029
2	Standard	Loperamide (3 mg/kg)	196.5 ± 24.137**	4.0 ± 0.966**	3.0 ± 0.577*	0.158 ± 0.028**
3	EEAC1	100 mg/kg	144.5 ± 25.407	6.667 ± 0.667	4.667 ± 0.615	0.267 ± 0.027
4	EEAC2	200 mg/kg	182.5 ± 11.494*	4.667 ± 0.667*	3.333 ± 0.615*	0.187 ± 0.035*
5	AEAC1	100 mg/kg	129.67 ± 22.618	7.0 ±0.775	5.333 ± 0.76	0.307 ± 0.041
6	AEAC2	200 mg/kg	172.83 ± 20.862*	4.5 ±0.992*	3.667 ± 0.803	0.192 ± 0.045*

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

Table 3: Distance travelled by charcoal meal in different groups

S. No.	Treatment	Dose (mg/kg)	Avg. % distance travelled by charcoal meal
1	Control	Distilled water	68.69%
2	Standard	Atropine Sulphate (0.1 mg/kg) i.p.	38.82%
3	EEAC1	100 mg/kg	55.50%
4	EEAC2	200 mg/kg	43.92%
5	AEAC1	100 mg/kg	58.82%
6	AEAC2	200 mg/kg	48.79%

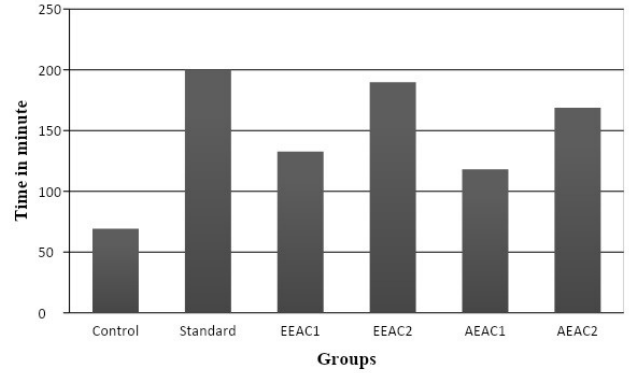

Fig.1: Average onset time of diarrhea of different groups in castor oil induced diarrhea

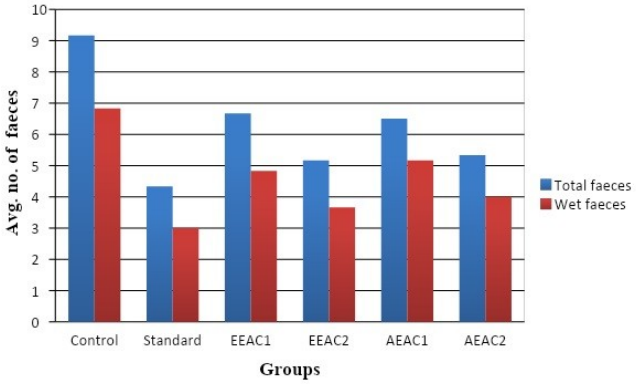

Fig. 2: Number of faeces (total &wet) of different groups in castor oil induced diarrhea

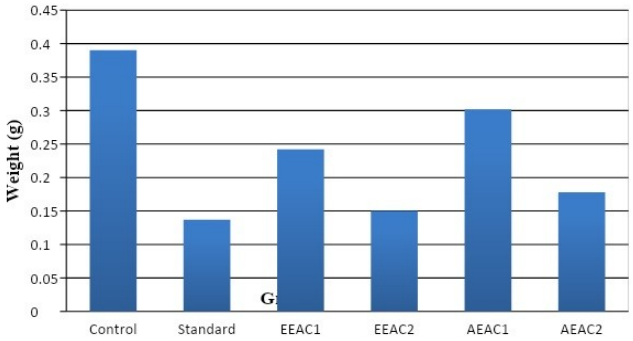

Fig. 3: Average weight of wet faeces of different groups in castor oil induced diarrhoea

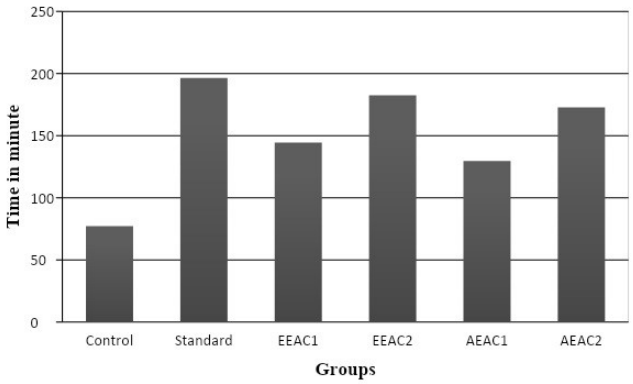

Fig.4: Average onset time of diarrhea of different groups in prostaglandin induced diarrhoea

