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Evaluation of Antiulcer Activity of *Amaranthus dubius* Leaves in Swiss Albino Mice

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KEYWORDS

Amaranthus dubius, Antioxidant activity, Ethanol induced ulcer, Ulcer index

ABSTRACT:

Objectives: The purpose of this study was to assess the antiulcer activity of *Amaranthus dubius* leaves extract (Amaranthaceae) in Swiss albino mice.

Methods: The effect of *Amaranthus dubius* leaves on gastric secretion and effect of gastric cytoprotection were evaluated using ethanol induced gastric ulcer. In mice, oral doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg of ADAE were given. Pantoprazole (40mg/kg) was used as standard drug. In-vitro antioxidant activity was measured using two assays: DPPH radical scavenging and alkaline DMSO methods. Gastric damage was evaluated by scoring ulcers, measuring ulcer index, and determining gastric juice pH. In addition, biochemical tests for nitric oxide and malondialdehyde (MDA) concentrations as well as histopathological analysis were carried out.

Results: Preliminary phytochemical screening revealed the presence of bioactive compound. In comparison to the control group, the ADAE resulted in a significant decrease in ulcer index and an increase in the pH of gastric juice. At highest dose, the extract exhibited 56.04% ulcer inhibition. Histopathological investigation it was observed that the group with highest dose of test extract showed mild haemorrhages and cellular infiltrations as well as less structural damage. Biochemical tests indicated that ADAE reduced gastric MDA levels and increased nitric oxide levels. An IC50 value of 131.16 µg/ml was found using the DPPH assay, and the alkaline DMSO method showed strong inhibition of superoxide radicals.

Conclusions: The present study concluded that *Amaranthus dubius* leaves extract shows a good anti-ulcer effect at 200 mg/kg dose with its gastroprotective mechanism and antioxidant property.

1. Introduction

A peptic ulcer is an acid-induced lesion of the digestive tract that usually affects the stomach or the proximal duodenum. The mucosa is cleared, and the damage extends across the muscularis propria or submucosa. The general population is estimated to be affected by peptic ulcer disease (PUD) in 5–10% of cases [1]. An imbalance in mucosal defensive factors, such as mucus secretion, bicarbonate efflux, endogenous antioxidant, cell regeneration, and ongoing production and emission of prostaglandin E2 (PGE2), nitric oxide (NO), and sulfhydryl compounds (SH), is the cause of these ulcers along with aggressive substances like smoking, alcohol consumption, dietary factors, stress, and prolonged and excessive use of nonsteroidal anti-inflammatory drugs (NSAIDs). Peptic ulcer symptoms may include a range of issues, including burning or gnawing pain, nausea, vomiting, bloating, and changes in appetite. Generally, some gastroprotective medicines used to treat peptic ulcer disease includes H2 receptor antagonists, proton pump inhibitors and drugs used in triple and quadruple therapy [2].

Amaranthus dubius commonly known as Red Spinach or Spleen Amaranth, belongs to the Amaranthaceae family. Although it originated in the West Indies, Mexico, and South America, it has spread across the globe. It is usually used as folk medicine in many places of India [3]. The plant has been reported to exhibit antidiabetic, anticancer, anti-obesity, antiviral, antioxidant and anti-inflammatory activities. Bioactive plant components

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include alkaloids, flavonoids, glycosides, phenolic acids, steroids, amino acids, terpenoids, lipids, and saponins. Being an important source of minerals, the consumption of *Amaranthus dubius* may prevent and improve conditions such as osteoporosis and anemia. It has also been traditionally used to alleviate stomach aches, bleeding, and fever [4].

2. Materials and Methods

Collection and Authentication of Plant

The leaves of *Amaranthus dubius* were collected from Pune, Maharashtra and were authenticated by Mr. Mahesh Atale, MSc. Botany, Alarsin Pioneers in Ayurvedic Research, Andheri (E), Mumbai – 400 093 and specimens were submitted to the Pharmacology department, Konkan Gyanpeeth Rahul Dharkar College of Pharmacy, Karjat, Maharashtra- 410201.

