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Anti Gastric Ulcer Activity of *Amaranthus spinosus* Linn. Leaves in Aspirin Induced Gastric Ulcer in Rats and the Underlying Mechanism

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Abstract

Problems: Search for new drugs of gastric ulcer. **Experimental approach:** Anti gastric ulcer activity of *Amaranthus spinosus* Linn. in aspirin induced gastric ulcers was studied in rats. **Findings:** Results showed that *Amaranthus spinosus* Linn. leaves significantly reduced ulcer index induced by aspirin. The plant leaves produced gastric anti secretory effect by decreasing gastric volume and acidity. Elevated level of gastric pepsin during aspirin induced ulcers was found lowered by the leaves indicating its involvement in offensive mechanism in production of gastric ulcer. Leaves further increased gastric mucin which showed its gastric cytoprotective effect. The plant leaves prevented loss of gastric protein, DNA and the increased lipid peroxidation during ulceration by aspirin. Activities of the anti oxidant enzymes were enhanced during ulceration by this plant leaves. Results were comparable to that of ranitidine, a standard anti ulcer drug.

Conclusion: Anti ulcerogenic activity of *Amaranthus spinosus* Linn. leaves was mediated through anti oxidant defense mechanism.

Keywords: *Amaranthus spinosus* Linn, aspirin, ranitidine, gastric ulcer, SOD, CAT, GSH.

Introduction

The term “Peptic ulcer” refers to an ulcer in the lower oesophagus, stomach or duodenum, in the jejunum after surgical anastomosis to the stomach or, rarely in the ileum adjacent to a Meckel’s diverticulum. Ulcer in the stomach (gastric ulcer) may be acute or chronic . Quincke [1] was probably the first to use the term ‘Peptic ulcer’. Because of its frequency and worldwide distribution, peptic ulcer continues to be a subject of numerous investigations, both experimental and clinico pathological. In this respect peptic ulcer occupies a place secondary to carcinoma in the field of gastroenterology.

There are medicines to treat peptic ulcer [2] . These drugs have brought about remarkable changes in peptic ulcer therapy but the efficacy of these drugs is still debatable. Reports on clinical evaluation of these drugs show that there are incidences of relapses and adverse effects and danger of drug interactions during ulcer therapy [3-4]. Hence, the search for an ideal anti –ulcer drug continues and has also been extended to medicinal plants / herbs in search for new and novel molecules, which afford better protection and decrease the incidence of relapse.

Numerous medicinal plants showed anti gastric ulcer activity. Sanyal *et al.*[5] found that vegetable banana is efficacious not only for experimentally induced gastric ulcers in albino rats, guinea pigs etc. but also for human being suffering from gastric ulcers. Akah *et al.*[6] demonstrated anti gastric ulcer activity of the herb *Cassampelos mucronata*. Likewise Shetty *et al.*[7] , Sairam *et al.*[8] , Maity *et al.* [9,10] and Dharmani and Palit [11] confirmed anti gastric ulcer activities of *Ginkgo biloba*, *Convolvulus pluricaulis Chois*, tea root extract and *Vernonia lasiopus* respectively. We also reported anti gastric ulcer activity of few medicinal plants in different experimental ulcer models [12-17] .

Recently we observed anti ulcer activity of the leaves of *Amaranthus spinosus* Linn. against

ethanol induced gastric ulcer in albino rats [18]. Tempted by this observation we undertook studies to note role of *Amaranthus spinosus* Linn. against aspirin induced gastric ulcer in rats. In present communication effect of *Amaranthus spinosus* Linn. leaves on aspirin induced gastric ulcer in albino rats and the possible mechanism involved therein are reported.

Materials and methods

Plant material

Leaves of *Amaranthus spinosus* Linn.were collected from the medicinal plants garden of the University of North Bengal during August – September, 2011 and authenticated by the taxonomist of the department of Botany of the said University. A voucher specimen was kept in



***Amaranthus spinosus* Linn.**

the department for future reference.

Preparation of the Test Drug

Leaves of *Amaranthus spinosus* Linn.were shade dried and powdered. The powder was used as the test drug.

