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Comparative Evaluation of Anti Gastric Ulcer Activity of Root, Stem and Leaves of *Amaranthus spinosus* Linn. in Rats

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Anti gastric ulcer activity of root, stem and leaves of *Amaranthus spinosus* Linn. was studied against ethanol, hydrochloric acid, indomethacin, stress and pyloric ligation induced gastric ulceration in albino rats. Omeprazole was used as standard anti gastric ulcer drug. Significant anti gastric ulcer activity was noted in root, stem and leaves of *Amaranthus spinosus* Linn. Root of the plant, however, showed highest activity which was comparable to that of omeprazole.

Keyword: Amaranthus Spinosus Linn, Antiulcer Activity, Ethanol, Stress, Indomethacin, Pyloric Ligation.

1. Introduction

A*maranthus spinosus* Linn. (Family : Amaranthaceae), one of the medicinal plants of Eastern Himalaya specially of Sikkim Himalaya, is known as "prickly amaranthus" in English and "ban lure" or "dhuti ghans" in Nepali. The plant is distributed in Common Temperate Himalaya at a range of 3000 – 5000 feet. It is also found on sloppy waste place. Leaves of *Amaranthus spinosus* Linn. are stacked and alternate. Hussain *et al*.[1] showed that ethanol extract of whole plant of *Amaranthus spinosus* Linn. has anti diarrheal and anti ulcer activity in experimental animals. Besides, *Amaranthus spinosus* Linn. is used as laxative, diuretic, digestive and anti pyretic. It is also used to treat anorexia, leprosy, blood diseases, burning sensation, bronchitis, piles and leucorrhoea. The plant is further reported having anti-inflammatory properties, immunomodulatory activity and has effect on hematology[2-6] . Recent studies showed antidiabetic property of *Amaranthus spinosus* Linn[7,8].

Ethnic use of A*maranthus spinosus* Linn, as reported in literature[9,10] is mainly in peptic ulcer. Leaf juice of the plant, two tea spoonful thrice a day, is given to patients suffering from peptic ulcer.

It was thus thought worthwhile to undertake study on the anti gastric ulcer activity of *Amaranthus spinosus* Linn. In this communication results of experiments of anti gastric ulcer activity of root, stem and leaves of *Amaranthus spinosus* Linn. in experimental ulcer models are reported.

2. Materials and Methods

2.1 Plant materials

A*maranthus spinosus* Linn. was collected from the medicinal plant garden of the University of

North Bengal sometimes in August, 2012 and authenticated by the experts of the department of Botany of the said University. Voucher specimens of the plant was kept in the department for future use.

A*maranthus spinosus* Linn.

2.2 Test drug

Root, stem and leaves were separated from the plant A*maranthus spinosus* Linn. They were washed thoroughly, sundried and powdered. Powdered materials were used as test drugs.

2.3 Experimental Animals

Wistar strain albino rats of both sex were used for the study. The animals were housed in colony cages (4 rats/cage) and were kept for at least a week in the experimental wing of the animal house (room temperature 25–28^{0}C and humidity 60–65% with 12 h light and dark cycle) before experimentation. Animals were fed on laboratory diet with water *ad libitum*. For each set of experiment ten animals were used. For toxicological study mice were employed. The animal experiment had approval of the institutional ethics committee.

2.4 Chemicals

Indomethacin (Torrent Research Centre, Gandhinagar), ethanol (Baroda Chemical industries Ltd., Dabhoi), HCl LR (Thomas baker, Mumbai), omeprazole (Kopran Pharma Ltd. Mumbai).

2.5 Production of gastric ulcers

2.5.1 Ethanol Induced Gastric Ulcer (Sairam *et al.* 2001)[11]

Rats were fasted for 18 h when no food but water was supplied *ad libitum*. Gastric ulcers were induced by administering ethanol (95%, 1 mL/200 g body weight) orally through a feeding tube. 1h after administration of ethanol, animals were sacrificed by cervical dislocation and the stomach was taken out and incised along the greater curvature. Stomach was then examined for the presence of ulcers.

2.5.2 HCl Induced Gastric Ulcer (Parmar and Desai, 1993)[12]

0.6M HCl (1 mL/200 g body weight) was orally administered to all rats. Rest part is same to that of ethanol induced gastric ulcer group.

2.5.3 Indomethacin Induced Gastric Ulcer (Parmar and Desai, 1993)[12]

Indomethacin(10 mg/kg) was given orally to rats in two doses at an interval of 15 hour. Rest part is same to that of ethanol induced gastric ulcer group.