Preparation of extract

The leaves of *Amaranthus dubius* were washed with tap water and shade-dried at normal room temperature. After drying, they were milled into a fine powder. Then, 50g of *Amaranthus dubius* leaves powder was mixed with 500ml distilled water. This mixture was heated to 100°C for 90 min. After boiling, the mixture was allowed to cool down to 25°C. The cooled mixture was separated, and the liquid was filtered through whatman grade 1 filter paper to remove solid particle. Filtered liquid, which contains the extract from the *Amaranthus dubius*, was then freeze-dried. (lyophilized) to remove the water and concentrate the extract. Finally, the concentrated extract was stored at 4°C [5].

Phytochemical screening:

Preliminary chemical tests were carried out on aqueous extract of *Amaranthus dubius* leaves for determining the existence of phytoconstituents like alkaloids, saponins, tannins, flavonoids, carbohydrates, glycosides [6].

IN-VITRO antioxidant studies:

DPPH radical scavenging assay:

Antioxidant activity in the sample compounds was estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals.1 ml of test compounds water were taken in the test tube. 1 ml of 0.1% ethanolic DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively and the absorbance of the mixture was measured at 517 nm [7].

DPPH radical scavenging activity (%) = [(Absorbance of control – Absorbance of test sample) / (Absorbance of control)] x 100

Alkaline DMSO method:

In this method, superoxide radical is generated by the addition of sodium hydroxide to air saturated DMSO. The generated superoxide remains stable in solution and reduces nitro blue tetrazolium (NBT) into formazan dye at room temperature which can be measured at 560 nm. To the reaction mixture containing 1000 µL of alkaline DMSO, 300 µL of the drug samples in concentration 1000µg/ml and standard ascorbic acid 1000µg/ml was added in DMSO at various concentrations followed by 100 µL of NBT (0.1 mg). The absorbance was measured at 560 nm [8].

Scavenging of Superoxide radical by Alkaline DMSO Method (%) = [(Absorbance of control – Absorbance of test sample) / (Absorbance of control)] x 100

Experimental animal

Healthy Swiss Albino Mice, 8-9 weeks old were used for the study. The animals were procured from the central animal facility of the institute. The use of these animals and the study protocols were approved by the Institutional Animal Ethics Committee of college. Mice were kept at the animal house in polypropylene cages, at 22 ± 2ºC, with 12:12 hrs dark: light cycle. they were provided with commercial mice feed and water ad libitum

Acute oral toxicity

As per the literature survey, it was found that aqueous extract of *Amaranthus dubius* leaves was safe for animal use as LD50 was considered as 2000 mg/kg. Thus, for the study the doses of *Amaranthus dubius* leaves extract were finalized to be 200 mg/kg high dose, 100 mg/kg median dose, 50 mg/kg low [9].

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Ethanol induced gastric ulcer model:

The principle of the cytoprotective action makes the ethanol-induced gastric ulcer model, which is one of the most popular models, applicable to mice. In addition to known to inhibit prostaglandin release, ethanol is known to cause damage to the superficial epithelial layers. Additionally, it raises blood neutrophil concentrations, which disrupts the microcirculation and produces reactive oxygen species. These events trigger the activation of H+/K+ ATPase, or the proton pump, and cause acid hypersecretion that damages the GI mucosa.

One of the most commonly prescribed medications for treating gastric ulcers is pantoprazole. It's a member of the class of drugs known as proton pump inhibitors, which stop stomach acid from being secreted. Proton pump inhibitors are prodrugs that convert to the sulfonamide cation form and bind to the H+/K+ ATPase sulfhydryl group irreversibly to completely inhibit acid secretion. This activity on nocturnal acid secretion is also provided. Any chemical that inhibits ethanol-induced ulcer formation may also have cytoprotective properties and work by inducing the release of mucin and endogenous prostaglandins [10].

Procedure:

Animals were randomly assigned to 6 groups (n=06).
Group 01: Normal-Vehicle 10 ml/kg p.o.
Group 02: Ulcer Control: Vehicle 10 ml/kg + Ethanol (1 ml/kg) p.o.
Group 03: Standard: Pantoprazole 40 mg/kg + Ethanol (1 ml/kg) p.o.
Group 04: Test dose 1: ADAE 50 mg/kg + Ethanol (1ml/kg) p.o.
Group 05: Test dose 2: ADAE 100 mg/kg + Ethanol (1 ml/kg) p.o.
Group 06: Test dose 3: ADAE 200 mg/kg Ethanol (1 ml/kg) p.o.

