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In-Vivo Screening of Acid-Neutralising Capacity of Methanolic Extract of Achyranthes aspera

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ABSTRACT

Background and Aim: The possible antiulcer qualities of the Amaranthaceae family have drawn interest. Alternative treatments for peptic ulcers, a common gastrointestinal ailment, must be investigated; Amaranthaceae species may provide encouraging leads in this respect. As a result, we have chosen Achyranthes aspera from the Amaranthaceae family to assess its antiulcer and antioxidant qualities.

Methodology: Using petroleum ether, chloroform, methanol, and water, the plant components were extracted by the successive Soxhlet extraction procedure. The stable "1,1-diphenyl-2-picrylhydrazyl" (DPPH) free radical action and hydrogen peroxide process were taken into account while evaluating the antioxidant activity of the plant extracts and the conventional medications. The methanolic extract of Achyranthes aspera (MEAA) was tested for its antiulcer properties using pyloric ligation and ulcers caused by non-steroidal anti-inflammatory drugs.

Result: Triterpenoids, flavonoids, alkaloids, tannins, glycosides, and proteins are all detected by phytochemical analysis of methanolic extracts. According to the findings of the antioxidant investigation, the MEAA has the highest potential for antioxidant activity among the four extracts. The extract-treated classes and the standard class showed a substantial ($p<0.05$) decrease in stomach volume, total, and free acidity as compared to the control. With 500 mg/ml PL and NSAID, the MEAA showed significant ($p<0.05$) percent inhibition, i.e., 59.42 and 69.59 percent, respectively. Conclusion: The results of this study showed that Achyranthes aspera's methanolic extract had strong antiulcer and antioxidant properties.

Keywords: *Achyranthes aspera, Amaranthaceae, Antioxidant, Antiulcer, Extraction*

1. INTRODUCTION

Traditional knowledge of Indian herbal therapy has contributed greatly to the global development of highly reliable, contemporary and alternatively healthcare systems. Peptic ulcers are a recurring wound that typically affects middle-aged to older persons, limiting their quality of life. They are the main cause of gastro-intestinal surgery, with high prevalence and death rate[1]. The development of ulcers is primarily driven by several competitive factors, including acid, pepsin, bile and helicobacter pylori infection. Additionally, various lifestyle and environmental factors, such as stress, alcohol consumption, smoking, use of steroid and non-steroidal anti-inflammatory drugs (NSAIDs), and low socioeconomic conditions, can contribute to the formation of ulcers[2,3,4,5]. Herbal treatments, especially those made from flowers, are becoming more and more popular in the anti-ulcer drug market in addition to allopathic capsules because of their perceived safety, effectiveness, and user-friendliness. Although ulcers often do not pose a threat to life, they can cause major side effects include bleeding in the gastrointestinal tract, perforation, penetration into adjacent organs, and obstruction of the gastric outlet[6]. Some drugs can treat ulcers, reduce pain, and stop them from coming back. These consist of proton pump inhibitors, antacids, and antibiotics[7]. Many drugs are available to treat stomach ulcers, however they frequently have unfavourable side effects[8].

The flowering plant family Amaranthaceae has many species with a wide range of traditional folk medicinal use. Several Amaranthaceae family members have drawn interest over time due to their possible antiulcer qualities. Alternative treatments for peptic ulcers, a common gastrointestinal ailment, must be investigated; Amaranthaceae species may provide encouraging leads in this respect[9]. As a result, we have chosen Achyranthes aspera from the Amaranthaceae family to assess its antiulcer and antioxidant qualities.

2. MATERIALS AND METHODS

Chemicals

The medication and chemical utilised were DPPH (1,1-diphenyl-2-picrylhydrazyl) from Sigma Chemical Co. All of the analytical-grade reagents used in the investigation procured from Mumbai-based S.D. Fine Chemicals Ltd. and Hi Media.

Plant Materials

The plant materials were collected from roadside areas of Maharashtra, India. They were positively identified by Department of Botany, S.S.S. K. R. Innani Mahavidyalaya, Karanja (Lad), Dist: Washim (M.S.) and the voucher specimen was deposited in a laboratory.