2.5.4 Stress Induced Gastric Ulcer (Alder, 1984)[13]

Rats were fasted for 24h when no food but water was supplied *ad libitum*. Stress ulcer was induced by forced swimming in the glass cylinder (height 45 cm, diameter 25 cm) containing water to the height of 35 cm maintained at 25^{0}C for 3h. Rats were then sacrificed. Rest part was same to that of ethanol induced gastric ulcer group.

2.5.5 Induction of Gastric Ulcer by Pyloric Ligation Method (Parmar and Desai, 1993)[12]

Rats were fasted for 24h when no food but water was supplied *ad libitum*. Under light ether anesthesia, abdomen was opened and the pylorus was ligated. The abdomen was then sutured. After 4h the rats were sacrificed with excess of anesthetic ether and the stomach was dissected
out. Rest part was same to that of ethanol induced gastric ulcer group.

2.6 Acute Oral Toxicity Study (Ghosh, 2005)[14]

Acute toxicity studies were carried out on Swiss albino mice. In separate experiments the test drugs i.e. powdered root, stem and leaves of A*maranthus spinosus* Linn. were given orally at doses of 100, 200, 500, 1000 and 3000 mg/kg to different groups of mice each group containing six animals. After administering the test drug, the animals were observed for the first three hours for any toxic symptoms followed by observation at regular intervals for 24 hours up to seven days. At the end of the study, the animals were also observed for general organ toxicity, morphological behavior and mortality.

2.7 Anti Gastric Ulcer Study

Rats were divided into 5 groups .
Group 1 : Ulcerogenic drug or Method (Ethanol / HCl / Indomethacin / Stress / Pyloric ligation)
Group 2 : Ulcerogenic drug or method + powdered root of A*maranthus spinosus* Linn.
Group 3 : Ulcerogenic drug or method + powdered stem of A*maranthus spinosus* Linn.
Group 4 : Ulcerogenic drug or method + powdered leaves of A*maranthus spinosus* Linn.
(Test drug was given orally 30 minutes prior to administration of ulcerogenic drug or method. Dose of the test drug was kept 1 g/kg body weight of the animal as per our earlier work[15])
Group 5 : Ulcerogenic drug or method + Omeprazole (8 mg/kg orally 30 minutes prior to administration of ulcerogenic drug or method). Omeprazole was used as per the method of Malairajan *et al*., 2008[16].

2.8 Evaluation of Ulcer Index (Szelenyi and Thiemer,1978)[17]

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.

2.9 Statistical Analysis

The values were expressed as mean ± SEM and were analyzed using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS). Differences between means were tested employing Duncan's multiple comparison test and significance was set at $p < 0.05$.

3. Results and Discussion

3.1 Acute Toxicity Studies

Acute toxicity studies revealed that the test drugs (roots, stems and leaves of A*maranthus spinosus* Linn.) did not produce any toxic symptoms when administered orally to mice in doses of 100, 200, 500, 1000 and 3000 mg/kg. Animals were healthy, cheerful and behaved normal throughout the experimental period. No death of animal was recorded during seven days of experiment.

3.2 Effect of root, stem and leaves of *Amaranthus spinosus* Linn. on Ethanol Induced Gastric Ulcer in Albino Rats

Result is given inTable-1

Table-1. Effect of root, Leave And Stem of A*maranthus Spinosus* Linn. on Ethanol Induced Gastric Ulcer

Group	Ulcer index (mean ± SEM)	% Ulcer protection
Control	Nil	--
Ethanol	30.8 ± 1.51	--
Ethanol+ root of AS(1g/kg)	12.7 ± 1.23**	58.44
Ethanol+ leave of AS(1g/kg)	18.1 ± 1.39**	41.23
Ethanol+ stem of AS(1g/kg)	16.7 ± 1.45**	45.78
Ethanol+Omeprazole (8mg/kg)	10.3 ± 1.08**	66.55

Results were in mean ± SEM, Each group had ten rats, ** p<0.001. AS : A*maranthus spinosus* Linn.

Massive gastric ulcers were developed in all rats by ethanol. Ulcers were superficial associated with bleeding. Ulcer index came 30.8 ± 1.51. Adhesion and dilatation of the stomach were also seen. Pretreatment with root, leave or stem of A*maranthus spinosus* Linn. gave significant protection to the animals from formation of ethanol induced gastric ulcer. Ulcer index came 12.7 ± 1.23 (protection : 58.44%), 18.1 ± 1.39 (protection : 41.23%) and 16.7 ± 1.45 (protection : 45.78%) respectively with root, leave and stem of A*maranthus spinosus* Linn.. Omeprazole gave further protection to the animals from formation of ethanol induced gastric ulcer. Ulcer index came 10.3 ± 1.08 that means 66.55% protection was achieved.