Mice were fasted for 12 hrs. before the start of the experiment. Then the first group received an oral dose of the vehicle (1 ml/kg) and the third group pantoprazole (40 mg/kg), 4, 5th and 6 groups received 50, 100, 200 mg/kg body weight of *Amaranthus dubius* aqueous extract (ADAE) respectively. This treatment was given for 7 days. On the 8th day of the experiment ethanol was administered to the animals and euthanasia was carried but in a Co2 chamber followed by extraction of gastric tissue collection of gastric content and microscopical evaluation of tissue for abnormalities in the mucosal lining Samples were then forwarded for histopathological evaluation.

The stomachs of each animal were extracted and opened along the greater curvature, and for examination of lesions. Lesions were counted by analysis in Image) software. The ulcers were scored according to

0 - Normal, No ulcer, 0.5 - Red Coloration, 1 - Spot Ulcer, 1.5 - Haemorrhagic Streak, 2 - Deep Ulcer, 3 – Perforations

Determination of ulcer index:

After scoring ulcers according to their severity, the mean ulcer score for each animal was expressed as ulcer index. The ulcer index was measured by using the following formula.

$$UI = UN+US+UP\times 10\text{-}1$$

Where, UI = Ulcer Index, UN = Average number of ulcers per animal, US = Average number of severity score, UP = Percentage of animal with ulcer.

Percentage inhibition of ulceration was calculated as below:

% Inhibition of Ulceration = (Ulcer Index Control – Ulcer Index Test) 100/Ulcer Index Control

Histopathological Evaluation

The mice stomach tissues were preserved using a 10% buffered formalin solution for histopathology assessment. Samples were analysed by "Unique Bio Diagnostics Enterprises" in Mumbai, India.

Biochemical Tests

Determination of malondialdehyde (MDA):

Stomach ulcer tissue homogenate was prepared, taken a mixture of 0.4 ml of 10% stomach ulcer homogenate added 1.5 ml of 8.1% Sodium dodecyl sulphate. 1.5 ml of 20% acetate buffer (pH 3.5) and 1.5 ml of 0.8% TBA solution were added to above mixture. The mixture was heated at 95°C for 60 min and cooled to room temperature. After cooling added 5 ml of n-butanol-pyridine (15:1). Vortexed the mixture thoroughly and allowed to stand until the organic and aqueous layers will not get separated. Recorded absorbance of organic layer at 532 nm on UV-visible spectrophotometer [12].

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Determination of total nitrite/nitrate contents (N.O):

Stomach ulcer tissue homogenate was prepared, taken a mixture of 0.4 ml of 10% stomach ulcer homogenate added 50µl 30% ZnSO4 for protein precipitation. Then, precipitated protein was removed by centrifugation for 15min. 100µl of the resulting supernatant was diluted to 300µl with water and treated with 300µl vanadium trichloride (0.8 g % in 1 M HCl), followed by rapid addition of 150µl sulphanilamide (2% in 5% HCl) followed by 150µl N-1-(naphthyl) ethylenediamine dihydrochloride (0.1%). The mixtures were then incubated at 37°C for 30 min. and then cooled. Recorded absorbance at 540 nm on UV-visible spectrophotometer [13].

Statistical Analysis

GraphPad Prism (version 10.2.2) was used to analyze the data gathered from the animal trials. The outcomes are expressed as Mean ± SEM (standard error of the mean) over each category. The data were subjected to Dunnett's test after analysis of variance (ANOVA) for a statistical analysis. Test with a p-value ≤ 0.05 was interpreted as statistically significant.

3. Results

Phytochemical screening

Based on the results of various chemical tests, the phytochemical analysis of the aqueous extract of *Amaranthus dubius* leaves revealed the presence of alkaloids, carbohydrates, flavonoids, tannins, glycosides and saponins (see Table 1).

