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Evaluation of Antiulcer and Anti-Diarrheal Activity of *Amaranthus spinosus*

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ABSTRACT

Background: *Amaranthus spinosus* is a widely used plant in traditional medicine for the treatment of various gastrointestinal disorders. The present study was undertaken to scientifically evaluate the antiulcer and antidiarrheal activities of the methanol extract of Amaranthus spinosus leaves (MEAS) in experimental animal models. **Methods:** Antiulcer activity was assessed using the aspirin + pyloric ligation model in rats, evaluating parameters such as ulcer index, gastric volume, pH, acidity, protein content, and mucin components (total carbohydrates, hexose, hexosamine, fucose). For antidiarrheal activity, castor oil-induced diarrhea and charcoal meal transit tests were employed. MEAS was tested at doses of 100 and 200 mg/kg, and compared to standard drugs Ranitidine (50 mg/kg) and Loperamide (3 mg/kg). **Results:** MEAS demonstrated significant, dose-dependent antiulcer activity, evident by a reduction in ulcer index, gastric acidity, and protein leakage, alongside increased pH and mucosal protective factors. In the castor oil-induced diarrhea model, MEAS significantly decreased fecal output, with up to 55.73% inhibition at 200 mg/kg. The extract also significantly delayed gastrointestinal transit time in the charcoal meal test, indicating an antimotility effect. **Conclusion:** The findings confirm the gastroprotective and antidiarrheal potential of Amaranthus spinosus, validating its traditional use in gastrointestinal disorders. The observed effects may be attributed to its anti-secretory, mucosal protective, and antimotility properties. Further studies are recommended to isolate and characterize the active phytoconstituents responsible for the observed activities.

Keywords: *Amaranthus spinosus*, antiulcer, antidiarrheal, MEAS, gastric protection, castor oil-induced diarrhea

INTRODUCTION

Gastrointestinal disorders such as peptic ulcers and diarrhea remain major health problems worldwide, particularly in developing countries where access to adequate healthcare and sanitation is limited. [1] Peptic ulcer disease results from an imbalance between aggressive factors like gastric acid, pepsin, and Helicobacter pylori infection, and the defensive mechanisms of the gastric mucosa such as mucus secretion, bicarbonate production, and mucosal blood flow. [2] On the other hand, diarrhea is characterized by frequent passage of loose or watery stools and can lead to severe dehydration and electrolyte imbalance. Although several synthetic drugs are available for the management of these conditions, their long-term use is often associated with adverse effects, high cost, and the development of resistance. [3, 4] This has stimulated interest in the exploration of medicinal plants as safer, cost-effective, and easily accessible therapeutic alternatives.

Amaranthus spinosus Linn. (family: Amaranthaceae), commonly known as spiny amaranth or thorny amaranth, is a widely distributed herbaceous plant traditionally used in various indigenous systems of medicine. [5] It has been employed for the treatment of ailments such as dysentery, diarrhea, ulcers, inflammation, and skin diseases. Phytochemical investigations have revealed that *A. spinosus* contains several bioactive constituents including flavonoids, alkaloids, tannins, saponins, and phenolic compounds, many of which are known to exhibit antioxidant, anti-inflammatory, and cytoprotective properties that may contribute to its gastrointestinal benefits. [6, 7, 8] Given the traditional claims and the presence of potentially beneficial phytochemicals, it is essential to provide scientific validation for the pharmacological activities of *Amaranthus spinosus*. [9, 10] Therefore, the present study aims to evaluate the antiulcer and antidiarrheal activities of the plant using appropriate experimental models. [11] The outcomes of this investigation may substantiate its traditional use and contribute to the development of novel plant-based therapeutic agents for the management of gastrointestinal disorders.

MATERIALS AND METHODS

Collection of plant materials: The leaves of *Amaranthus spinosus* were collected from the local region of Gwalior, India.

Animals: Healthy adult albino rats of the Wistar strain, weighing between 150–200 grams, were procured from a CPCSEA-approved breeder for the purpose of this study. Upon arrival, the animals were housed in the institutional Animal House Facility, maintained under standard laboratory conditions. A controlled 12 ± 1 hour light/dark cycle was followed, with room temperature maintained between 15–25 ± 2 °C, ensuring proper ventilation and hygiene throughout the experimental period. The rats were housed in spacious, clean polypropylene cages, lined with sterilized bedding, which was changed regularly to maintain sanitary conditions. They were provided with a standard rat pellet diet, procured from Durga Feeds, Bangalore, India, and had free access to clean drinking water (ad libitum). [12, 13] Prior to the commencement of experiments, the animals were acclimatized for a suitable period to the laboratory environment to minimize stress and ensure reliability of results.