Experimental animals

Wistar strain albino rats (150 - 180 g) of either sex were used for the study. Rats were

housed in colony cages (5 rats / cage) and were kept for at least a week in the experimental wing of the animal house (room temperature 25 – 28 degree centigrade and humidity 60 – 65% with 12 h light and dark cycle) before experimentation. Animals were fed on laboratory diet with water *ad libitum*. 8 rats were used for each set of experiment. The animal experiment was approved by the ethics committee of the Institute.

Chemicals and Drugs

All chemicals used in this experiment were procured from Ranbaxy and SD Fine Chemicals, New Delhi, India. Aspirin (Indosal Chemical Corporation), ranitidine (Cipla pharmaceuticals) were used in the study.

Acute toxicity study

Acute toxicity study of *Amaranthus spinosus* Linn. was done by the method of Ghosh [19]. Rats were starved overnight (water is supplied *ad libitum*) and were divided into five groups of eight each.

Powdered leaves of *Amaranthus spinosus* Linn. in watery suspension was given to the rats orally through feeding tube in increasing dose levels of 0.1, 0.5, 1, 3, 5g/kg body weight. The animals were observed continuously for 2 hours for the following :

- a) *Behavioral profile* : alertness, restlessness, irritability and fearfulness.
- b) *Neurological profile*: spontaneous activity, reactivity, touches response, pain response and gait.
- c) *Autonomic profil* : defecation and urination

The number of deaths, if any, was recorded after 24 and 72 hours.

Production of gastric ulcer

Aspirin induced gastric ulcer was produced by the method of Parmar and Desai [20] with slight modification. Rats were fasted for 36 h when no food but water was supplied *ad libitum*. Aspirin is suspended in 1% carboxy methyl cellulose in water (20 mg/ml) and administered orally (gavage) in a dose of 500 mg/kg to the rats. Pylorus parts of the animals were then ligated. Four hours after pyloric ligation the animals were sacrificed by cervical dislocation. The stomach was taken out, gastric juice collected and the stomach was then incised along the greater curvature

to examine ulcers.

Antiulcer Study

Rats were divided into 4 groups.

1. Control: Rats took normal diet, water and vehicle of aspirin.
2. Drug treated : Rats were treated with aspirin.
3. Drug + *Amaranthus spinosus* Linn. : Powdered leaves of *Amaranthus spinosus* Linn. in watery suspension was given to the rats orally through feeding tube 30 minutes prior to administration of aspirin.
4. Drug + Ranitidine : Ranitidine was given in the dose of 50 mg/kg p.o. 30 minutes prior to administration of aspirin. Dose of ranitidine was selected based on report of Khare *et al.* [21].

Evaluation of Ulcer Index

This was done by the method of Szelenyi and Thiemer [22]. According to this method,

Gastric lesions were counted and the mean ulcerative index was calculated as follows :

I - Presence of edema, hyperemia and single sub mucosal punctiform hemorrhage.

II – Presence of sub mucosal hemorrhagic lesions with small erosions.

III – Presence of deep ulcer with erosions and invasive lesions.

Ulcer index = (number of lesion I) x1 + (number of lesion II) x2 + (number of lesion III) x 3.

Biochemical estimations

Collected gastric juice from the rat's stomach was centrifuged and its volume and pH were measured. Gastric juice was further used for the estimation of free and total acidity as described by Hawk *et al.*[23] , pepsin content by the method of Anson [24], mucin by our method [25] and total protein by the method of Lowry *et al.*[26].

The mucosal tissue from rat's stomach was scrapped and then homogenized (5%) in ice cold 0.99% saline with a Potter – Elvehjem glass homogenizer for 30 sec. The homogenate was used for the estimations of DNA [27], nitric oxide [28], lipid peroxides [29], superoxide dismutase [30], catalase [31], glutathione [32], and glutathione peroxidase [33] .

Statistical Analysis

The values were expressed as mean \pm SEM and were analyzed using one-way analysis of

variance (ANOVA) using Statistical Package for Social Sciences (SPSS) 20th versions.

Differences between means were tested employing Duncan's multiple comparison test and significance was set at $p < 0.05$.