Table 1: Phytochemical test of *Amaranthus dubius* extract

Phytochemical	Test	Inferences
Alkaloids	Mayer's Test	Present
	Wagner's Test	Present
Flavonoids	Shinoda Test	Present
	Ferric Chloride Test	Present
Glycosides	Keller- Kilani Test	Present
Tannins	Ferric Chloride Test	Present
	Lead Acetate	Present
Saponins	Foam Test	Present
Carbohydrate	Molisch's Test	Present
	Benedict's Test	Present

***IN-VITRO* tests on extract: (Antioxidant assay)**

DPPH radical scavenging assay:

The IC_{50} values of extracts and the control (ascorbic acid) were calculated as follows**.**

Table 2: IC_{50} Determination of extract

Sr no	Sample	IC_{50}(µg/ml)
1	Ascorbic acid	115
2	*Amaranthus dubius* leaves extract	131.16

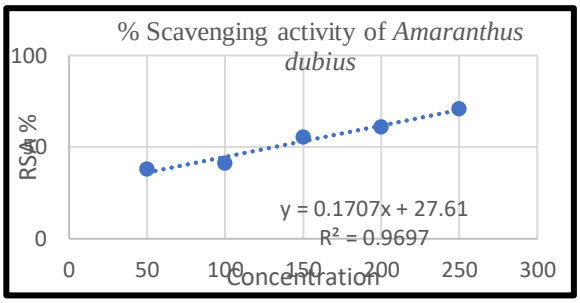

Figure 1: DPPH radical scavenging activity of *Amaranthus dubius* leaves extract

The DPPH radicles can be scavenged by these extracts. Stronger antioxidant activity was shown *by Amaranthus dubius* leaves extract (IC50 = 131.16µl/ml).

Alkaline DMSO method: The antioxidant activity of *Amaranthus dubius* leaves extract was carried out bt alkaline DMSO method using Ascorbic acid as reference standard antioxidant.

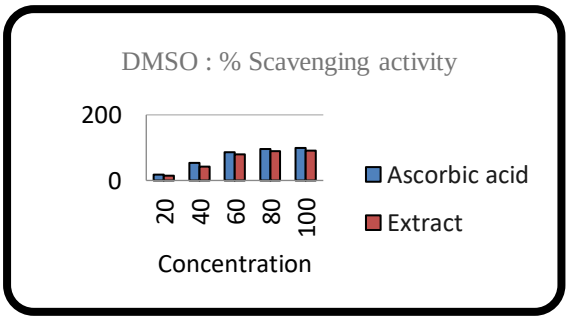

Figure 2: Superoxide Radical Savenging Activity of *Amaranthus dubius* leaves extract by Alkaline DMSO

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Antioxidants mediate their protective effect by directly reacting with free radicals, quenching them and thereby preventing damage to cellular components, thus consequently hindering diseases. The superoxide radical scavenging activity of *Amaranthus dubius* extract was assessed by the alkaline DMSO method. The extract sample strongly inhibited the superoxide radical generation.

Ethanol induced ulcer:

I. Ulcer index and % inhibition of ulcer

It was found that a 200 mg/kg dose of *Amaranthus dubius* extract shows significant inhibition of ulcers compared to the standard and ulcer control groups

Table no 03 show that the % occurrences of ulcer in stomach is significantly inhibited by the standard drug pantoprazole at doses of 50 mg per kg, 100 mg/kg, and 200 mg/kg of body weight [F (5,30) = 9.49; $p<0.05$ Fig no.3].

Table 3: Effect of ADAE on Ethanol Induced Ulcer

Treatment	Dose	Ulcer Index	% Inhibition
Ulcer control	1 ml/kg	46.64 ± 5.712	-
Pantoprazole	40 mg/kg	19.68 ± 3.687**	57.80%
ADAE	50 mg/kg	24.89 ± 5.617	46.63%
ADAE	100 mg/kg	22.76 ± 4.999	51.20%
ADAE	200 mg/kg	20.50 ± 6.087*	56.04%

