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Original Research Article

Evaluation of Anti-Ulcer Potential of Methanolic Extract of *Amaranthus viridis* L. In Experimental Rats

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Abstract: The research focuses on the anti-ulcerogenic potential of *Amaranthus viridis* L. evaluated through ethanol induced ulcer in Wistar albino rats of both sexes using omeprazole 0.6mg/kg as standard drug. Animals were divided into five groups i.e., normal, control, standard, and experimental groups (I & II). The experimental groups were treated with plant extract of doses 10µg/kg and 50µg/kg, respectively. Different physical and biochemical parameters were evaluated. The extract showed a graded dose response as gastro-protective shield. Macroscopic assessment of gastric lining showed that extract reduced the severity score and the number of lesions. Results revealed that gastric juice volume and total acidity were also reduced as dose of extract increases. However, gastric pH increased with an increase in dose showing the neutralizing of acidic environment. Biochemical parameters including total protein content and mucus adherence to gastric wall showed the gastro-protective effect of the plant. The assessed parameters proved that methanol extract of *Amaranthus viridis* L. leaves may have anti-ulcer activity due to its antioxidant, anti-radical and anti-secretory mechanisms due to the presence of several secondary metabolites including tannins, alkaloids, flavonoids, glycosides, steroids, and triterpenoids.

Keywords: Anti-Ulcerogenic, Medicinal Plants, Herbal Extract, Anti-Ulcer Effect, Gastroprotection.

INTRODUCTION

Gastric ulcers, or peptic ulcers, are lesions in the stomach lining caused by an imbalance between defensive systems and aggressive forces, including hydrochloric acid and pepsin (Laine and Peterson 1994). The risk factors of ulcers include, *Helicobacter pylori* (*H. pylori*), non-steroidal anti-inflammatory drugs (NSAIDs), smoking, alcohol, and stress (Konturek, Bielański *et al.,* 2003). The ulcers are currently treated with proton pump inhibitors, H_2-receptor blockers, and mucoprotective agents, but the risk of side-effects is promoting the need of natural remedies (Anmol, Jaspreet *et al*., 2023).

Many medicinal plants having antioxidant, anti-inflammatory, and cytoprotective attributes have historically been employed to combat stomach ulcers, and new research is being done to validate the effectiveness of these natural remedies (Gupta, Tangri *et al*., 1974). *Amaranthus viridis* L., popularly referred to as "green amaranth," belongs to the Amaranthaceae family. This plant has been recognized for its varied therapeutic uses in different cultures, comprises bioactive components like flavonoids, tannins, saponins, and alkaloids, which show antioxidant, anti-inflammatory, hepatoprotective, antimicrobial, and anti-ulcer effects (Ruth, Unathi *et al*., 2021). Its anti-ulcerogenic activity, however, has not been fully scientifically explained (Ahmed, Rabbee *et al*., 2021).

This research study is designed to evaluate the gastroprotective potential of methanol extract of *Amaranthus viridis* L. against alcohol-induced gastric ulcers in Wistar albino rats. To prove the effectiveness of extract and its use as a traditional remedy, parameters including ulcer index, gastric pH, mucus adhesion were calculated. This study examines the anti-ulcerogenic properties of methanol extracts of *Amaranthus viridis* L. leaves and assesses its effectiveness in ulcer prevention and stomach tissue protection.

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MATERIALS AND METHODS

Plant Collection and Authentication

Amaranthus viridis L. leaves were collected from the footpath of Lawrence Garden, Lahore and authenticated by Dr. Zaheer-ud-din Khan, Assistant Professor Botany, Government College University, Lahore, Pakistan. Voucher number 3796 was assigned to the sample. The collected leaves were washed, shade dried and powdered.

Preparation of Extract

Powder was cold macerated with methanol with continuous stirring every day for five days. Then, it was passed through muslin cloth and collected liquid was further filtered using Whattman's filter paper. The solvent was evaporated under vacuum using rotary evaporator (Heidolph Lab 4002 Sigma Aldrich, Germany) temperature exceedingly not more than 40ºC. The collected extract was then refrigerated at (2-8ºC) for further experimental work (Khazaei and Salehi 2006).

Solubility Analysis

A pinch of extract was tested for its solubility in six solvents i.e., distilled water, normal saline, ethanol, polysorbate 20, polysorbate 80, and dimethyl sulfoxide (DMSO). The test tubes were then sonicated in the electronic sonicator for 15 minutes and then centrifuged for 10 minutes at 5000rpms. Relative solubility of the extract was checked with naked eye. The greater the residue left at the bottom the lower will be the solubility in respective solvent (Laboukhi-Khorsi, Daoud *et al*., 2017).

Evaluation of Gastroprotection

The experiment was carried out as devised by Morimoto (Mozafar and Salehi 2007). Rats were divided into five groups (n=5) and kept fasted for a day. The normal group (G-I) was administered with water whereas the control group (G-II) was given ethanol only (200µg). Absolute ethanol was given to all groups to induce ulcers followed by oral doses of omeprazole (0.6 mg/kg B.W.) in standard group (G-III), and methanol extract of *Amarathus viridis* L. at doses 10ug/kg B.W. (G-IV) to 50ug/kg, B.W. (G-V) respectively. After an hour, rats were sacrificed, and stomach were removed, opened along the greater curvature and washed with normal saline to access lesions.

Macroscopic Examination of the Stomach

The stomachs were incised, leading to the opening of stomach and emptying of the gastric contents. The gastric juice was then carefully obtained, and the stomachs were washed with normal saline. The number as well as the severity of ulcers was then accessed using a 10X magnifying glass as per method described by Kulkarni. The numbers of ulcers were counted with the help of the scale i.e., perforation = 3, deep ulcer = 2, hemorrhagic streak = 1.5, spot ulcer = 1, red coloration = 0.5, normal colored stomach = 0 (Adinortey, Ansah *et al*., 2013).