Results and discussion

Effect of powdered leaves of *Amaranthus spinosus* Linn. on acute toxicity study

Acute toxicity studies revealed the non toxic nature of powdered leaves of *Amaranthus spinosus* Linn. No toxic reactions were found in any one of the selected doses till the end of study. All rats were healthy and active during the experimental period. Not a single rat died during the study.

Effect of *Amaranthus spinosus* Linn on aspirin induced gastric ulcers in rats

Results were shown in Table -1.

Aspirin produced massive ulcers in glandular part of rat's stomach. Incidence of ulceration was 100% . Acute dilatation and hemorrhage were seen in stomach. In two rats perforation of the stomach was noted. Ulcer index came 30.1 ± 1.22 . Pretreatment of rats with *Amaranthus spinosus* Linn. leaves gave significant protection (25.25%, 63.12% and 66.78% by the doses 0.5 g/kg, 1.0 g/kg and 1.5 g/kg respectively) to the animals from forming ulcers by aspirin. Ranitidine (50 mg/kg), however, gave 72.76% protection.

Effect of *Amaranthus spinosus* Linn. leaves on volume and pH of gastric juice during aspirin induced gastric ulcer in rats.

Results were shown in Table – 2. Aspirin increased volume of gastric juice and decreased its pH. In control rats volume and pH of gastric juice were 1.29 ± 0.06 and 2.95 ± 0.06 respectively. In aspirin group the same values came 3.44 ± 0.09 and 1.70 ± 0.05 respectively. Changes were statistically significant ($p < 0.001$). Pretreatment of rats with *Amaranthus spinosus* Linn. leaves, however, could decrease volume of gastric juice (1.36 ± 0.06) and increase its pH (2.71 ± 0.05). Effects were comparable to that of ranitidine.

Effect of *Amaranthus spinosus* Linn. leaves on free and total acidity of gastric juice during aspirin induced gastric ulcer in rats.

Table – 3 showed effects of *Amaranthus spinosus* Linn. leaves and ranitidine on free and total acidity of gastric juice during aspirin induced gastric ulcer in rats. Aspirin significantly increased both free and total gastric acidity. Free and total gastric acidity of control rats were



Aspirin induced gastric ulcer in albino rats.



Effect of *Amaranthus spinosus* Linn. on aspirin induced gastric ulcer in albino rats.

10.42 ± 0.45 and 30.92 ± 0.49 respectively. For aspirin group values came 23.71 ± 1.25 (free acidity) and 78.72 ± 2.62 (total acidity). *Amaranthus spinosus* Linn. leaves could decrease free and total gastric acidity (13.13 ± 0.63 and 47.36 ± 1.39 respectively). Ranitidine also decreased raised free and total gastric acidity during aspirin induced gastric ulcers.

Effect of *Amaranthus spinosus* Linn. leaves on gastric pepsin and mucin during aspirin induced gastric ulcer in rats.

Effects of *Amaranthus spinosus* Linn. and ranitidine on gastric pepsin and mucin during aspirin induced gastric ulcer in rats were shown in Table - 4. It appears from the table that aspirin elevated activity of gastric pepsin (46.32 ± 1.53, control - 30.66 ± 1.07) and lowered mucin content of gastric juice (1.52 ± 0.16, control - 5.33 ± 0.31).Changes were statistically significant (p<0.001). *Amaranthus spinosus* Linn. could decrease gastric pepsin activity and increase mucin content of gastric juice during aspirin induced gastric ulcer in albino rats. Results were statistically significant and comparable to that of ranitidine.

Table-1. Effect of *Amaranthus spinosus* Linn. leaves (ASL) on aspirin (ASP) induced gastric ulcer in albino rats.

Group	Ulcer index (mean ± SEM)	% Ulcer protection
Control	Nil	--
ASP	30.1 ± 1.22	--
ASP + ASL (0.5g/kg)	22.5 ± 1.11*	25.25
ASP + ASL (1.0g/kg)	11.1± 1.14**	63.12
ASP + ASL (1.5g/kg)	10.0 ± 1.01**	66.78
ASP + Ranitidine (50mg/kg)	8.2 ± 1.03**	72.76

Results were in mean ± SEM, Each group had eight rats, *p<0.05, ** p<0.001

Table-2. Effect of *Amaranthus spinosus* Linn. leaves (ASL) on volume and pH of gastric juice during aspirin (ASP) induced gastric ulcer in albino rats.