Preparation of plant extracts

The plant material was cleaned and rinsed twice in tap water and once in distilled water as soon as it was collected in order to remove any unwanted materials and outside soil. For 72 hours, the entire plant was kept in the shade to dry. Using Soxhlet's apparatus, the plant material was extracted in stages using petroleum ether, chloroform, methanol, and water. A rotary evaporator was used to dry out the Soxhlet extract at 60°C, and the yield was noted. The extracted materials were marked as follows:PEAA: Petroleum ether extract of *Achyranthes aspera*; CEAA: chloroform extract of *Achyranthes aspera*; MEAA: Methanol extract of *Achyranthes aspera*; WEAA: Water extract of *Achyranthes aspera*

3. ANTIOXIDANT STUDY

DPPH Solution (0.1 mM)

The DPPH solution was made by dissolving 33 mg of DPPH in one litre of analytical-grade methanol, and it was then stored in an amber-colour bottle to protect it from the sun.

Ascorbic Acid

Ten milligrams of ascorbic acid were dissolved in one hundred millilitres of distilled water to create a stock solution containing 100 µg/ml. Ascorbic acid concentrations of 10, 20, 40, 80, 100, and 200 µg/ml were produced by this solution.

Sample Preparation

10 mg of various Achyranthes aspera extracts were mixed with 10 ml of methanol to create a 1 mg/ml stock solution. After that, solutions with varying extract concentrations 10, 20, 40, 80, 100, and 200 µg/ml were made from the stock solution.

DPPH Radical Scavenging Assay Method

The modified procedure of stable DPPH-free radical activity was used to assess the antioxidant activity of plant extracts based on their ability to scavenge radicals[10]. The optical density was recorded, and the percentage of inhibition was computed using the formula shown below[11].

$$Percentage\ inhibition\ of\ DPPH\ activity = \left[\frac{A-B}{A}\right] * 100$$

Where A = the blank absorbance and B = the sample absorbance. Compared to positive controls, the real reduction in absorption caused by the test was compared. The IC50 (50 percent inhibition concentration) values were determined using the linear dose inhibition curve by plotting the concentration of the extract versus the corresponding scavenging effect.

Hydroxyl peroxide method

A 0.1 M phosphate buffer with a pH of 7.4 (2.4 mL) and 43 mM hydrogen peroxide solution (0.6 mL) were mixed with different amounts of methanolic extract (10-200 µg/mL). After 10 minutes, the optical density was measured at 230 nm. There was a blank sample used. Ascorbic acid was the typical medication utilised.

4. ANTIULCER PROPERTY

Animals

Male Wistar albino rats weighing 160–200 g were gathered from the animal house's institutional facility and divided into five groups of six animals at random. Unless otherwise noted, they were given regular pellet meal and water "ad libitum" while being kept in "polypropylene cages" over husk bedding. The animals were kept at 25 ± 2 °C with a 12-hour dark and light interval. The "Institutional Animal Ethics Committee" (IAEC) gave its approval and all of its rules were followed before any animal experiments were carried out.

Antiulcer activity Evaluation

Experimental setup for “pyloric ligation induced gastric ulcer”. The animals were allocated into 5 classes, each consisting of

6 rats.

Class I: Vehicle (0.9% w/v, p.o normal saline.) administered 1 h prior to pyloric ligation on the day of the procedure.

Class II: For ulcer induction, rats are subjected to pyloric ligation.

Class III: MEAA (250 mg/kg, p.o.) administered 1 h before pyloric ligation on the day of the procedure.

Class IV: MEAA (500 mg/kg, p.o.) administered 1 h before pyloric ligation on the day of the procedure.

Class V: Standard administration (ranitidine 50 mg/kg, p.o.) 1 h before pyloric ligation on the day of experimentation.

Following an 18-hour fast, ulcers developed, and the experiment was carried out in accordance with the guidelines provided in the studies by Beena et al. (2011)[10] and Devhare and Gokhale (2022)[11]. Similarly, the animals were divided into five groups, each consisting of six rats, for the "NSAID-induced ulcer model" experimental design:

Class I: Vehicle administered (0.9 percent w/v, p.o normal saline) 30 min prior ulcers caused by Indomethacin

Class II: Indomethacin-administered disease control group (25 mg/kg, p.o.) for gastric ulcers induction

Class III: 250 mg/kg, p.o. MEAA was administered 30 min before ulcers caused by Indomethacin

Class IV: 500 mg/kg, p.o MEAA administered 30 minutes before ulcers caused by Indomethacin

Class V: Standard administration (50 mg/kg, p.o. ranitidine) 30 min prior ulcers caused by Indomethacin

According to Buzlama et al (2021)[12] the experimental design was carried out for the NSAID-induced ulcer model.

Estimation of gastric volume

Four hours following ligation, stomachs were dissected, and material was collected to calculate the amount of gastric content in the measuring cylinder.