Calculation of Ulcer Index

Ulcer index was calculated as,

$UI = (UN + US + UP) \times 10^{-1}$

where, UI = ulcer index, UN = average of number of ulcers per rat, US = average of severity score calculated, and UP = percentage of rats with ulcers (Adinortey, Ansah *et al*., 2013)

Percentage Protection

Using Basile method, the ulcer protective effect was calculated using formula (Basile, Sertié *et al*., 1990),

$$\text{Percentage Protection (\%)} = \frac{\text{Ethanol group UI} - \text{Treated group UI}}{\text{Ethanol group UI}} \times 100$$

Analysis of Gastric Secretions

Stomach secretions were analyzed through method described by Shay with little modifications (SHAY, Komarov *et al*., 1949). The stomach contents were emptied into test tubes, centrifuged at 3000 rev/min for ten minutes, and volume of gastric secretions was calibrated using graduated measuring cylinders. The pH of juice was measured using pH meter. In addition, total acidity was measured by adding 2ml of distilled water to the same volume of gastric juice using phenolphthalein as an indicator and titration was performed using 0.01N NaOH until persistent light pink colour obtained. Total acidity in mEq/L was determined using following formula:

$$Total\ Acidity = \frac{Normality\ of\ NaOH\ \times Volume\ of\ NaOH\ used}{0.1} \times 100$$

Histopathological Examination

The stomachs, after dissection of animals, were washed with the normal saline and stored in the 10% formalin solution buffered to maintain the pH for histopathological examination. The fine segments of tissues were cut into 3 microns with the help of a microtome. Slides were made by staining the tissues with dyes. The microscope was used to interpret the results from prepared slides (Umamaheswari, Asokkumar *et al*., 2007).

Biochemical Parameters

Mucus Adherence to Gastric Mucosa

The ability of mucus to adhere to the gastric lining has an essential role in the etiology of ulcers; therefore, estimation of mucus attached to the gastric

walls is necessary. The glandular part of stomach was separated, weighed and processed in 10ml of 0.1% w/v solution of alcian blue dye suspended in 0.16M sucrose solution (buffered using 0.05M sodium acetate to maintain a pH of 5). This mixture was sonicated for two hours and incubated for 5 minutes. The excess dye was washed out twice, using 0.25M sucrose solution. After the removal of excess dye, the gastric mucus that remained attached with the alcian blue was extracted with 10ml of 0.5M solution of magnesium chloride for thirty minutes with vigorous shaking. A 4ml of this mixture was forcibly shaken with equal quantity of diethyl ether in a test tube. The emulsion was centrifuged for ten minutes at 3000 rev/min. Finally, the absorbance of aqueous layer was measured using a spectrophotometer at 580nm (UV-2500, Schimadzu Corporation, Koyoto, Japan). The extent of alcian blue mined per thousand milligrams from the glandular tissue was calculated from the standard curve. The standard curve of alcian blue was made by preparing different concentrations of alcian blue dye as 200, 400, 600, 800 and 1000 µg/ml in the same manner. Absorbance of samples were taken through spectrophotometer at 580nm against blank (Corne 1974).

Total Protein Contents

Lowry method was used to estimate the total protein binding of stomachs. Bovine Serum Albumin (BSA) standard curve was prepared for this purpose using BSA stock solution (1mg/10ml). 25, 50, 75, 100, 125 and 150 ug/ml concentrations of BSA stock solution were prepared in six test tubes and volume was made up to 1ml with 0.02M solution of tris-HCl buffer. 1ml of 0.02M solution of tris buffer (pH 7.6) was used as a blank. 4.5ml of reagent I (1ml of 0.5% copper sulfate solution in water + 1ml of 1% NaK tartarate solution in water + 48ml of 2% sodium carbonate solution in 0.1N NaOH) was added in six test tubes and incubated for ten minutes. Then 0.5ml of reagent II (1:1 Folin phenol and water solution) was added and the mixture was incubated for thirty minutes. The absorbance was noted at 750nm and then a standard curve was prepared between the absorbance and concentration of the solution. To prepare tissue homogenate, the glandular part of the stomach was weighed i.e., 100mg and mixed with 1ml of tris buffer. It was then chopped in a petri-dish by using scissors and blade. The chopped tissue sample was then homogenized by the homogenizer (WiseStir, HS30E, Daihan Scientific Co., Ltd., Korea) and centrifuged at 12000rpm in 4ºC for 30 minutes. Finally, the absorbance of the supernatant layer was calculated for each sample. This absorbance value was then fitted into BSA standard curve and amount of protein was determined as mg/100 mg stomach tissue.

Statistical Analysis

The readings acquired from experiments were analyzed through statistical analysis; one way analysis of variance (ANOVA) preceded by Dunnett' test in Graph Pad Prism version 8.0. Probability levels found to be lower than 0.05 were considered statistically significant.

RESULTS

Solubility Analysis

The solubility of extract was checked in six different solvents (table 1). Distilled water was used to dissolve the extract.

Table 1: Solubility studies of extract

Solvent	Solubility
Distilled water	Soluble
Normal saline	Soluble
Ethanol	Soluble
Polysorbate 20	Insoluble
Polysorbate 80	Insoluble
Dimethylsulfoxide (DSMO)	Insoluble

Macroscopic Examination of the Stomach

The rats were sacrificed after the experiment and their stomachs were obtained. Figure 1(a) shows the normal internal lining of stomach with no ulcer. Figure 1(b) represents the stomach of control animal treated with ethanol only, showing ethanol-induced ulcer. It is evident that ethanol caused severe ulceration in the form of perforations, and hemorrhagic streaks. Omeprazole (0.6mg/kg) group showed better protection against ethanol, where no major ulcer was seen, figure 1(c). Methanol extract of *Amranthus viridis* L. in doses starting from 10µg/kg to 50µg/kg showed better result of percentage protection as compared to control with only few spot ulcers were seen figure 1(d) and (e) respectively. The percentage protection against ethanol increases with the increasing doses of methanol extract of *Amaranthus viridis* L. High doses of extract had shown a remarkable reduction in ulcer index and severity score at 50µg/kg.