Group	Volume of gastric juice (ml)	pH of gastric juice
Control	1.29 ± 0.06	2.95 ± 0.06
ASP	3.44 ± 0.09**	1.70 ± 0.05**
ASP + ASL (1.0 g/kg)	1.36 ± 0.06**	2.71 ± 0.05**
ASP + Ranitidine (50mg/kg)	1.30 ± 0.07**	2.83 ± 0.05**

Results were in mean ± SEM, Each group had eight rats, ** p<0.001

Table-3. Effect of *Amaranthus spinosus* Linn. leaves (ASL) on free and total gastric acidity during aspirin (ASP) induced gastric ulcer in albino rats.

Group	Free acidity (mEq/l/100g)	Total acidity (mEq/l/100g)
Control	10.42 ± 0.45	30.92 ± 0.49
ASP	23.71 ± 1.25**	78.72 ± 2.62**
ASP + ASL (1.0 g/kg)	13.13 ± 0.63**	47.36 ± 1.39**
ASP + Ranitidine (50mg/kg)	10.65 ± 0.51**	33.23 ± 1.11**

Results were in mean ± SEM, Each group had eight rats, ** p<0.001

Table-4. Effect of *Amaranthus spinosus* Linn. leaves (ASL) on gastric pepsin and mucin content during aspirin (ASP) induced gastric ulcer in albino rats.

Group	Pepsin (micromole/ml)	Mucin (microgram/g)
Control	30.66 ± 1.07	5.33 ± 0.31
ASP	46.32 ± 1.53**	1.52 ± 0.16**
ASP + ASL (1.0 g/kg)	31.63 ± 1.19**	4.76 ± 0.22**
ASP + Ranitidine (50mg/kg)	33.47 ± 1.42**	4.59 ± 0.13**

Results were in mean ± SEM, Each group had eight rats, ** p<0.001

Table-5. Effect of *Amaranthus spinosus* Linn. leaves (ASL) on gastric protein and DNA content during aspirin (ASP) induced gastric ulcer in albino rats.

Group	Protein (mg/ml)	DNA (microgram/mg protein)
Control	35.92 ± 1.52	137.13 ± 5.21
ASP	10.67 ± 1.15**	84.19 ± 3.32**
ASP + ASL (1.0 g/kg)	29.88 ± 1.01**	121.66 ± 3.52**
ASP + Ranitidine (50mg/kg)	32.73 ± 1.61**	127.11 ± 4.63**

Results were in mean ± SEM, Each group had eight rats, ** p<0.001

Table-6. Effect of *Amaranthus spinosus* Linn. leaves (ASL) on gastric nitric oxide and lipid peroxides during aspirin (ASP) induced gastric ulcer in albino rats.

Group	Nitric oxide (micromole/g wet tissue)	Lipid peroxides (nm/mg protein)
Control	116.15 ± 7.91	4.68 ± 0.05
ASP	195.64 ± 8.33**	9.94 ± 0.06**
ASP + ASL (1.0 g/kg)	129.73 ± 7.56**	4.82 ± 0.07**
ASP + Ranitidine (50mg/kg)	120.12 ± 6.34**	4.58 ± 0.06**

Results were in mean ± SEM, Each group had eight rats, ** p<0.001

Table-7. Effect of *Amaranthus spinosus* Linn. leaves (ASL) on superoxide dismutase and catalase activity during aspirin (ASP) induced gastric ulcer in albino rats.