Measurement of total acidity and free acidity:

To determine the total and free acidity, the stomach contents were centrifuged and subjected to titration. After dissolving 1 ml of the liquid supernatant in distilled water, 10 mL was pipetted out. The liquid was then titrated against 0.01N NaOH to the equivalency point, where the solution turned orange, using "Topfer's reagent" as an indicator. The titration process was continued after adding a 1 percent phenolphthalein solution until the liquid turned pink. The right amount of NaOH was noted and interpreted as a reference to the total acidity. Two titrations were added to indicate absolute acidity[13].

Ulcer index:

The number of ulcers was counted and scored using the methodology described by Al-Thobaiti et al., 2022)[14]. The formula provided by Joshi et al (2022)[15]was used to calculate the percentage of ulcer defence.

Statistical analysis:

The "p value<0.05" was deemed significant, and the data was interpreted as "mean ± SEM." An "one-way analysis of variance" (ANOVA) was performed on the data. Using a statistical package of SPSS statistics ((Version 7.5), "Tukey's multiple range tests" assessed data to determine the significance level of mean differences between several treated classes.

5. RESULT

Preliminary phytochemical analysis

Triterpenoids, flavonoids, alkaloids, tannins, glycosides, and proteins are all detected by phytochemical analysis of methanolic extracts. Flavonoids, triterpenoids, tannins, and carbohydrates are all present in the chloroform extracts. Alkaloids, glycosides, flavonoids, and triterpenoids are all present in the water extracts. Thus, it was further investigated for various properties such as antiulcer and antioxidants.

Antioxidant Activity

Using in vitro models of hydrogen peroxide and DPPH radical techniques, the antioxidant activity of various solvent extracts of Achyranthes aspera was tested at doses ranging from 10 to 200 µg/ml. The DPPH method results showed that the scavenging capacity of MEAA compared to the standard resulted in a substantial ($p<0.05$) drop in the DPPH radical concentration, with the percent inhibition for MEAA extracts at 200 µg/ml being 66.55±0.09 (table 1). As shown in Table 2 and Figure 1, the H2O2 method's ideal scavenging capability was 58.69±0.23 at 200 µg/mL. When these two approaches were compared, the MEAA's antioxidant capability was significantly ($p<0.05$) higher.

The results of the antioxidant analysis showed that the methanolic extract (MEAA) has the highest antioxidant potential among the four extracts. Therefore, the same extract is being studied further for its antiulcer potential.

Table 1: Percent inhibition of extracts by using DPPH radical scavenging assay

SN	Samples	Percent Inhibition					
		Drug Concentration (µg/ml)					
		10	20	40	80	100	200
1	PEAA	1.28±1.01	3.26±1.25	7.25±0.19	9.28±0.84	11.91±0.64	15.73±0.43
2	CEAA	2.25±1.45	4.42±0.85	5.65±1.45	10.45±1.45	14.45±2.12	19.78±1.48
3	**MEAA**	9.66±1.01	19.44±2.04	29.99±0.96	46.55±0.96	56.76±0.11	66.55±0.09
4	**WEAA**	5.006±0.75	12.3±5.66	33.46±0.68	41.46±0.66	50.38±1.23	53.18±1.47
		Drug Concentration (µg/ml)					
		10	20	40	80	100	200
5	**Ascorbic Acid**	27.96±0.21	37.14±0.38	50.51±3.21	57.29±0.25	63.42±1.27	72.13±1.5

The values are expressed as Mean±SEM

Table 2: Percent inhibition of extracts by using Hydroxyl peroxide method

Sr. No.	Samples	Percent Inhibition					
		Drug Concentration (µg/ml)					
		10	20	40	80	100	200
1	PEAA	1.62±1.29	2.12±1.23	4.29±0.99	6.66±0.36	10.58±0.66	14.32±1.89
2	CEAA	1.19±0.97	3.65±0.79	7.65±1.63	11.42±0.99	16.5±1.98	21.23±1.53
3	**MEAA**	15.26±0.98	24.45±0.7	32.26±0.98	37.85±0.36	45.23±1.15	58.69±0.23
4	**WEAA**	4.45±0.89	15.65±1.23	25.66±0.22	37.46±0.65	45.68±0.58	49.28±1.35
		Drug Concentration (µg/ml)					
		10	20	40	80	100	200
5	**Ascorbic Acid**	28.76±0.23	38.54±0.28	51.61±4.31	58.28±0.15	61.52±1.17	71.83±1.6