Extraction: The leaves of *Amaranthus spinosus* were shade-dried to preserve their phytoconstituents and subsequently coarsely powdered using a mechanical grinder. The total weight of the powdered material was approximately 200 grams. The coarse powder was then subjected to hot continuous successive extraction using a Soxhlet apparatus, with solvents applied in order of increasing polarity—chloroform followed by methanol. The extraction process was carried out under controlled temperatures ranging from 50–60 °C to ensure optimal yield of bioactive compounds. The resulting solvent extracts were concentrated at temperatures below 40 °C using a rotary evaporator to prevent degradation of heat-sensitive constituents. Final drying was performed under reduced pressure to obtain the chloroform and methanol extracts in solid form. [14] These dried extracts were carefully stored in a desiccator until further pharmacological and phytochemical evaluations were conducted. The percentage yields of the extractives were calculated using the formula:

$$\% \text{ Yield} = \frac{\text{Weight of the extractives}}{\text{Weight of the crude drug}} \times 100$$

Qualitative Phytochemical Screening: The preliminary phytochemical screening of the herbal extractives was carried out using standard qualitative tests to detect the presence of various phytoconstituents, such as alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, phenolic compounds, and others. These tests were performed according to established protocols to determine the broad chemical classes present in the chloroform and methanolic extracts of Amaranthus spinosus. [15].

Acute Oral Toxicity and Gross Behavioural Study (14 Days): A single-dose oral acute toxicity study was conducted over a period of 14 days to evaluate the safety profile and gross behavioural effects of different extractives of *Amaranthus spinosus*. The study followed the OECD Guideline 425 (Up-and-Down Procedure) and was performed using healthy adult female albino Wistar rats weighing between 150–200 g. The extracts were suspended in 0.1% (w/v) sodium carboxymethyl cellulose (CMC) and administered orally. A total of four sets of rats (each consisting of three animals) were used. Prior to dosing, the animals were fasted for 18 hours, with water withdrawn 4 hours before administration, and body weights were recorded both before and after dosing Body weights were measured again on the 8th and 14th days post-dosing. Throughout the study, no signs of acute toxicity or mortality were observed, indicating a favourable safety profile of the extractives up to 2000 mg/kg. The same experimental design was replicated to assess the acute oral toxicity of leaf extracts of *Amaranthus spinosus*, using identical parameters and procedures. All experimental protocols were conducted in compliance with ethical guidelines and OECD Test Guidelines 425. [16]

Evaluation of Anti-Ulcer Activity in Rats

Aspirin Induced Modified Pylorus Ligated Model: The selected methanolic extract of *Amaranthus spinosus* (MEAS) was evaluated for its antiulcer potential using the aspirin-induced modified pylorus ligation model in Wistar albino rats. Healthy adult rats of either sex, weighing between 150–200 g, were used for the study. Prior to the experiment, animals were fasted for 48 hours with free access to water to ensure an empty stomach, and housed in cages with grated floors to prevent coprophagy. The rats were randomly divided into six groups, each comprising six animals. The experimental design was as follows: Group 1 served as the ulcer control and received solvent (10 ml/kg) + aspirin (200 mg/kg); Group 2 received ranitidine (50 mg/kg); Groups 3 and 4 were administered MEAS at doses of 100 mg/kg and 200 mg/kg, respectively; and the standard group received omeprazole (20 mg/kg, p.o.). Aspirin (200 mg/kg) was administered orally as a suspension in 0.1% CMC, one hour prior to pyloric ligation to induce

ulcerogenesis. The test and standard drugs were administered orally once daily for seven consecutive days, with the final dose given one hour before aspirin administration on the day of the experiment. [17, 18]

Pyloric ligation was carried out under light ether anesthesia, ensuring that the pylorus and surrounding blood vessels were not injured. After ligation, the stomach was carefully repositioned in the abdominal cavity, and the incision was sutured. The rats were deprived of food and water for four hours post-surgery to avoid interference with gastric secretion. After this period, the animals were sacrificed using an overdose of ether, and the stomachs were excised. Gastric contents were collected and centrifuged for biochemical estimation of gastric volume, pH, free acidity, and total acidity in all groups. [19] The stomachs were then rinsed with normal saline, soaked for five minutes, and pinned onto boards for morphological examination. The ulcer index was determined based on visible lesions, and photographs of each stomach were taken to document and compare the extent of ulceration among the different treatment groups.