3.3 Effect of root, stem and leaves of *Amaranthus spinosus* Linn. on HCl Induced Gastric Ulcer In Albino Rats

Result is shown in Table – 2

Table 2: Effect of root, leave and stem of A*maranthus spinosus* Linn. on hydrochloric acid (HCl) induced gastric ulcer

Group	Ulcer index (mean ± SEM)	% Ulcer protection
Control	Nil	--
HCl	29.7 ± 1.31	--
HCl+ root of AS(1g/kg)	14.1 ± 1.32**	52.52
HCl+ leave of AS (1g/kg)	17.2 ± 1.05**	42.09
HCl+ stem of AS(1g/kg)	18.4 ± 1.70**	38.04
HCl+Omeprazole (8mg/kg)	10.2 ± 1.44**	65.65

Results were in mean ± SEM, Each group had ten rats, ** p<0.001. AS : A*maranthus spinosus* Linn.

All rats developed gastric ulcer by hydrochloric acid (HCl). Ulcers were deep and penetrating. There were adhesion, dilatation and bleeding in the stomach. Ulcer index came 29.7 ± 1.31.

Pretreatment of rats with root, leave or stem of A*maranthus spinosus* Linn. gave significant protection to the animals from formation of HCl induced gastric ulcers. Protections were 52.52%, 42.09% and 38.04% respectively with root, leave and stem A*maranthus spinosus* Linn. Omeprazole produced more protection (65.65%) in course of production of gastric ulcer by HCl.

3.4 Effect of Root, Stem and Leaves of *Amaranthus spinosus* Linn. on Indomethacin Induced Gastric Ulcer in Albino Rats

Result is shown in Table – 3

Table-3. Effect of root, leave and stem of A*maranthus spinosus* Linn. on indomethacin (INDO) induced gastric ulcer

Group	Ulcer index (mean ± SEM)	% Ulcer protection
Control	Nil	--

INDO	30.5 ± 1.51	--
INDO+ root of AS(1g/kg)	15.9 ± 1.13**	47.87
INDO+ leave of AS (1g/kg)	19.2 ± 1.22**	37.05
INDO+ stem of AS(1g/kg)	17.3 ± 1.43**	43.28
INDO+Omeprazole (8mg/kg)	11.0 ± 1.34**	63.93

Results were in mean ± SEM, Each group had ten rats, ** p<0.001 AS :A*maranthus spinosus* Linn.

Indomethacin produced massive ulcers in stomachs of all rats. Adhesion and dilatation of the stomach were seen. There was bleeding in few stomach. Ulcer index came 30.5 ± 1.51. Pretreatment with root, leave or stem of A*maranthus spinosus* Linn. gave significant protection to the animals from formation of indomethacin induced gastric ulcers. Ulcer index came 15.9 ± 1.13 (protection : 47.87%), 19.2 ± 1.22 (protection : 37.05%) and 17.3 ± 1.43 (protection : 43.28%) respectively with root, leave and stem of A*maranthus spinosus* Linn.. More protection (63.93%) was noted in omeprazole group. Ulcer index came 11.0 ± 1.34

3.5 Effect of Root, Stem and Leaves of *Amaranthus Spinosus* Linn. on Swimming Stress Induced Gastric Ulcer in Albino Rats

Result is shown in Table – 4

Table-4. Effect of root, leave and stem of A*maranthus spinosus* Linn. on Swimming Stress (SS) induced gastric ulcer

Group	Ulcer index (mean ± SEM)	% Ulcer protection
Control	Nil	--
SS	31.4 ± 1.22	--
SS+ root of AS(1g/kg)	16.5 ± 1.31**	47.45
SS+ leave of AS (1g/kg)	18.0 ± 1.33**	42.67
SS+ stem of AS(1g/kg)	19.1 ± 1.54**	39.19
SS+Omeprazole (8mg/kg)	10.5 ± 1.26**	66.56

Results were in mean ± SEM, Each group had ten rats, ** p<0.001, AS : A*maranthus spinosus* Linn.

Swimming stress produced massive gastric ulcers in all the rats under study. Most of the ulcers were superficial in nature. Few ulcers were penetrating. There was bleeding in the stomach. Adhesion and dilatation were also noticed in stomach. Ulcer index came 31.4 ± 1.22. Pretreatment of rats with root, leave or stem of A*maranthus spinosus* Linn. gave significant (p<0.001) protection of the animals from swimming stress induced ulcers by 47.45% (ulcer index : 16.5 ± 1.31), 42.67% (ulcer index : 18.0 ± 1.33) and 39.19% (ulcer index : 19.1 ± 1.54) respectively. Omeprazole gave more protection (66.56%) to the rats from swimming stress induced gastric ulcers. Ulcer index came 10.5 ± 1.26.