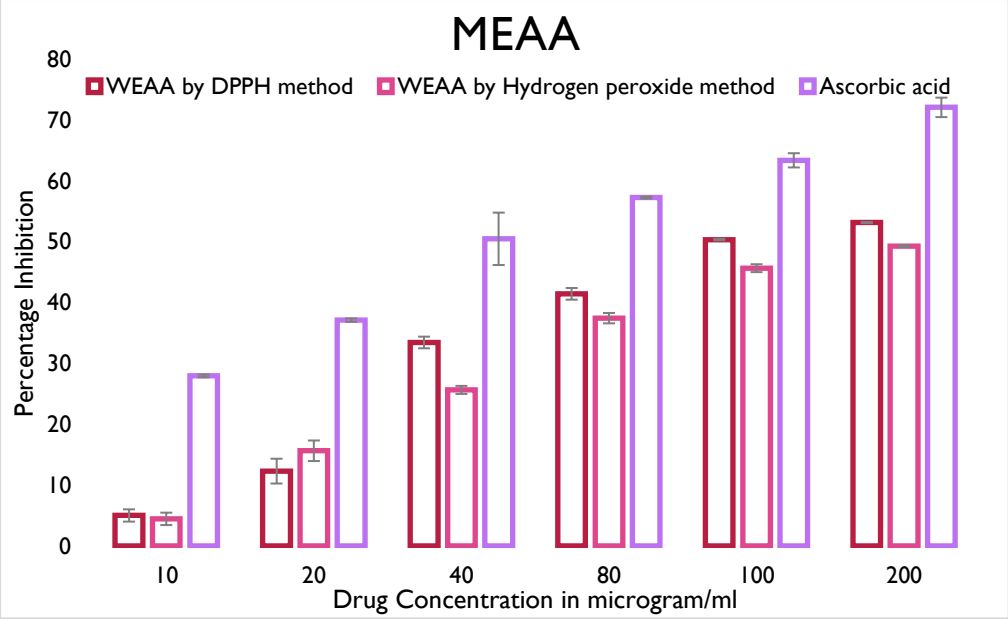

Figure 1: Percent inhibition of MEAA by Hydrogen peroxide and DPPH method

Acute toxicity study

An acute oral toxicity study was conducted in accordance with OECD 423 standards. The Achyranthes aspera solvent extract was tested for acute toxicity (LD50) in rats. The extract did not cause any deaths in the acute toxicity testing up to 5000 mg/kg.

Antiulcer activity:

The anti-ulcerogenic effect of MEAA was examined using two models of pyloric ligation-mediated ulcers and non-steroidal anti-inflammatory ulcers. Gastric volume, free, and total acidity were noted in the control group in the event of pylorus ligation. In comparison to the control score, the extracts significantly ($p<0.05$) decreased the ulcer score (Table 2). The ulcer index was reduced by ranitidine (250 mg mL^{-1}) and MEAA (500 mg mL^{-1}).

When compared to the control group, a statistically significant ($p<0.05$) decrease in stomach volume, total acidity, and free acidity suggests that the MEAA treatments were successful in lowering ulcer-causing variables. According to the considerable inhibition percentages (59.42% for PL and 69.59% for NSAIDs), ulcer development was effectively prevented by both the extract and the conventional treatment. Table 3 shows the effects of MEAA on the ulcerative index and % inhibition in PL and NSAID.

Table 3: Influence on gastric volume, total acidity and free acidity in PL induced ulcers

Group	Dose (mg/kg)	Gastric Volume (ml/100 gm)	Total Acidity (mEq/L/100gm)	Free Acidity (mEq/L/100gm)
Normal	-	4.29±0.68	74.68±0.93	42.36±0.56
Control	-	8.01±0.98[a]	98.56±1.25[a]	55.02±0.91[a]
MEAA	250	3.02±0.25[b]	70.89±1.02[b]	39.23±0.37[b]
MEAA	500	1.97±0.56[c]	62.85±1.49[c]	35.26±0.45[c]
Ranitidine	50	1.49±0.85[b]	57.29±1.78[b]	29.49±0.39[b]

"Data presented as Mean±SEM, n = 6; [a]$p<0.05$ compared to control group, [b]$p<0.05$ compared to treated group, [c]$p<0.05$ compared to ranitidine group"

Table 4: Influence of MEAA on % inhibition and ulcerative index in PL and NSAID

Group	Dose (mg/kg)	Ulcer index		Percentage inhibition	
		PL	NSAID	PL	NSAID
Normal	-	0.78 ± 0.05	0.78 ± 0.05	-	-
Control	-	8.28 ± 0.42[a]	15.23 ± 1.29[a]	-	-
MEAA	250	5.27 ± 0.64[b]	7.56 ± 0.87[b]	36.23	50.36
MEAA	500	3.36 ± 0.52[c]	4.63 ± 0.61[c]	59.42	69.59
Ranitidine	50	3.03 ± 0.49[b]	4.29 ± 1.19[b]	63.40	71.83