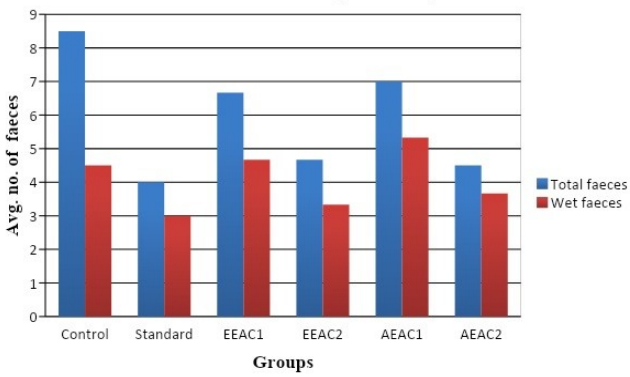

Fig.5: Number of faeces (total & wet) of different groups in prostaglandin induced diarrhea

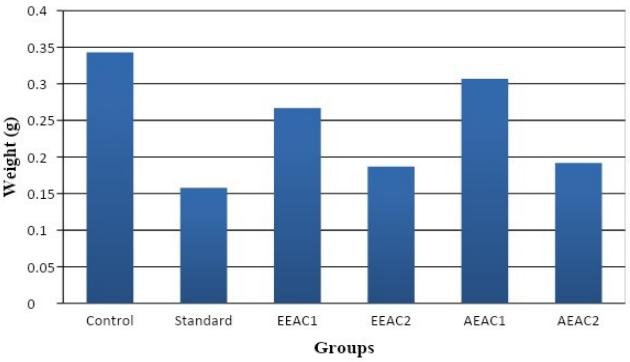

Fig.6: Average weight of wet faeces of different groups in prostaglandin induced diarrhoea

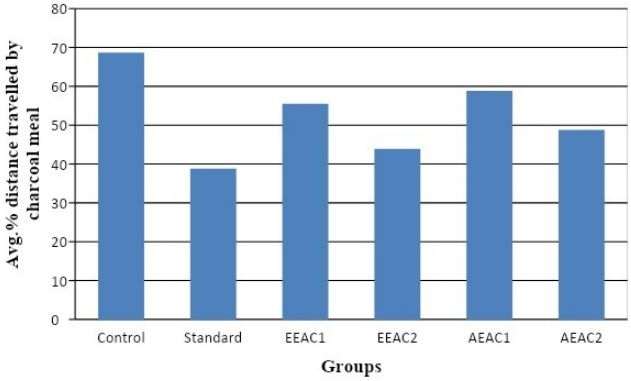

Fig.7: Movement of charcoal meal in small intestine in different groups

4. Discussion

In traditional medicine, many herbs are claimed for their anti-diarrhoeal activity. In the present study, the dried leaves of *Averrhoa carambola* are selected for that study. Previous reports have demonstrated antidiarrhoeal activity of tannins and flavonoids containing plant extracts. Tannins can evoke an antidiarrhoeal effect since these substances may precipitate proteins of the electrolytes and reduce peristaltic movement and intestinal secretions. The antidiarrhoeal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydroelectrolytic secretion which are known to be altered in

this intestinal condition. The preliminary phytochemical investigation of the ethanolic extract and aqueous extract of *Averrhoa carambola* leaves showed the presence of tannins and flavonoids. These phytochemicals may be responsible for the significant antidiarrhoeal activity in this study. The present study is carried out to evaluate the anti-diarrhoeal activity of ethanolic and aqueous extract of *Averrhoa carambola* Linn leaves against different animal models of diarrhea like - Castor oil, Prostaglandin induced induced-diarrhea and Gastro-intestinal motility test model

Ricinolic acid is the active component of castor oil which induces the permeability changes in the mucosal fluid and electrolyte transport that results in hypersecretion and causes diarrhea. Ethanolic and aqueous extract of *Averrhoa carambola* Linn leaves inhibit the action of ricinoleic acid and shows anti-diarrhoeal activity. Prostaglandins induced intestinal fluid accumulation and increased fluid movement in secretory diarrheas. PG_s also inhibit the absorption of glucose, a major stimulus to intestinal absorption of water and electrolytes. These causes diarrhea which is controlled by administration of ethanolic and aqueous extract of *Averrhoa carambola* Linn leaves in the rat in the present study. In gastrointestinal motility test model, the charcoal meal was used as a marker. The experiment is carried out on the gastrointestinal tract motility of charcoal meal. Motility after charcoal meal administration showed a reduction in the propulsive movement of the small intestine after pre-treatment with the ethanolic and aqueous extract of *Averrhoa carambola* Linn leaves compared to control group. If we compare between the above two extracts then the result showed that the ethanolic extract have more potent anti-diarrhoeal activity than aqueous extract.

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 the different solvent extract. The 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. Oxidation is also responsible for the damage of our organs in our body like stomach, kidney, 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. Both ethanolic and aqueous extracts have high portion of tannins which may be responsible for anti-diarrhoeal activity

Conflict of interest

The authors declare that there are no conflicts of interest.

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