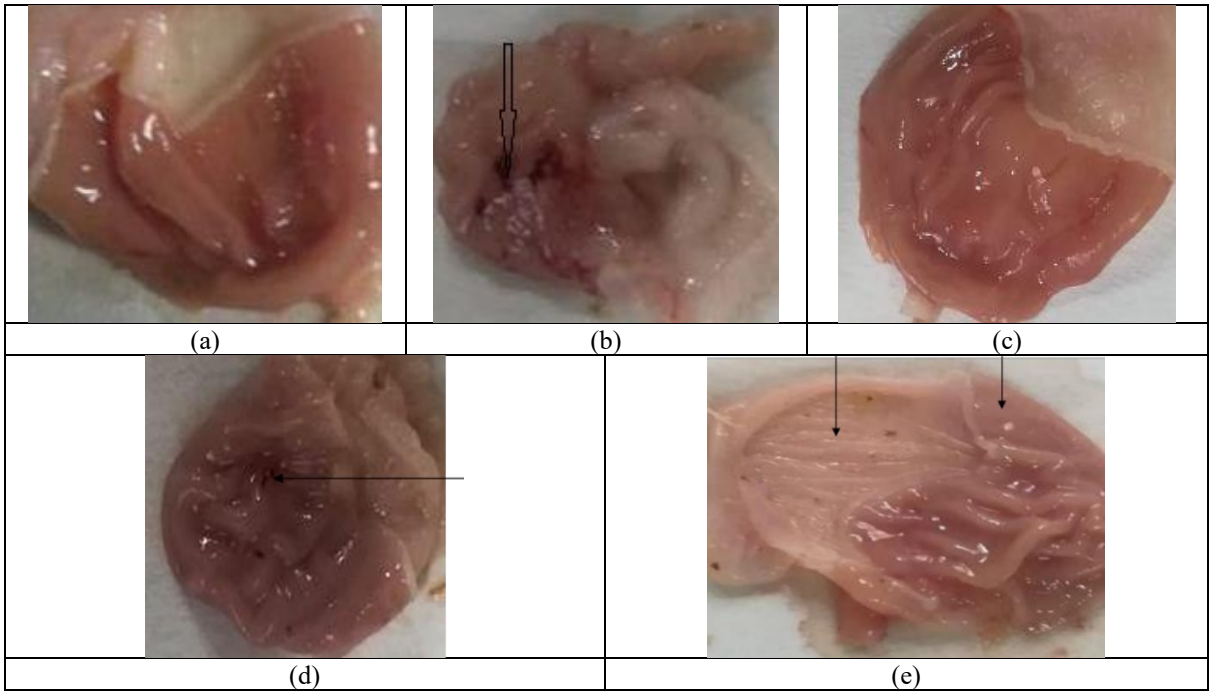

Figure 1: Microscopic view of stomach. (a) normal, (b) deep ulceration in ethanol-treated rat, (c) omeprazole-treated rat, (d) hemorrhagic steaks in rats treated with 10µg/kg extract, (e) ulcers and flattening of mucosal folds in rats treated with 50µg/kg extract. Each ulcer has severity score used to calculate ulcer index as; normal (0), red coloration (0.5), spot ulcer (1.0), hemorrhagic streak (1.5), deep ulcer (2) and perforation (3)

Calculation of Ulcer Index

Figure 2 shows the comparison of ulcer index of control with other groups. Both doses of methanol extract and standard drug show significant difference from the ulcer index of control ($p < 0.0001$) group, increasing dose of extract decreases the ulcer index though having significant difference from the control. The results are summarized in table 2.

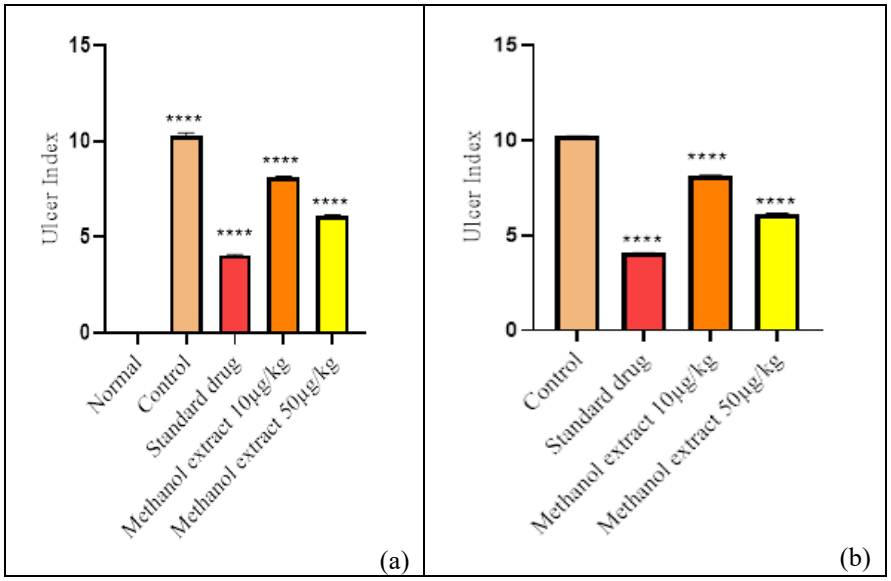

Figure 2: Effect of *Amaranthus viridis* L. leaf extract on ulcer index against ethanol-induced ulcers in rats when compared to; (a) normal, (b) control (mean ± SD; n = 5; p < 0.0001 = significant*

Percentage Protection

Percentage protection was measured from ulcer indices of different groups. Results of gastric ulcer protection are shown in table 2 for each group. Percentage protection of different groups is also represented graphically in figure 3 that elaborates lower doses of extract show minor protection against ulcer whereas significant percentage ulcer protection is noted at highest dose of 50µg/kg i.e. 41.9.