Evaluation of Ulcer Index and Inhibition: The ulcer index was calculated by counting the lesions with the aid of hand lens (10 X) and graded as follows. [20]

- 0 = Normal coloured stomach
- 0.5 = Red colouration
- 1 = Spot ulcer
- 1.5 = Haemorrhagic streaks
- 2.0 = ulcers > 3 but < 5
- 3.0 = ulcers > 5

Mean ulcer score for each animal was expressed as ulcer index.

$$\% \text{ Ulcer protection} = \frac{\text{Ulcer Index in Control} - \text{Ulcer index in Test}}{\text{Ulcer Index in Control}} \times 100$$

The volume and pH of the collected gastric juice was recorded. Free acidity and total acidity was calculated. Various bio-chemical estimations like total proteins, ttotal carbohydrate and carbohydrate/protein ratio of the gastric juice were performed using standard methods. [21]

Anti-diarrhoeal Activity

Castor Oil Induced Diarrhea: Castor oil induced diarrhea model was carried out using the method described by Shoba and Thomas (2001). [22] The animals were screened initially by giving 1ml of castor oil and those showing diarrhea were selected for the final experiment. Twenty five albino rats were randomly divided in to five equal group (n=5) divided in to control group, standard group and test groups. The control group received vehicle (1ml/rat). The standard group received loperamide at the dose of 3mg/kg orally. The test group received methanol extract of *Amaranthus spinosus* leaves 100, 200mg/kg orally. Each animal was placed in individual cage, the floor of which was lined with bloating paper. The floor lining was changed for every hour. Diarrhea was induced by oral administration of 1.0 ml castor oil to each rat. 1hour after the above treatment during an observation period of 4hours, the total numbers of faeces excreted by the animals were recorded. A numeric score based on the stool consistency was assigned as follows: normal stool=1, semi solid stool= 2 and watery stool =3. The number of diarrhoeal faeces and percentage of Inhibition of diarrhoeal faeces were calculated. Percentage inhibition was calculated as follows.

PI= Mean defecation (control-treated group) Mean defecation of control group x100

Gastro intestinal transit time: Rats were fasted for 24hr and divided in to five groups of five rats each. Each animal was given 1ml of 1% charcoal suspension orally 60 min after an oral dose of the test drug, standard and vehicle. Group I was administered 0.5ml distilled water, and Group II received Loperamide 3mg/kg, Group III and IV received extract at the dose of 100mg/kg and 200mg/kg body weight respectively. The faecal bolus was expelled were collected. Each faecal bolus was pressed on a white sheet of paper examine the presence of char coal meal. The time for the appearance of the 1st faecal bolus with char coal meal was recorded. [23]

Statistical Analysis: The results were expressed as mean ± SEM and analyzed statistically to find out significance difference between control groups against each test group separately. The value of $P< 0.05$ was considered statistically significant. [24]

RESULTS AND DISCUSSION

Extraction and Qualitative Phytochemical Studies: The leaves of Amaranthus spinosus were subjected to solvent extraction using methanol and chloroform to evaluate their extractive yields and phytochemical constituents. The results of the extraction and preliminary phytochemical screening are presented in Table 1. The methanol extract yielded a greenish-brown solid with a yield of 0.4% w/w, whereas the chloroform extract gave a brown solid with a significantly higher yield of 1.5% w/w. This suggests that non-polar to moderately polar phytoconstituents are more abundantly present in *A. spinosus* leaves, as chloroform—a moderately polar solvent—was more effective in extracting compounds than methanol, which is more polar.