Group	Superoxide dismutase (SOD) (Unit/g wet tissue)	Catalase (CAT) (Unit/g wet tissue)
Control	136.79 ± 5.35	24.94 ± 2.16
ASP	88.92 ± 2.11**	9.84 ± 1.23**
ASP + ASL (1.0 g/kg)	120.87 ± 4.32**	18.67 ± 2.11**
ASP + Ranitidine (50mg/kg)	128.96 ± 4.43**	20.56 ± 2.32**

Results were in mean ± SEM, Each group had eight rats, ** p<0.001

Table-8. Effect of *Amaranthus spinosus* Linn. leaves (ASL) on glutathione and Glutathione per oxidase activity during aspirin (ASP) induced gastric ulcer in albino rats.

Group	Glutathione(GSH) (micro mole/mg protein)	Glutathione per oxidase (Micro mole of GSH consumed/min/mg protein)
Control	4.94 ± 0.88	235.12 ± 7.22
ASP	1.59 ± 0.04**	190.61 ± 5.05**
ASP + ASL (1.0 g/kg)	4.93 ± 0.06**	218.13 ± 6.56**
ASP + Ranitidine (50mg/kg)	4.77 ± 0.07**	225.13 ± 6.44**

Results were in mean ± SEM, Each group had eight rats, ** p<0.001

Effect of *Amaranthus spinosus* Linn. leaves on gastric protein and DNA content of gastric mucosa during aspirin induced gastric ulcer in rats.

Results are given in Table – 5. Gastric protein and DNA content of gastric mucosa were found decreased by aspirin. Values were statistically significant (p<0.001). Pretreated group of rats by *Amaranthus spinosus* Linn. leaves showed increased values of gastric protein and DNA content of gastric mucosa which were more close to the normal values. Results were comparable to that of ranitidine.

Effect of *Amaranthus spinosus* Linn. leaves on gastric nitric oxide and lipid peroxides during aspirin induced gastric ulcer in rats.

Nitric oxide levels and Lipid peroxides were increased significantly (p<0.001) in aspirin treated rats. Pretreatment with *Amaranthus spinosus* Linn. leaves reversed this enhanced free radicals. Values came down almost to controls. Ranitidine also decreased nitric oxide levels and Lipid peroxides during aspirin induced gastric ulcers. Results are shown in Table – 6.

Effect of *Amaranthus spinosus* Linn. leaves on gastric superoxide dismutase and catalase activity during aspirin induced gastric ulcer in rats.

Gastric superoxide dismutase and catalase activity was found significantly decreased during

aspirin induced gastric ulcer. But in group of rats pretreated with *Amaranthus spinosus* Linn. leaves activities of these enzymes were maintained near normal. Same trend was also noticed for ranitidine (Table – 7).

Effect of *Amaranthus spinosus* Linn. leaves on gastric glutathione and glutathione peroxidase activity during aspirin induced gastric ulcer in rats.

Results are given in Table – 8. Aspirin caused significant decrease in gastric glutathione level and glutathione peroxidase activity. In control rats levels of glutathione and glutathione peroxidase were 4.94 ± 0.88 micro mole/mg protein and 235.12 ± 7.22 micro mole of GSH/min/mg protein respectively. These values came down to 1.59 ± 0.04 and 190.61 ± 5.05 respectively after aspirin treatment. Rats pretreated with *Amaranthus spinosus* Linn. leaves, however, showed increased glutathione level and glutathione peroxidase activity in aspirin group and the values were near to control values and statistically significant.

Amaranthus spinosus Linn., a medicinal plant under the family of amaranthaceae, is distributed in lower to middle hills (3000–5000 ft) of entire north eastern Himalayas. The plant grows in cultivated areas as well as in waste places. Leaves of *Amaranthus spinosus* Linn. are stacked and alternate. The plant is known as “prickly amaranthus” in English and “ban lure” or “dhuti ghans” in Nepali. Medicinal uses of *Amaranthus spinosus* Linn. as mentioned in Ayurvedic text [34,35] are: Leaf infusion is diuretic and used in anemia. Root paste is used in gonorrhoea, eczema, menorrhoea etc. Ethnic use of *Amaranthus spinosus* Linn. is mainly with village-people of Sikkim who use leaf infusion of the plant in stomach disorder specially in case of indigestion and peptic ulcer [36].