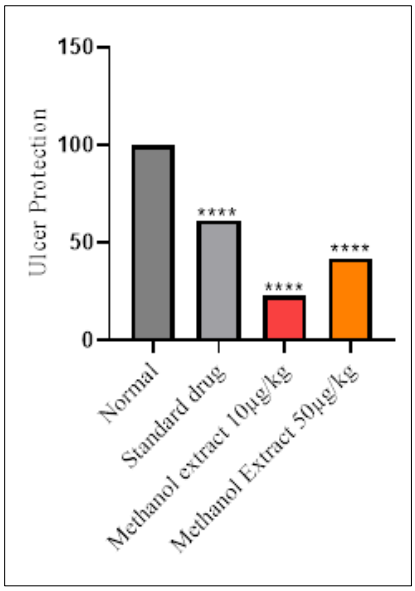

Figure 3: Effect of *Amaranthus viridis* L. leaf extract on ulcer protection (%) against ethanol-induced ulcers in rats when compared to toxic group (mean ± SD; n = 5; p < 0.0001 = significant*

Table 2: Effect of *Amaranthus viridis* L. leaf extract on ulcer index (mean ± SD) and percentage protection in rats

Group	Ulcer Index	Percentage Protection %
G-I (Normal)	0.0 ± 0.0	100
G-II (Control)	10.32 ± 0.2362	0
G-III (Standard)	4.054 ± 0.07470	61.6
G-IV (Extract 10µg/kg)	8.126 ± 0.1074	22.9
G-V (Extract 50µg/kg)	6.102 ± 0.1314	41.9

Determination of Gastric Juice Volume (ml)

The results (table 3, figure 4) show that gastric juice volume decreases in the descending order with the increasing dose of extract when compared to the control group. However, it was noted that volume of gastric secretions in all extract treated groups decreased from lower doses to higher doses.

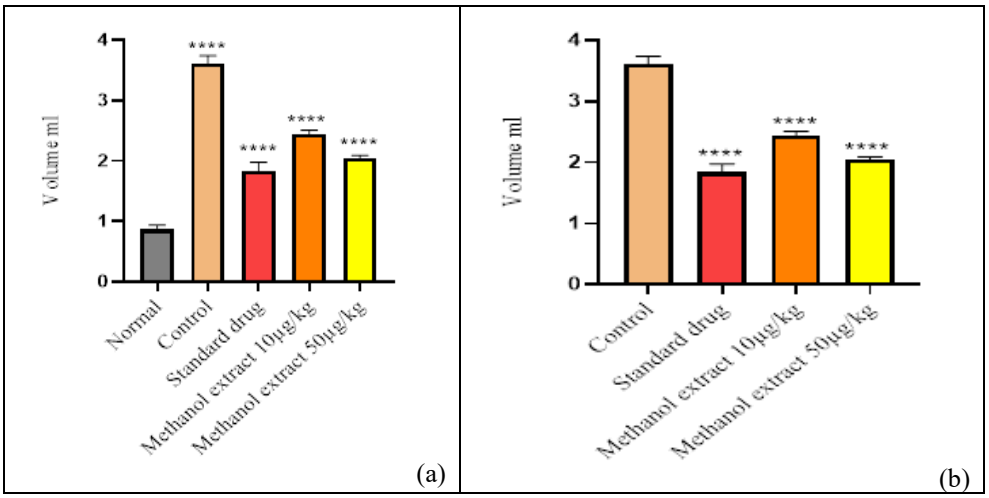

Figure 4: Effect of *Amaranthus viridis* L. leaf extract on gastric juice volume (ml) against ethanol-induced ulcers in rats when compared to; (a) normal, (b) control (mean ± SD; n = 5; p < 0.0001 = significant*

Determination of Gastric pH

Results of gastric pH are summarized in table 3 along with the graphical representation in figure 5. It was illustrated that standard groups and control have shown significant difference ($p < 0.0001$) in gastric pH while treated groups have also shown significant difference ($p = 0.0003$, $p = 0.0006$) respectively when compared to normal groups. It was observed that pH increases in ascending order with increasing doses of extracts.

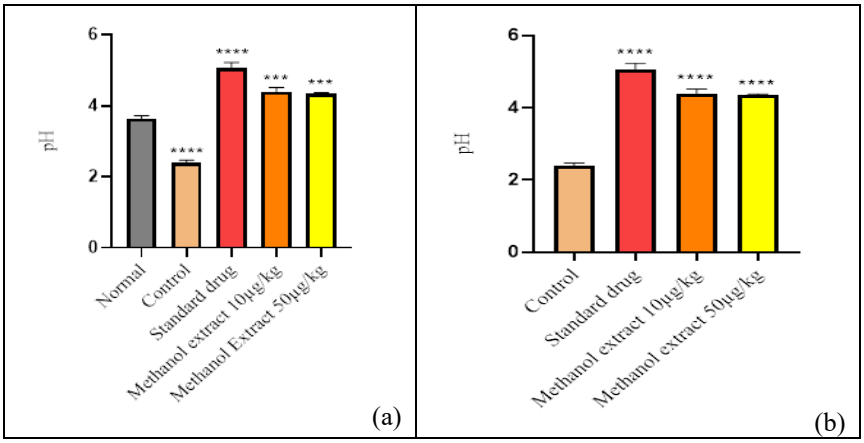

Figure 5: Effect of *Amaranthus viridis* L. leaf extract on gastric pH against ethanol-induced ulcers in rats when compared to; (a) normal, (b) control (mean ± SD; n = 5; p < 0.0001 = significant*

Determination of Total Acidity

Total acidity decreases with the increasing doses of extract of *Amaranthus viridis* L. as shown in figure 6. The results are summarized in table 3.