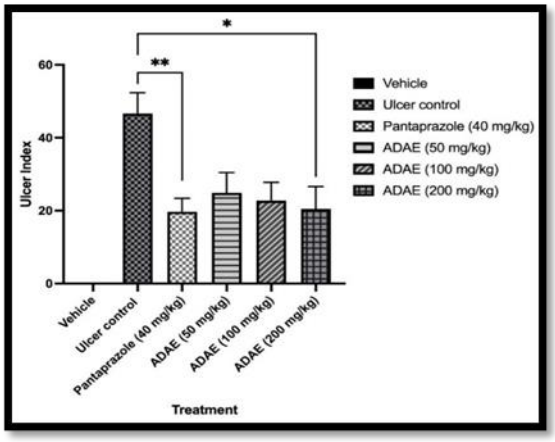

Figure 3: Effect of ADAE on ulcer index in Ethanol-Induced Ulcer

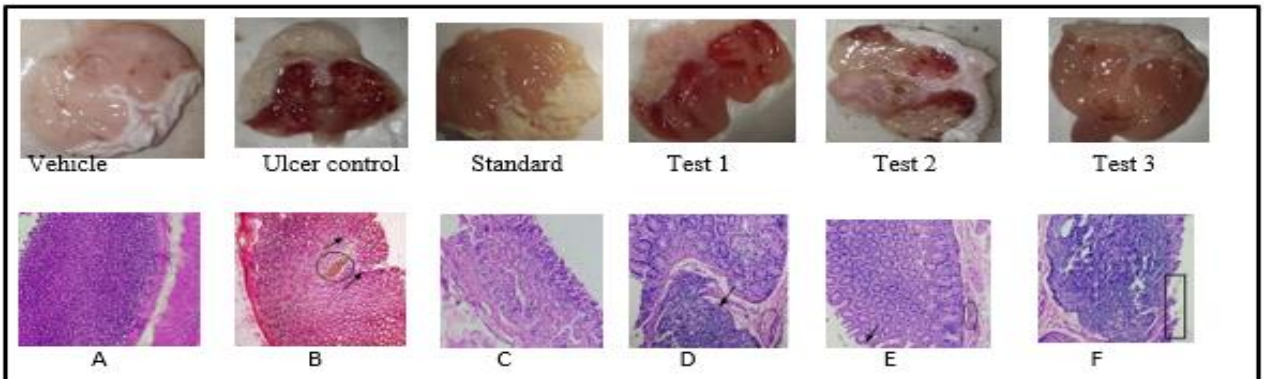

Figure 4: Histopathological samples illustrating stomach tissue from various experimental groups: Normal control (A); Ulcer control (B); Standard (Pantoprazole) (C); ADAE 50 mg/kg (D); ADAE 100 mg/kg (E); and ADAE 200mg/kg (F). Note: Structural loss of tissue (arrows), Hyperaemia (Circles).

Ethanol-induced gastric damage showed gross mucosal lesions, including haemorrhage bands and other structural damage. Ethanol increases vascular permeability and exposing gastric mucosa to proteolytic and hydrolytic effect. The pantoprazole (Std) group showed fewer haemorrhages and less cellular infiltration showing nearly normal gastric mucosa. ADAE (50 mg/kg) and ADAE (100 mg/kg) showed moderate haemorrhages, and structural damage, while ADAE (200 mg/kg) showed mild haemorrhages and cellular infiltrations as well as less structural damage.

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II. Determination of pH of gastric content

Low pH is responsible for more damage and abrasion in the mucosal lining. In this study, the pH is increase by the standard (Pantoprazole) and test (50mg/kg, 100mg/kg, 200mg/kg of *Amaranthus dubius* leaves extract) group as compared to the ulcer control group [F (4,25) = 7.880; p<0.05]

Table 4: Observation of pH Gastric Juice

Sr.no	Treatment	Dose (mg/kg)	pH of gastric content
1	Ulcer control	1 ml/kg	1.410±1.1200
2	Pantoprazole	40 mg/kg	5.210±0.1300****
3	ADAE	50 mg/ kg	3.600±0.1800
4	ADAE	100 mg/ kg	4.200±0.2100
5	ADAE	200 mg/ kg	4.800±0.2500**