"Data presented as Mean±SEM, n = 6; [a]p<0.05 compared to control group, [b]p<0.05 compared to treated group, [c]p<0.05 compared to ranitidine group"

6. DISCUSSION

An imbalance between defensive mechanisms, such as blood flow and mucosal integrity, and aggressive factors, such as gastric acid output, leads to peptic ulcers, which include gastric and duodenal ulcers.One Historically, high acid secretion that caused mucosal injury was the main contributing reason. However, our knowledge of ulcer causation was broadened by the identification of Helicobacter pylori infection and the extensive use of NSAIDs. Because the mucosal barrier is disrupted by H. pylori colonisation and NSAID-induced mucosal injury, acid and other aggressive factors can destroy the underlying tissue and cause ulcers.[16]

Conventional drugs like proton pump inhibitors (PPIs), antibiotics, and cytoprotective medicines are frequently used to treat peptic ulcers. Nonetheless, traditional medical systems around the world have long used herbal medications to treat ulcers.

Pre-treating pylorus-ligated rats with 500 mg per kg MEAA (group IV) and ranitidine (group V) had comparable antiulcer activity in the current study, as evidenced by a reduction in the increase in stomach volume, pH, and free and total acidity. "The methanol bark extract of *Mimusopselengi L. (Sapotaceae)*[17] and flower extract of *Hemidesmus indicus R. Br. (Asclepiadaceae)*[18] were reported to reduce total acidity and volume of gastric acid secretion in gastric ulcer-induced rats, and this antiulcer activity was attributed to the strengthening of the mucosal defence mechanism by these plant extracts". According to Suzuki and Ishii (1996),[19] "The gastric and duodenal mucosa are protected against hydrochloric acid by an increase in bicarbonate ion concentration. The gastric mucosa's epithelial cells, which are impermeable to hydrogen ions, may be the cause of this mucosal defence mechanism[20].Additionally, as prostaglandins in parietal cells increase mucosal resistance, possibly by boosting the synthesis of mucus and bicarbonate and strengthening the mucosal barrier, protection against experimental ulcers may be linked to their activity[21,22].Based on these results, it is hypothesised that the strengthening of the mucosal defence system is responsible for the anti-ulcer activity of MEAA as revealed in the current study.

Previous research has demonstrated that the entire plant possesses a variety of pharmacological properties, including anti-inflammatory[23], antibacterial[24], and antifertility properties[25].A phytochemical analysis revealed that amaranthine is one of the marker constituents, along with quercetin, kaempferol, and isoquercitrin[26].Several researchers' extraction of flavonoids from Achyranthes aspera created the foundation for confirming the plant's overall antioxidant capacity[27,28]. It is unclear exactly which mechanisms of action the MEAA uses to protect cellular functions from oxidative stress. Therefore, the antioxidant function of Achyranthes aspera was assessed using models like DPPH and hydrogen peroxide. The free radical DPPH is purple and has high maximum absorption at 517 nm.When the radical electron combines with hydrogen, DPPH is reduced to DPPH-H, resulting in a hue shift from purple to yellow[29]. In the current study, the hydrogen peroxide procedure had the maximum activity, which was 200 µg/mL. The results showed that the MEAA by DPPH technique had the best antioxidant capacity, which was 66.55±0.009. The hydrogen peroxide technique showed 58.69±0.23 percent inhibition by MEAA, indicating that the extract exhibited exceptional free radical scavenging activity.

7. CONCLUSION

The results of this study showed that Achyranthes aspera's methanolic extract had strong antiulcer and antioxidant properties. MEAA's antiulcer activity reduces the production of stomach acid, inhibits the production of free radicals or prevents lipid peroxidation, protects the mucosal barrier and restores mucosal secretions, and has antioxidant or free radical scavenging qualities. Additionally, the antiulcer activity of "non-steroid anti-inflammatory induced ulcer and pylorus ligation induced gastric ulcer" was investigated; the results demonstrated a strong antiulcer effect in comparison to normal. The strong antioxidant and antiulcer properties of Achyranthes aspera are supported by these findings, which also contain pharmacological evidence. Additionally, more research can be done to ascertain whether the medicine is dangerous or non-toxic.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

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