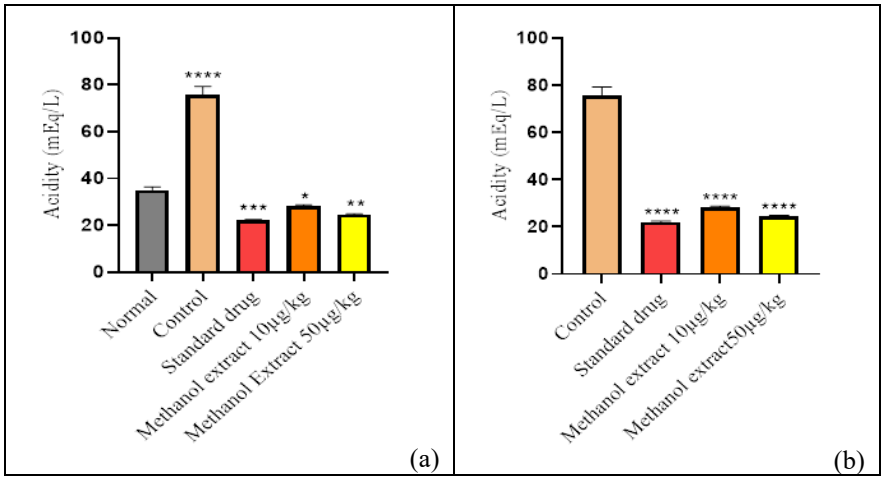

Figure 6: Effect of *Amaranthus viridis* L. leaf extract on total acidity against ethanol-induced ulcers in rats when compared to; (a) normal, (b) control (mean ± SD; n = 5; p < 0.0001 = significant*

Table 3: Effect of *Amaranthus viridis* L. leaf extract on gastric juice volume, gastric pH, and total acidity (mean ± SD) in rats

Group	Gastric Juice Volume (ml)	Gastric pH	Total Acidity (mEq/L)
G-I (Normal)	0.86 ± 0.1673	3.640 ± 0.1834	35 ± 3.317
G-II (Control)	3.60 ± 0.3162	2.388 ± 0.1921	75.8 ± 7.791
G-III (Standard)	1.84 ± 0.3050	5.066 ± 0.3596	22 ± 1.0
G-IV (Extract 10µg/kg)	2.44 ± 0.1517	4.390 ± 0.2842	28.4 ± 0.8944
G-V (Extract 50µg/kg)	2.04 ± 0.1140	4.342 ± 0.08075	24.6 ± 0.5477

Histopathological Studies

The histopathological findings of stomach samples were visualized using prepared slides under the lens of 10×10 magnification (figure 6). The gastric mucosa of normal group was found intact (figure 7, a-c). The stomach membranes of control group rats were found ulcerated because of the corrosive action of ethyl alcohol on stomach wall. All the layers were heavily damaged. Tissue congestion and sloughing of gastric epithelial cells can also be seen though (figure 7, d-f). The gastric membranes of standard group were found intact. The standard drug omeprazole (0.6mg/kg) retained mucosal integrity against acid by increasing mucus and bicarbonate production (figure 7, g-i). The mucosa of treated groups (10µg/kg & 10µg/kg) was found intact. The extract retained the integrity of stomach mucosa. No gastric pits and ulcers were observed (figure 7, j-o).