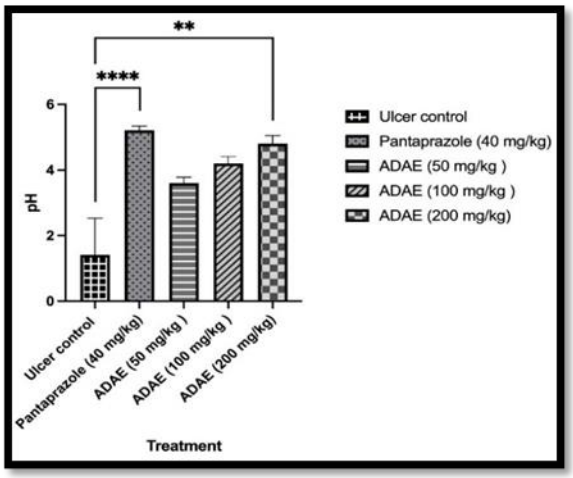

Figure 5: Effect of ADAE on pH in ethanol induce Gastric ulcer

Biochemical test

Determination of malondialdehyde (MDA):

As shown in Fig 6, Ulcer control group increased the gastric MDA level compared to the vehicle group [F (5, 30) = 19.85 p < 0.05, Fig No.6]. Administration of extract at doses of 50, 100 and 200mg/kg, similar to the pantoprazole group exhibited a dose-dependent decrease in MDA level.

Table 5: Observation table of MDA level

Sr. no	Treatment	MDA
1	Vehicle	0.010±0.001
2	Ulcer control	0.036±0.0003
3	Pantoprazole(40mg/kg)	0.0153±0.0004****
4	ADAE (50mg/kg)	0.0301±0.0007
5	ADAE (100mg/kg)	0.0261±0.0006
6	ADAE (200mg/kg)	0.0235±0.0006**

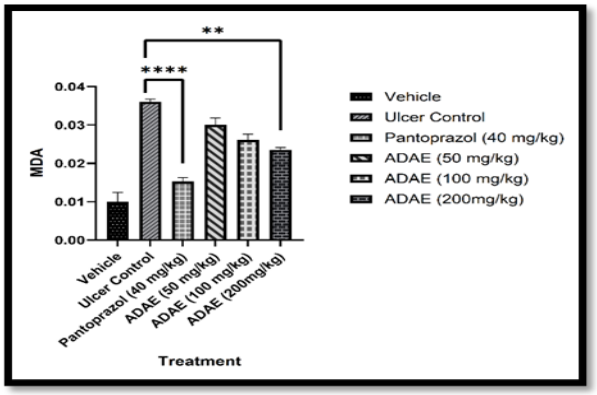

Figure 6: Effect of ADAE (50, 100 and 200 mg/kg) on the level of MDA in gastric tissue.

Determination of nitric oxide:

As shown in Fig No 7, Ulcer control group decreases the gastric nitric oxide level compared to the vehicle group [F (5,30) = 11.8; p < 0.05, Fig no.7]. Administration of extract at doses of 50, 100, and 200mg/kg, similar to the pantoprazole group exhibited a dose-dependent increase in nitric oxide level.

Table 6; Observation of NO level

Sr.no	Treatment	Nitrate level
1	Vehicle	20.10±0.2430
2	Ulcer control	7.110±0.0210
3	Pantoprazole(40mg/kg)	14.95±0.128***
4	ADAE (50mg/kg)	10.02±0.014
5	ADAE (100mg/kg)	11.68±0.087
6	ADAE (200mg/kg)	12.97±0.2030**

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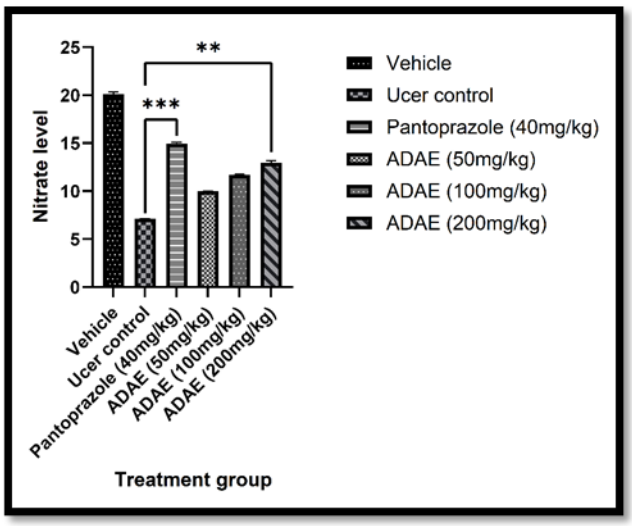