Table 1. Extraction and Phytochemical Analysis of *Amaranthus spinosus* Leaves extracts (Methanol and Chloroform)

Extractives	Nature	Yield (% w/w)	Phytochemical analysis
Methanol	Greenish brown solid	0.4	Steroids, terpenoids
Chloroform	Brown solid	1.5	Alkaloids, phenols, terpenoids

Acute Toxicity Study: The administration of various extractives derived from Amaranthus spinosus to rats via oral gavage at doses of 50 mg/kg, 300 mg/kg, and up to 2000 mg/kg did not result in any observable signs of toxicity or adverse effects. Throughout the 14-day observation period, animals were monitored twice daily, and no clinical symptoms such as behavioral changes, weight loss, or signs of distress were noted. Importantly, there were no instances of mortality recorded at any of the administered doses. Based on these findings, it was concluded that the acute oral LD_{50} (lethal dose for 50% of the population) of the Amaranthus spinosus extractives exceeds 2000 mg/kg body weight, which was the highest dose tested in this study. The absence of toxicological manifestations at this dose suggests that the extractives are relatively safe for oral consumption in the tested range, supporting their potential for further pharmacological evaluation and therapeutic application.

Effect of the MEAS on Aspirin + Pyloric Ligation Induced Gastric Ulceration in Rats: The effect of MEAS (Methanolic Extract of *Amaranthus spinosus*) on ulcer inhibition was evaluated in aspirin + pyloric ligation-induced gastric ulceration in rats. The results are summarized in Table 2. The control group showed a high ulcer index of 5.231 ± 0.35. Treatment with MEAS at doses of 100 mg/kg and 200 mg/kg significantly reduced the ulcer index to 2.543 ± 0.36 and 2.134 ± 0.32, respectively, with ulcer inhibition percentages of 55.56% and 63.98% ($p < 0.01$). The standard anti-ulcer drug, Ranitidine (50 mg/kg), produced the most significant reduction in ulcer index (0.904 ± 0.2), showing 87.54% inhibition ($p < 0.01$).

Anti-secretory Parameters: Gastric volume in the MEAS treated groups indicated that there was no significant decrease in the volume of the gastric juice at 100 mg/kg, but at 200 mg/kg there was a significant decrease in comparison to the control group ($p<0.05$). Ranitidine at 50 mg/kg caused a significant decrease in gastric volume (Table 3). Administration of MEAS at 100 mg/kg significantly increased the gastric pH (2.652 ± 0.65, $p < 0.01$) and significantly reduced free and total acidity to 45.11 ± 3.11 and 57.11 ± 3.54 mEq/L/100g, respectively ($p < 0.01$), though the reduction in gastric volume was not statistically significant. At a higher dose of 200 mg/kg, MEAS produced a significant reduction in gastric volume (1.79 ± 0.02 ml/100g, $p < 0.05$*), a further increase in pH to 3.16 ± 0.04 ($p < 0.01$), and a marked decrease in free acidity (37.11 ± 2.64) and total acidity (41.41 ± 2.45) ($p < 0.01$). The standard anti-secretory agent Ranitidine (50 mg/kg) showed the most pronounced effects, with a gastric volume of 1.47 ± 0.01, a significantly elevated pH (5.23 ± 2.55), and a sharp decline in free (22.43 ± 2.51) and total acidity (30.54 ± 3.21), all statistically significant at $p < 0.01$.

Table 2. Effect of MEAS on Ulcer Inhibition in Aspirin + Pyloric Ligation Induced Gastric Ulceration in Rats

Treatment	Dose (mg/kg b.w.)	Ulcer Index (Mean ± SEM)	% Ulcer Inhibition	Significance
Control	10 ml/kg	5.231 ± 0.35	-	-
MEAS	100	2.543 ± 0.36	55.56	**p<0.01
MEAS	200	2.134 ± 0.32	63.98	**p<0.01
Ranitidine	50	0.904 ± 0.2	87.54	**p<0.01

Values are mean ± S.E.M, n=6, *p < 0.05 and **p < 0.01 Vs control- One way ANOVA followed by Dunnett's test

Table 3: Effect of MEAS on Anti-secretory Parameters

Treatment	Dose (mg/kg b.w.)	Gastric Volume (ml/100g)	pH (Mean ± SEM)	Free Acidity (mEq/L/100g)	Total Acidity (mEq/L/100g)	Significance
Control	10 ml/kg	2.55 ± 0.23	1.67 ± 0.32	57.23 ± 3.13	79.11 ± 3.23	-
MEAS	100	2.01 ± 0.65	2.652 ± 0.65 **	45.11 ± 3.11**	57.11 ± 3.54**	NS, **p<0.01
MEAS	200	1.79 ± 0.02 *	3.16 ± 0.04 **	37.11 ± 2.64**	41.41 ± 2.45**	*p<0.05, **p<0.01
Ranitidine	50	1.47 ± 0.01 **	5.23 ± 2.55 **	22.43 ± 2.51**	30.54 ± 3.21**	**p<0.01