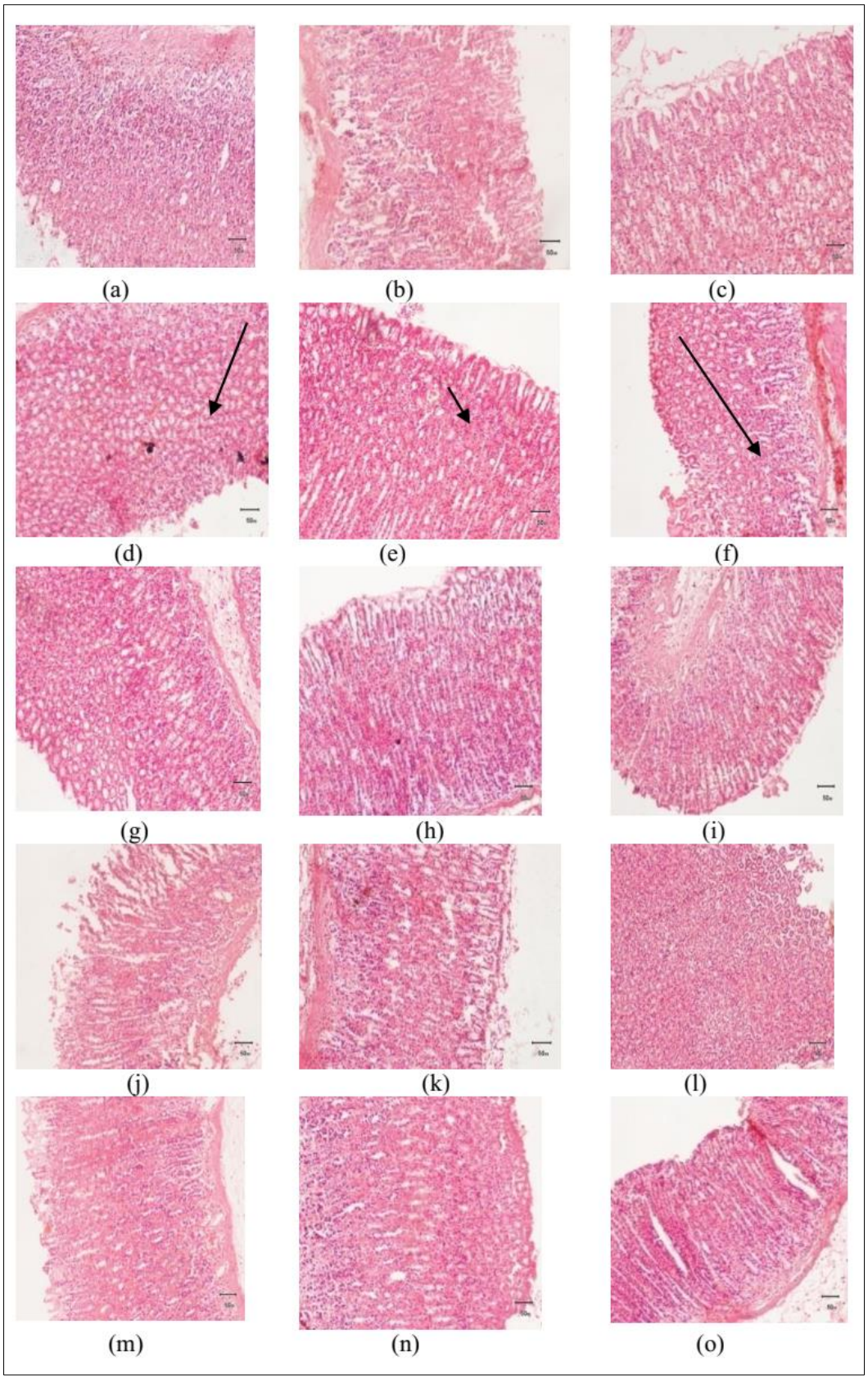

Figure 7: Effect of *Amaranthus viridis* L. leaf extract on gastric mucosa, (a-c) intact gastric mucosa of normal group, (d, e) edema with neutrophils in control group, (f) gastric pits of control group, (g-i) intact gastric mucosa of standard group, (j-l) tissue regeneration in gastric mucosa of experiment group-I (10µg/kg), (m-o) tissue regeneration in gastric mucosa of experiment group-I (50µg/kg)

Total Mucous Adherence

The mucus content of gastric wall calculated in µg/ml of tissue was determined from the alcian blue standard curve (figure 8). Gastric wall mucus decreased significantly ($p < 0.0001$) with ethanol because it enhances the production of stomach acid having a drastic effect on gastric mucosa and it increased with the increasing doses of extract in experimental animals (table 4). It depicts the shielding effect of extract against the corrosive action of stomach acid. The graphical illustration of mucus adherence is shown in figure 9.

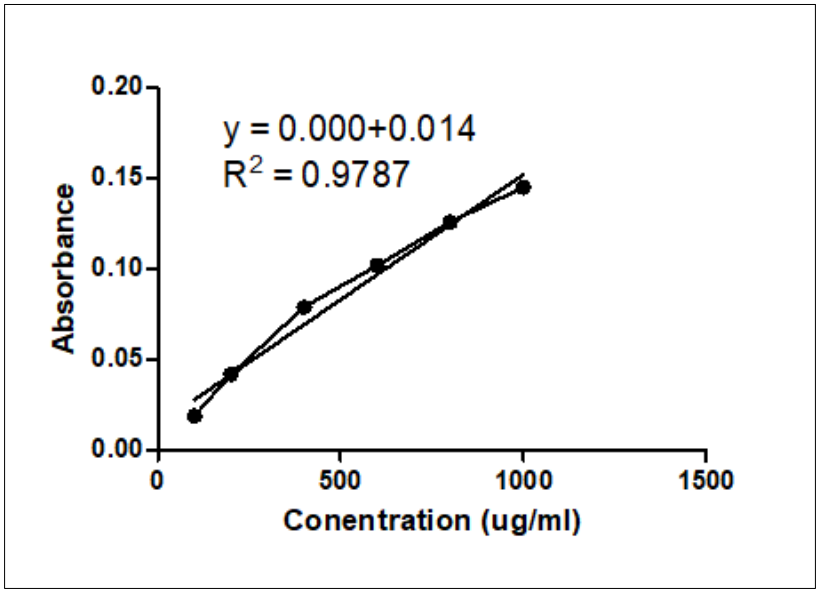

Figure 8: Standard curve of alcian blue

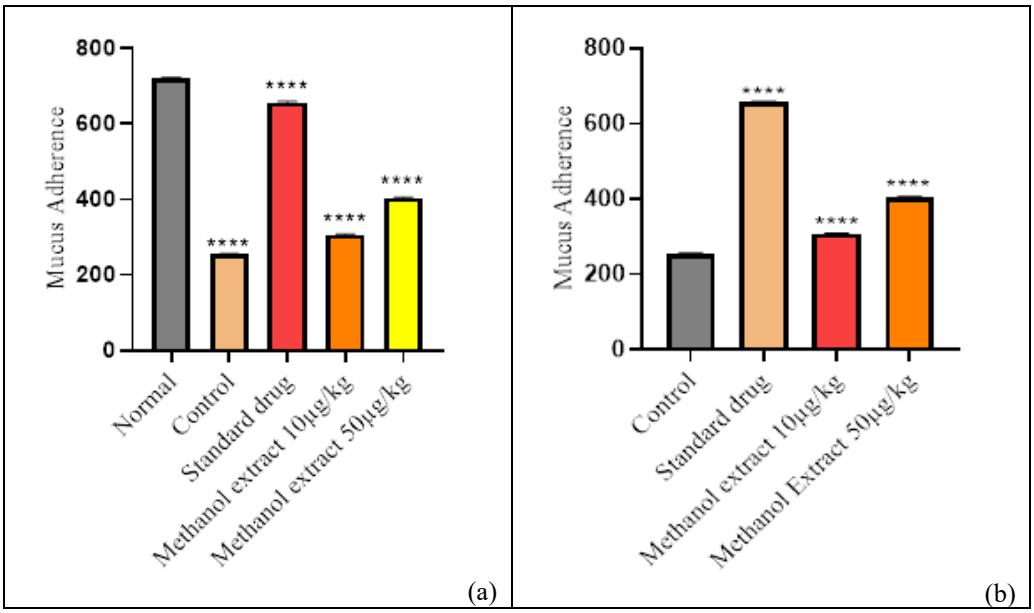

Figure 9: Effect of *Amaranthus viridis* L. leaf extract on total mucus adherence against ethanol-induced ulcers in rats when compared to; (a) normal, (b) control (mean ± SD; n = 5; p < 0.0001 = significant*

Total Protein Binding

Total protein content was calculated from the standard curve of bovine serum albumin (BSA) (figure 10). It was seen that gastric protein content decreased significantly ($p < 0.0001$) with ethanol (figure 11) because it enhances the production of stomach acid having a drastic effect on gastric mucosa and it increased with the increasing doses of extract in experimental animals (table 4). It demonstrates the shielding effect of the extract of plant against the corrosive action of stomach acid.

