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RESEARCH ARTICLE

ANTI PEPTIC ULCER ACTTIVITY OF AN ISOLATED COMPOUND (AS–1) FROM THE LEAVES OF Amaranthus spinosus L.

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ABSTRACT

An active compound (AS-1) was isolated from the leaves of Amaranthus spinosus L. and its antiulcer activity was studied against ethanol induced gastric ulcer and cysteamine induced duodenal ulcer in albino rats. Significant antiulcer activity of AS-1 was observed in all the models. AS-1 thus provides a scientific rationale for the use as antiulcer drug.

Key words: Amaranthus spinosus L., isolated compound (As-1), anti ulcer activity.

INTRODUCTION

Numerous medicinal plants showed anti gastric ulcer activity. Sanyal *et al.* as early as 1961 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 [1]. Akah *et al.* Demonstrated anti gastric ulcer activity of the herb *Cassampelos mucronata* [2]. Likewise Shetty *et al.* [3] Sairam *et al*. [4], Maity *et al.*[5,6] and Dharmani and Palit [7] 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 [8-15].

Recently we observed anti ulcer property of the leaves of *Amaranthus spinosus* L. against experimental peptic ulcer models. Tempted by this observation we undertook studies to isolate the active compound present in *Amaranthus spinosus* L. and to know its antiulcer activity against ethanol induced gastric ulcers and cysteamine induced duodenal ulcers in albino rats.

MATERIALS AND METHODS

Plant materials

Leaves of *Amaranthus spinosus* L. were collected from the medicinal plant garden of the University of North Bengal and identified by the experts of the department of Botany. A voucher specimen of the leaf was kept in the department for future references.

Isolation of the active principle (AS-1) from the leaves of *Amaranthus spinosus* L.

Fresh plant leaves were shade dried at room temperature, ground into fine powder. 50g of this

Powder was then extracted with 500 ml methanol for 24 hours using the soxhlet apparatus at a

Temperature of 60 degree centigrade. The extract was concentrated under reduced pressure using a rotary evaporator to a volume of 10 ml. This was then subjected to column chromatographyusing alumina as adsorbent. Elution was done by 50% methanol-chloroform mixture. Eluted material was evaporated to dryness and extracted with 10 ml ethyl acetate. The ethyl acetate extract was further subjected to column chromatography using silica gel mesh (200-400 size) as adsorbent. The fraction obtained after elution with ethyl format: formic acid mixture (100:5, v/v) was subjected to repeated crystallization when a compound was crystallized. The compound was given a trivial name AS-1. The compound was preserved for acute toxicity study as well as for anti peptic ulcer activity.

Test drug

Isolated compound (AS-1) was used as the test drug.

Acute oral toxicity study

This was done by the method of Ghosh [16]. Acute toxicity studies were carried out on Swiss albino mice. Isolated compound (AS-1) from the leaves of *Amaranthus spinosus* L.was given orally at doses of 100, 500, 1000 and 3000 mg/kg to five groups of mice, each group containing six animals. After administration of the compound, 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.

Experimental animals

Wistar strain albino rats of both sexes were used for the study. The animals 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*. For each set of experiment 8 animals were used. The animal experiment was approved by the ethics committee of the Institute.

Chemicals and drugs

Ethanol (Baroda Chemical industries Ltd., Dabhoi), cysteamine (Sigma Chemical Co., USA) and omeprazole (Kopran Pharma Ltd. Mumbai) were used in the study.

Production of peptic ulcer

Ethanol induced gastric ulcer

This was done by the method of Sairam et al [4]. 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. 1 h 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 bleeding, adhesion, dilatations and ulcers.

Cysteamine induced duodenal ulcer

This was done by the method of Parmar and Desai [17]. To 18 h fasted rats (water was supplied *ad libitum*) cysteamine hydrochloride (400 mg/kg, p.o. in 10% aqueous solution) was administered in two doses at an interval of 4 h to produce duodenal ulcers. After 24 h of the first dose of cysteamine, animals were sacrificed by cervical dislocation and the duodenum was excised carefully and opened along the antimesenteric side. Duodenum was then examined for the presence of ulcers.

Anti ulcer study

Rats were divided into 3 major groups.