3.6 Effect of Root, Stem and Leaves of *Amaranthus spinosus* Linn. on Pyloric Ligation Induced Gastric Ulcer In Albino Rats

Result is shown in Table – 5

Table-5. Effect of root, leave and stem of A*maranthus spinosus* Linn. on Pyloric Ligation(PL) induced gastric ulcer

Group	Ulcer index (mean ± SEM)	% Ulcer protection
Control	Nil	--
PL	28.0 ± 1.45	--
PL+ root of AS(1g/kg)	14.3 ± 1.31**	48.93
PL+ leave of AS (1g/kg)	15.9 ± 1.23**	46.96
PL+ stem of AS(1g/kg)	18.2 ± 1.13**	35.00

PL+Omeprazole (8mg/kg)	10.8 ± 1.24**	61.43

Results were in mean ± SEM, Each group had ten rats, ** p<0.001, AS : A*maranthus spinosus* Linn.

Pyloric ligation induced gastric ulcers in all albino rats. There were adhesion, dilatation and bleeding in the stomach. Ulcers were superficial in nature. Ulcer index came 28.0 ± 1.45. Pretreatment of rats with root, leave or stem of A*maranthus spinosus* Linn. produced significant (p<0.001) protection to the animals from formation of pyloric ligation induced gastric ulceration. Protections were 48.93% (ulcer index : 14.3 ± 1.31), 46.96% (ulcer index : 15.9 ± 1.23) and 35.00% (ulcer index : 18.2 ± 1.13) respectively for root, leave and stem of A*maranthus spinosus* Linn. Omeprazole produced more protection (61.43 %) in course of formation of gastric ulcer by pyloric ligation. Ulcer index in this group came 10.8 ± 1.24

In gastric ulcer disorder a discontinuity in the gastric mucosa is observed. There are medicines to treat ulcer[18]. These include drugs inhibiting proton pump, receptor blocking drugs, drugs affecting central nervous system and drugs that affect the mucosal barrier[19-22] . Many of these drugs, however, do not fulfill all requirements and reports on clinical evaluation of these drugs show that there are incidences of relapses and adverse effects such as impotency, arrhythmias and haematopoietic changes occur[23] . Hence, the search for an ideal anti – ulcer drug continues and has also been extended to vegetables, medicinal plants, herbs etc. in search for new and novel molecules, which afford better protection and decrease the incidence of relapse.

In this direction medicinal plants were extensively screened by researchers. They found that many medicinal plants have anti gastric ulcer activity[24-29] . Report from this laboratory also claimed anti gastric ulcer activity of several plants of this region[30-32] .

In the present study anti gastric ulcer activity of root, stem and leaves of A*maranthus spinosus* Linn. was noted against a variety of experimental ulcer models. It was observed that root had highest anti gastric ulcer activity but stem and leaves of the plant also possessed significant anti ulcer property.

Ayurvedic medicine from India, Traditional Chinese Medicine and various other traditions around the world primarily use plants as the basis of their treatments. According to the World Health Organization 80% of the world's population relies on traditional medicine, which is largely plant-based. And, in this way medicinal plants save millions of lives worldwide. Thus there is increasing demand of medicinal plants. In the year 2011 only, 2000 tonnes of extract of *Coscinium fenestratum*, the medicinal plant used in cure of diabetes, was used by the pharma industry to prepare a wide range of formulations. Unfortunately, this tree is only found in the States of Orissa, Karnataka and Goa. Thus *Coscinium fenestratum* is now in the list of endangered species. Few more medicinal plants like *Saraca Asoca, Taxeus Wallchiana* and *Decilipis Hamiltoni* etc. are also in the red-light category. In fact, a total of 315 medicinal plants
are now under threat of extinction This is due to habitat loss and degradation and obviously due to increasing demand of these plants for consumption in the name of medicine[33- 39] .

Further, in ethnic use roots of the medicinal plants are usually consumed. Thus roots of the medicinal plants are being continuously utilized by village people as well as by the herbal practitioners. This practice destroys the plant which may be another cause of putting them in the list of endangered species.

Under the circumstances, the present study is significant as it gives the message that not only root but stem and leaves of A*maranthus spinosus* Linn. also possessed anti gastric ulcer activity. Thus stem and leaves of the plant can be used, root may be escaped and the plant will be saved.

4. Conclusion

Root, leave and stem of A*maranthus spinosus* Linn. showed anti ulcer effect against ethanol, hydrochloric acid, indomethacin, swimming stress and pyloric ligation induced gastric ulcer in albino rats. Anti ulcer activity in all cases was found statistically significant though root of the plant had maximum anti gastric ulcer effect. The study thus gave a message that to treat gastric ulcer stem or leaves of A*maranthus spinosus* Linn. may be used. Roots may be escaped to save the plant from extinction.

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