Values are mean ± S.E.M, n=6, NS-not significant, *p < 0.05 and **p < 0.01 Vs control, One way ANOVA followed by Dunnett's test

Anti-diarrhoeal activity

Castor oil induced diarrhea: The antidiarrheal effect of the methanol extract of A. spinosus leaves (MEAS) was evaluated using the castor oil-induced diarrhea model, and the results are presented in Table 4. In the control group, animals treated with castor oil alone exhibited a high number of fecal droppings (11.65 ± 1.43) over 4 hours, confirming the diarrheagenic effect of castor oil. Treatment with the standard drug Loperamide (3 mg/kg) significantly reduced the number of fecal droppings to 3.25 ± 0.05, resulting in 89.41% inhibition of defecation ($p < 0.01$ vs control). Administration of MEAS at 100 mg/kg led to a significant decrease in fecal output (5.51 0.76) with 49.94% inhibition, while MEAS at 200 mg/kg further reduced fecal droppings to 4.75 ± 0.56, corresponding to 55.73% inhibition. Both doses showed statistically significant effects compared to the control ($p < 0.01$).

Table. 4: Effect of Methanol Extract of Leaves of A. spinosus on Castor oil

Group	Treatment	No. of Fecal Droppings (in 4 hrs)	% Inhibition of Defecation	Significance
I (Control)	Castor oil (1 ml p.o) + Normal saline (1 ml p.o)	11.65 ± 1.43	0%	-
II (Standard)	Castor oil (1 ml p.o) + Loperamide (3 mg/kg)	3.25 ± 0.05	89.41%	**p<0.01
III	Castor oil (1 ml p.o) + MEAS (100 mg/kg)	5.51 ± 0.76	49.94%	**p<0.01
IV	Castor oil (1 ml p.o) + MEAS (200 mg/kg)	4.75 ± 0.56	55.73%	**p<0.01

Values are presented as Mean ± SEM, (n=5); ** p<0.05, Dun net's t-test as compared to Control.

Gastro intestinal transit time: The impact of MEAS on gastrointestinal transit time, assessed by the time taken for the first appearance of a fecal bolus following charcoal suspension administration, is shown in Table 5. In the control group, the first fecal bolus was observed at 50.11 ± 1.71 minutes. In contrast, the standard drug Loperamide (3 mg/kg) significantly delayed the onset of the first fecal bolus to 148.55 ± 5.66 minutes ($p < 0.01$), confirming its well-established inhibitory effect on intestinal motility. MEAS also significantly delayed the gastrointestinal transit time at both doses: At 100 mg/kg, the first fecal bolus appeared at 68.11

± 4.87 minutes ($p < 0.01$ vs control). At 200 mg/kg, the time further increased to 102.4 ± 3.43 minutes ($p < 0.01$ vs control), indicating a dose-dependent inhibition of GI motility.

Table. 5: Effect of Methanol extract of leaves of Amaranthus spinosus on Charcoal suspension stimulated gastrointestinal transit time

Group	Treatment	Time (minutes) for 1st Fecal Bolus	Significance
I (Control)	Distilled water (1 ml)	50.11 ± 1.71	-
II (Standard)	Loperamide (3 mg/kg)	148.55 ± 5.66	**p<0.01
III	MEAS (100 mg/kg)	68.11 ± 4.87	**p<0.01
IV	MEAS (200 mg/kg)	102.4 ± 3.43	**p<0.01

Values are presented as Mean ± SEM, (n=5); ** p<0.05, Dunnet's t-test as compared to Control.

CONCLUSION

The methanol extract of *Amaranthus spinosus* leaves exhibits significant anti-ulcer and antidiarrheal activities in experimental models. Its ability to protect the gastric mucosa and reduce intestinal motility and secretion supports its traditional use in treating gastrointestinal disorders. These findings provide a scientific basis for the therapeutic potential of *A. spinosus* and warrant further studies, including isolation of active constituents, mechanistic investigations, and clinical validation.

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