1. Drug treated control : In this group either ethanol or cysteamine was given.
2. AS-L and drug : Powdered AS-L collected from the leaves of *Amaranthus spinosus* L. was given to the rats orally 30 minutes prior to administration of ethanol and 30 minutes before each dose of cysteamine hydrochloride. *Amaranthus spinosus* L. was used in two doses - 100 mg/kg and 200 mg/kg.
3. Omeprazole and drug : Omeprazole was given in the dose of 8 mg/kg p.o. 30 minutes prior to administration of ethanol and 30 minutes before each dose of cysteamine hydrochloride. Dose of omeprazole was used as per the method of Malairajan *et al*. [18]

Evaluation of ulcer index

1. Evaluation of ulcer index was done by the method of Szelenyi and Thiemer [19]. Gastric /duodenal lesions were counted and the mean ulcerative index was calculated as follows :
2. Presence of edema, hyperemia and single sub mucosal punctiform hemorrhage.
3. Presence of sub mucosal hemorrhagic lesions with small erosions.
4. 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.

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) 20th versions.

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

RESULTS

Acute toxicity studies

Acute toxicity studies revealed that AS-1 did not produce any toxic symptoms when administered orally to mice in doses of 100, 500, 1000 and 3000 mg/kg. Animals were found healthy, cheerful and behaved normal throughout the experimental period. No death of animal was recorded during seven days of experiment.

Effect of AS-1 on experimental peptic ulcers

Results are shown in Table:1.

Ethanol produced massive gastric ulcers in all albino rats. Ulcers were superficial. There was bleeding in the stomach. Adhesion and dilatation were also seen. Ulcer index was 28.2 ± 1.25. Cysteamine produced profuse ulcer in the upper part of duodenum. Ulcer index came 20.8 ± 1.02

Pretreatment of rats with AS-1 collected from the leaves of *Amaranthus spinosus* L. (100 mg/kg, 200 mg/kg) produced dose dependent reduction of ulcer index in ethanol as well as cysteamine treated rats when compared to control. Omeprazole produced significant protection (68.8% for gastric ulcer and 82.2% for duodenal ulcer) in coarse of ulcer formation. The anti ulcer activity of AS-1 in the dose of 200 mg/kg was comparable to that of omeprazole. In this case protection was 63.8% for gastric ulcer and 76.9% for duodenal ulcer.

Table 1 : Showing effects of AS-1 and omeprazole against ethanol induced gastric ulcer and cysteamine induced duodenal ulcers in rats.

Group & Dose	Ethanol (1 mL/200 g) Ulcer index(mean ± SEM)	Cysteamine(400 mg /kg) Ulcer index(mean ± SEM)
Drug treated control	28.2 ± 1.25	20.8 ± 1.02
AS-1 (100 mg / kg)	15.3 ± 0.69*	8.6 ± 0.43*
AS-1 (200 mg / kg)	10.2 ± 0.68*	4.8 ± 0.51*
Omeprazole (8 mg / kg)	8.8 ± 0.21*	3.7 ± 0.32*

Values were mean ± SEM of 8 animals in each group. * p < 0.001 when compared to drug control.

DISCUSSION

Amaranthus spinosus L., 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* L. 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* L. as mentioned in Ayurvedic text [20] are: Leaf infusion is diuretic and used in anemia. Root paste is used in gonorrhea, eczema, menorrhea etc. Ethnic use of this plant is mainly with village people of Sikkim who use leaf infusion of *Amaranthus spinosus* L. in stomach disorder specially in case of indigestion and peptic ulcer [21].

Recently we observed anti ulcer activity of the leaves of *Amaranthus spinosus* L. against ethanol and cysteamine induced peptic ulcer in albino rats. Tempted by this observation we undertook studies for isolation of the active compound present in *Amaranthus spinosus* L. and to know the antiulcer activity of the isolated compound against different experimental ulcer models.

By various solvent extraction processes and chromatographic experiments an active compound was isolated from the leaves of *Amaranthus spinosus* L. A trivial name of the compound was given as AS-1. Anti gastric ulcer activity of AS-1 was studied against ethanol induced gastric ulceration and cysteamine induced duodenal ulceration in albino rats. Two doses of AS-1 (100 mg/kg and 200 mg/kg) were used. Results were compared with omeprazole, a known anti peptic ulcer drug.

Significant anti peptic ulcer activity of AS-1 was observed in the models employed. Results showed that pretreatment of rats with AS-1 produced dose dependent protection. The protections were statistically significant ($p<0.001$) and comparable to that of omeprazole group.

It is known that peptic ulcer is formed either through offensive mechanism (acid – peptic secretion) or through defensive mechanism (mucus secretion) [22, 23]. Anti peptic ulcer activity of AS-1 may be related with any one of these two mechanisms. Work in this direction is now under progress.

CONCLUSION

An active compound (AS-1) was isolated from the leaves of *Amaranthus spinosus* L. The compound was found having anti peptic ulcer activity against experimental ulcer models. AS-1 thus provides a scientific rationale for the use as antiulcer drug.

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