Hussain *et al.* [37] showed that ethanol extract of whole plant of *Amaranthus spinosus* Linn. has anti diarrheal and anti ulcer activity in experimental animals. Recently we confirmed anti ulcer activity of *Amaranthus spinosus* Linn. leaves in ethanol induced gastric ulcer and cysteamine induced duodenal ulcer in albino rats [18]. Since more models are needed to confirm medicinal property of a material we studied anti gastric ulcer activity of *Amaranthus spinosus* Linn. leaves in aspirin induced gastric ulcer in albino rats.

Results showed that *Amaranthus spinosus* Linn. leaves in the dose of 1g/kg could prevent formation of aspirin induced gastric ulcer by 63.12%. The result was comparable to that of

ranitidine, the standard drug of ulcer, where inhibition rate was 72.76% (Table – 1).

In ulcer research emphasis has been given on rate of gastric secretion and gastric pH[38]. Aspirin increased gastric secretion in rats. *Amaranthus spinosus* Linn. leaves could lower the increased gastric secretion to almost normal level. This gastric inhibitory activity of the plant, perhaps, helps to prevent formation of gastric ulcer by aspirin. The plant leaves also increased pH of gastric juice (Table – 2).

Elevated gastric acidity is responsible for ulcer formation [2]. Aspirin increased gastric free and total acidity. Gastric acidity of rats pretreated with *Amaranthus spinosus* Linn. leaves showed more or less normal values. This helps to prevent ulcer formation by aspirin. Ranitidine also decreased gastric acidity (Table – 3).

Pepsin has relation with development of gastric ulcer. In many cases of gastric ulcers, gastric pepsin was found elevated[2]. The present study showed that gastric pepsin was elevated during aspirin induced gastric ulcer which came to almost normal level by the pretreatment with *Amaranthus spinosus* Linn. leaves . Gastric mucin which gives cyto protection to stomach[39] was found decreased by aspirin and reversed back to control level by pretreatment with *Amaranthus spinosus* Linn. leaves (Table – 4). This suggests cyto protective activity of the plant. In experimental ulcers levels of gastric protein and DNA of gastric mucosa are found to be decreased[39]. Aspirin could develop ulcer by decreasing gastric protein and DNA of gastric mucosa. Rats pretreated with *Amaranthus spinosus* Linn. leaves showed increased amount of these materials and thereby could prevent ulcer formation (Table – 5).

Gastric mucosal lipid peroxidation has been reported to increase incidence of experimental ulcers [40]. In this study we noted elevated levels of nitric oxide and lipid peroxides in gastric mucosa during aspirin induced gastric ulceration. Pretreatment of rats by *Amaranthus spinosus* Linn. leaves could decrease levels of gastric nitric oxide and lipid peroxides thus prevent aspirin induced ulcer formation (Table – 6)

Free radicals scavenging enzymes like superoxide dismutase, catalase, glutathione per oxidase are involved in development of gastric ulcer. If generation of free radicals exceeds the ability of free radical scavenging enzymes, gastric mucosa may be injured by the free radicals resulting

development of gastric ulcer [41]. Activities of all these free radical scavenging enzymes were found decreased by aspirin which was reversed by pretreatment with *Amaranthus spinosus* Linn. leaves (Tables - 7, 8)

From the results of this study, it may be stated that aspirin could increase gastric lipid peroxidation therefore generate reactive oxygen metabolites. This could damage gastric cells. This was reflected by decreased amount of DNA in gastric mucosa which, in turn, was responsible for decreased synthesis of gastric muco substances. In absence of proper protective layer of muco substances, ulcer developed in the stomach. *Amaranthus spinosus* Linn. leaves, on the other hand, could inhibit gastric lipid peroxidation thereby inhibit generation of reactive oxygen metabolites. This could protect the gastric cells from damage. DNA of gastric mucosa was, thus, found increased with concomitant increase in the level of muco substances. These muco substances gave proper protection in the stomach for which ulcer could not develop.

Conclusion

Leaves of *Amaranthus spinosus* Linn. could decrease aspirin induced gastric ulcers in rats and this anti ulcerogenic activity was mediated through anti oxidant defense mechanism.

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Authors Column



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