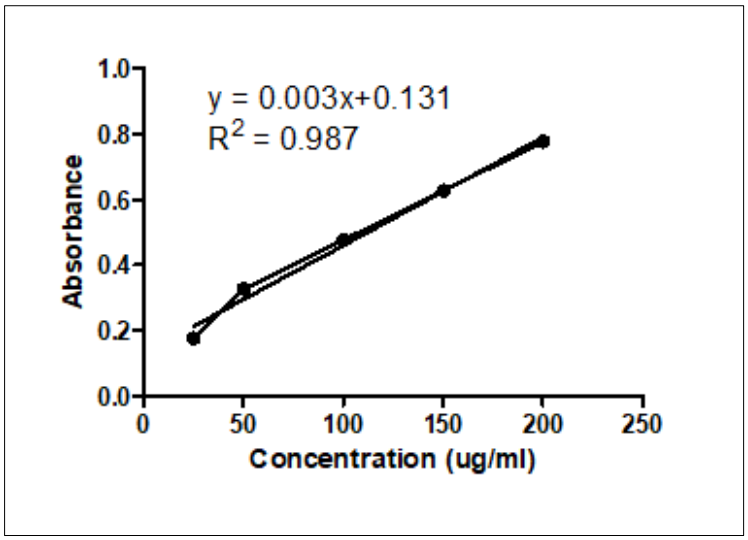

Figure 10: Standard curve of BSA

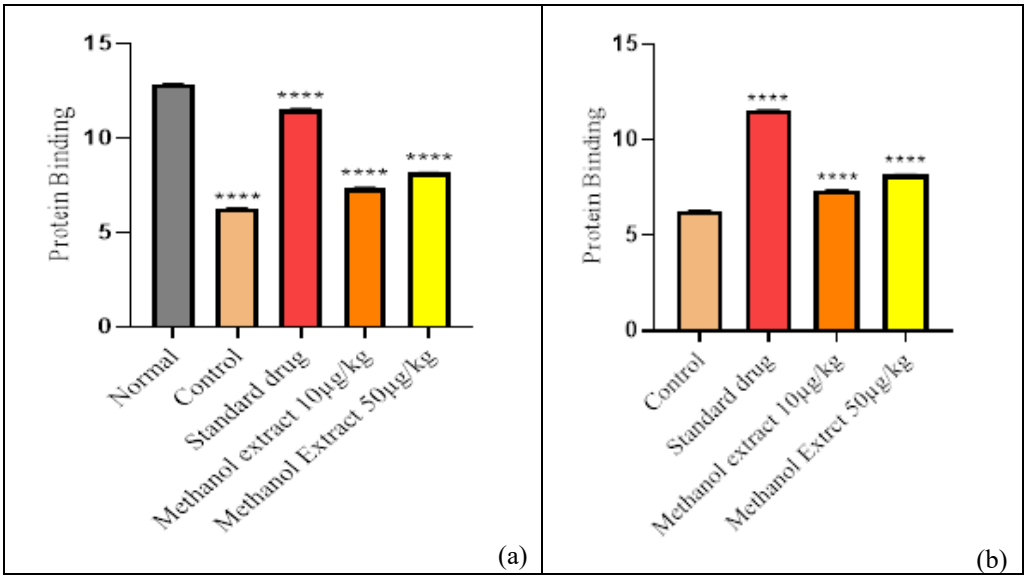

Figure 11: Effect of *Amaranthus viridis* L. leaf extract on total protein binding against ethanol-induced ulcers in rats when compared to; (a) normal, (b) control (mean ± SD; n = 5; p < 0.0001 = significant*

Table 4: Effect of *Amaranthus viridis* L. leaf extract on mucus adherence and total protein binding (mean ± SD) in rats

Group	Mucus Adherence (µg/ml)	Total Protein Binding
G-I (Normal)	720.0 ± 7.906	12.88 ± 0.06782
G-II (Control)	255.2 ± 3.114	6.276 ± 0.05413
G-III (Standard)	657.2 ± 4.970	11.52 ± 0.09418
G-IV (Extract 10µg/kg)	307.2 ± 4.604	7.340 ± 0.1153
G-V (Extract 50µg/kg)	404.0 ± 4.950	8.150 ± 0.07714

DISCUSSION

Gastrointestinal diseases have become a huge challenge to the modern world and a lot of research is employed to combat this global threat (Dharmani, Kuchibhotla *et al*., 2004). Gastric ulcers are the common gastrointestinal problem involving different etiologies such as smoking, hypersecretory acid conditions and alcohol intake. However, drinking contributes towards most cases (Ham and Kaunitz 2007). Recently, researchers have carried out many studies for developing new gastroprotective drugs free of side effects, commonly seen with traditional therapies. These days new drug therapies are mostly derived from natural origin (Balunas and Kinghorn 2005).

The ethnobotanical studies of plant *Amaranthus viridis* L. (family Amaranthaceae) claims its importance in the treatment of gastrointestinal problems (Muhammad and Amusa 2005). So, the current activity was planned to investigate the validity of methanolic extract of plant for the treatment of ulcers.

Ethanol administration in rats produced multiple-hemorrhagic red bands of various lengths. Ethanol exerts direct toxic action through inhibition in the secretion of bicarbonate ions and mucus production which results in necrotic lesions within the gastric mucosa (figure 1). Defect in the homeostatic mechanism for cellular integrity in gastric mucosa is the initial step in the ulcer formation. Research reveals that ethanol produces its action of cellular disruption through microvascular injury which results in increased vascular leakage and edema formation (Olawoye, Gbadamosi *et al*., 2017). Moreover, ethanol damages the mucosal lining by neutrophils infiltration. Neutrophils release oxygen free radicals that prolong the healing of ulcers in mucosa. So, the damaging effect of ethanol can also be attributed to the radical producing effect of ethanol in stomach lining (Chatterjee, Saluja *et al.*, 2007).