Figure 7: Effect of ADAE (50, 100 and 200 mg/kg) on the level Nitric Oxide in gastric tissue

4. Discussion

The current investigation was carried out to screen anti-ulcer activities using experimental models in Swiss albino mice. Secondary compounds that involve alkaloids, flavonoids, saponins, glycosides, tannins and carbohydrate were found in *Amaranthus dubius* during preliminary phytochemical screening in an aqueous solvent. The inhibition of ulcers is significantly aided by flavonoid presence. There is a suggestion that flavonoids can prevent mast cells from secretin acid, which lowers histamine release. Flavonoids also inhibit the H+/K+ ATPase pump, which reduces the amount of acid secreted by parietal cells.

The antioxidant capacity of ADAE was further confirmed through In-vitro assays, including the DPPH radical scavenging assay and alkaline DMSO assay. DPPH is a stable free radical and accepts an electron or hydrogen radical to turn into a stable diamagnetic molecule. The conversion of color from purple to yellow showed the decline in absorbance of DPPH radical at 517nm caused by reaction between antioxidants present in *Amaranthus dubius* and free radicals. *The Amaranthus dubius* leaves extract demonstrated stronger antioxidant activity, as indicated by its IC50 value.

Similarly, alkaline DMSO assay, the antioxidant activity of Amaranthus dubius leaves extract was assessed adopting ascorbic acid as reference standard. The scavenging activity of the extract has been demonstrated to be strongly equivalent as that of standard, reinforcing its potential as significant antioxidant.

The ulcer index parameter was employed to assess the anti-ulcer activity, as the development of ulcers is strongly related to variables like decreased stomach volume and decreased levels of both free and total acidity. The amount of gastric mucosal damage caused by ulcerogenic substances was decreased by aqueous extracts of *Amaranthus dubius* leaves at a concentration of 200 mg/kg.

In ethanol induced ulcer method, ulcers emerge through alterations of superficial epithelial cells. Particularly, the mucosal mast cells leading to the discharge of the vasoactive mediators that include histamine thus resulting in damage to gastric mucosa. Mucosal damage triggered by alcohol is regulated by prostaglandin. The potency of ADAE protection against mucosal damage triggered by ethanol is evidence for its effect on prostaglandins.

Histopathological analysis showed that ethanol-induced gastric damage showed gross mucosal lesions, including haemorrhage bands and other structural damage. Ethanol increases vascular permeability and exposing gastric mucosa to proteolytic and hydrolytic effect. The pantoprazole (Std) group showed fewer haemorrhages and less cellular infiltration showing nearly normal gastric mucosa. ADAE (50 mg/kg) and ADAE (100 mg/kg) showed moderate haemorrhages, and structural damage, while ADAE (200 mg/kg) showed mild haemorrhages and cellular infiltrations as well as less structural damage.

Additionally, gastric levels of MDA and nitrate were found. The result showed increase in NO that helps heling the ulcer by maintaining gastric mucosal credibility and monitoring of acid, mucus secretion and gastric mucosal flow of blood. In this study also performed malondialdehyde assay which results show extracts of Amaranthus dubius have decreased gastric MDA levels than ulcer control group, which also signifies that the it has higher antioxidant activity and can reduce oxidative stress caused by ulcer.

5. Conclusion

Preliminary phytochemical analysis of *Amaranthus dubius* leaves extract showed the presence of Saponins, flavonoids, alkaloids, carbohydrate, and tannins. The

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study suggests that *Amaranthus dubius* leaves prove to exhibit anti-ulcer activity. It has been shown to improve ulcer protective symptoms. During histopathological investigation it was observed that the group with highest dose of test extract showed mild haemorrhages and cellular infiltrations as well as less structural damage. These findings partially support the plant's traditional medicinal use by demonstrating the therapeutic potential of its active phytochemical compounds. However, further research is necessary to fully elucidate the mechanism of action underlying its anti-ulcer effects.

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