Ulcer index (UI) and ulcer protection (UP) are standard parameters used to confirm the gastroprotective potential of agents under question (Bhajoni, Meshram *et al*., 2016). It was observed that all doses of methanol extract (10, 50µg/kg) showed drastic reduction in UI and relative increase in UP (table 2). The greater the ulcer index the lower the percentage protection. It was observed that ulcer index of methanol extract of *Amaranthus viridis* L. decreased with increasing doses of extract (figure 2, 3). This was probably due to the anti-inflammatory actions of tannins and flavonoids present in the extract on stomach lining (Kumari, Elancheran *et al*., 2018). Flavonoids and tannins reduced the migration of neutrophils in mucosa, stopped the propagation of ulcers, and reduced oxygen stress through their antioxidant properties. The scavenge (ROS) which in turn also reduced the number of ulcers which led to reduced severity score, ulcer index and increased percentage protection (Macharla, Venkateshwarlu Goli *et al*., 2011).

Increased intestinal motility increases the risk of ulcer formation and vice versa. The pre-treatment with methanol extract reduced the intestinal motility by relaxing the smooth muscles accompanied with flattening of mucosal folds in stomach walls (figure 1). The methanol extract of *Amaranthus viridis* L. not only enlarged mucosal surface area to gastric secretions but also reduced the volume of gastric irritants on mucosal surface (Abdulla, Al-Bayaty *et al.*, 2010).

Ethanol administration stimulated the release of acid from the gastric parietal cells resulting decreased pH, increased total acidity and HCl available for neutralization in the lumen (Marhuenda, Martin *et al*., 1993). The results (table 3, figure 4-6) show that methanol extract of *Amaranthus viridis* L. decreased gastric juice volume and total acidity. The pH of the secretions was also found high for both doses (10, 50µg/kg) of plant extract. This was possibly due to the anti-secretory action of flavonoids mediated through the blockade of proton pumps H^+/K^+-ATPase present in stomach walls, leading to the decrease in gastric juice volume, decrease in total acidity and increase in pH within the lumen (Zayachkivska, Konturek *et al.*, 2005, Sarker and Oba 2019).

Mucin (a glycoprotein) plays its role as first line defense against all the gastric irritants. It is continually secreted from the mucous cells. Estimation of mucus adherence and protein content is the biologic indication for gastro-protective effect (Lee, Lee *et al*., 2006). Ethanol decreased mucus adherence and total protein content due to the decreased mucus production leading to the ulcerative lesions (Strous, Dekker *et al*., 1992).

It was demonstrated that pre-treatment with methanol extract of *Amaranthus viridis* L. showed comparable results (table 4) to that of omeprazole, but omeprazole showed better values for mucus adherence and total protein content in rats as depicted (figure 8, 10). The increases in values were probably due to the presence of flavonoids (Kumari, Elancheran *et al*., 2018). Flavonoids are famous for their antioxidant properties. They strengthen mucosal defense system through enhanced mucus production (Martin, Marhuenda *et al*., 1994). Therefore, the increase in mucus adherence and total protein content can be attributed to the cytoprotective, anti-secretory, and antioxidant properties of flavonoids (Kumari, Elancheran *et al*., 2018).

Histopathological evaluation revealed gastroprotective potential of plant extract (figure 6). Evaluation showed that gastric mucosa of normal groups was intact while mucosa of control group (treated with ethanol only) showed edema, congestion, and ulcerative lesions. Pre-treatment with *Amaranthus viridis* L. reduced congestion, edema, tissue necrosis, and hemorrhage in mucosal membranes, hence promoted tissue regeneration. The efficacy of extract in treating congestion and ulcerative lesion were found comparable to omeprazole. Studies confirm that flavonoids and tannins have shown to contain antisecretory and cytoprotective properties in ulcer models. Therefore, it can be safe to say that the extract protected the gastric mucosa possibly due to the cytoprotective and antisecretory mechanisms of tannins and flavonoids (Zayachkivska, Konturek *et al*., 2005). Moreover, methanol extract of *Amranthus viridis* L. also reduced the migration of neutrophils to the inflammatory site (figure 6). This resulted in reduced inflammation and ulcer inhibition (Macharla, Venkateshwarlu Goli *et al*., 2011, Al-Radahe, Ahmed *et al*., 2012).

Molecular mechanisms of gastric ulcers suggest the involvement of various cytokines including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) (Choi, Raghavendran *et al*., 2010, Yadav, Adhikary *et al*., 2012). Ethanol ingestion produces gastric lesions through the activation of these cytokines. TNF-α promotes leukocytes adherence and increases mucosal

damage through inflammation and hyperemia in mucosa (Yadav, Adhikary *et al*., 2012). IL-6 initiates neutrophil migration to inflamed sites and promotes tissue necrosis. These activated neutrophils eventually increase oxidative stress and pro-inflammatory mediators that cause ulceration in stomach walls (Mei, Xu *et al*., 2012). Moreover, IL-6 and TNF-α also activate nuclear factor-κB (NF-κB) (Verstrepen, Carpentier *et al*., 2009). Nuclear factor-κB is further involved in the transcription of multiple inflammatory mediators i.e. IL-6, IL1-β and TNF-α contributing towards ulcerative lesions (Wu, Fan *et al*., 2008). Studies have revealed that plants having gastroprotective activity reduce the expression of these inflammatory mediators at molecular level (Li, Huang *et al*., 2013). Since *Amaranthus viridis* L. is rich in flavonoids and tannins which contain anti-inflammatory and antioxidant properties (Kumari, Elancheran *et al*., 2018). Therefore, it might be possible that the presence of flavonoids and tannins reduce these pro-inflammatory cytokines and contributes towards mucosal integrity, serving as the possible mechanism for gastro-protection.

CONCLUSION

The methanol extract of *Amaranthus viridis* L. demonstrates potential as a natural antiulcer agent, providing protection via its anti-inflammatory, anti-secretory, and antioxidant properties. Additional research needs to be carried out to isolate and identify the exact active chemicals responsible for these benefits and to thoroughly analyze the potential mechanisms of action.

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Ethical Issue

The Punjab University Institutional Ethical Review Board reviewed and approved the experimental protocol with voucher number D/88/FIMS dated 28-08-2024.

Conflict of Interest: Authors declare no conflict of interest.

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