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EFFECT OF AN ISOLATED COMPPOUND (AS-1) FROM THE LEAVES OF *AMARANTHUS SPINOSUS* L. ON INDOMETHACIN INDUCED GASTRIC ULCER IN ALBINO RATS

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ABSTRACT

This study was aimed to know the anti-gastric ulcer effect of AS-1, a compound isolated from the leaves of *Amaranthus spinosus* L. Gastric ulcer was induced by oral administration of indomethacin (10 mg/kg in two doses) in albino rats and effect of AS-1 was examined. Results showed that compound AS-1 could decrease ulcer index in rats induced by indomethacin. Compound AS-1 produced gastric anti secretory effect by decreasing gastric volume and acidity. It further increased gastric mucin which showed gastric cytoprotective effect. Results were comparable to that of ranitidine, a standard anti-ulcer drug. AS-1, thus, provides a scientific rationale for the use as anti-gastric ulcer drug.

Keywords: *Amaranthus spinosus* L., Isolated compound (AS-1), Anti-ulcer activity, Ranitidine, Indomethacin.

INTRODUCTION

Because of its frequency and worldwide distribution, peptic ulcer occupies a place secondary to carcinoma in the field of gastroenterology. Quincke [1] was probably the first to use the term 'Peptic ulcer'.

There are medicines to treat peptic ulcer [2]. These drugs have brought about remarkable changes in peptic ulcer therapy; 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.

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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 activities of few medicinal plants in different experimental ulcer models [12-17]. Recently we observed anti gastric ulcer activity of the leaves of *Amaranthus spinosus* L. against ethanol induced gastric ulcer in albino rats [18]. We intended to isolate the active compound responsible for anti-gastric ulcer activity. A compound (AS-1) was isolated and found efficacious against ethanol induced gastric ulcers in rats [19]. In present communication we report effect of AS-1 on indomethacin induced gastric ulcer in albino rats.

MATERIALS AND METHOD

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 of Biochemistry, North Bengal Medical College, Dist. Darjeeling, West Bengal, India for future references.

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

Isolation of AS-1 from the Leaves of *Amaranthus spinosus* L. was described in our earlier communication [19]. In brief, 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 chromatography using 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 formate : 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 anti peptic ulcer activity.

Acute toxicity study

In our earlier communication [19] we have reported that AS-!, collected from the leaves of *Amaranthus spinosus L.*, was not toxic for rats.

Experimental animals

Wistar strain albino rats of both sex 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

All chemicals used in this experiment were procured from Ranbaxy and SD Fine Chemicals, New Delhi, India. Indomethacin (Torrent Research Centre, Gandhinagar), ranitidine (Cipla pharmaceuticals) were used in the study.

Production of gastric ulcer

Indomethacin induced gastric ulcer was produced by the method of Parmar and Desai [20] with slight modification. Rats were fasted for 18 h when no food but water was supplied *ad libitum*. Indomethacin (10 mg/kg) was given to rats orally through a feeding tube in two doses at an interval of 15 hour. 1h after administration of last dose, pylorus part of the animals was ligated. Four hours after the 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 the ulcers.

Antiulcer Study

Rats were divided into 4 groups.

1. Control: Rats took normal diet and water.
2. Indomethacin treated: Rats were treated with indomethacin.
3. Indomethacin + *AS-1*: AS-1 in 100 mg/kg and 200 mg/kg doses were given to the rats orally through feeding tube 30 minutes prior to each dose of indomethacin.
4. Indomethacin + Ranitidine: Ranitidine was given in the dose of 50 mg/kg p.o. 30 minutes before each dose of indomethacin. Dose of ranitidine was selected based on the report of Khare *et al* [21].

Evaluation of Ulcer Index

This was done by the method of Szelenyi and Thiemer [22].

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], mucin by our method [24] and total protein by the method of Lowry *et al*.[25].

Statistical analysis

The values were expressed as mean ± SEM and was analyzed using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) 20^{th} versions. Differences between means were tested employing Duncan's multiple comparison test and significance was set at $p < 0.05$.

RESULTS

Effect of AS-1 on indomethacin induced ulcers

Results are shown in Table - 1.

Indomethacin produced massive gastric ulcers in all rats. Ulcers were mostly superficial. Few were penetrating. There was bleeding in the stomach. Adhesion and dilatation were also seen. Ulcer index was 31.7 ± 2.12. Pretreatment of rats with AS-1 isolated from the leaves of *Amaranthus spinosus* L. produced dose dependent reduction of ulcer index in Indomethacin treated rats when compared to control. AS-1 in the dose of 100mg/kg and 200mg/kg gave ulcer index 23.2 ± 1.12 and 12.8 ± 1.23 respectively. Changes were statistically significant. Ranitidine produced significant protection (64.3%) in coarse of ulcer formation. The anti-ulcer activity of AS-1 in the dose of 200 mg/kg (59.6%) was comparable to that of ranitidine.

Anti-secretory effect of AS-1

Indomethacin increased significantly ($p<0.001$) volume of gastric juice, free and total gastric acidity. Control animals showed gastric juice volume 1.13 ± 0.04 ml which was increased to 3.88 ± 0.08 ml by indomethacin. AS-1 reduced significantly gastric juice volume (1.45 ± 0.07) and the value was comparable to that of ranitidine group (Table – 2). The same trend was also noted in case of gastric free and total acidity (Table – 3). pH of gastric juice was, however, found decreased by indomethacin which was elevated by pretreatment of rats with AS-1 (Table – 2).

Cytoprotective effect of AS-1

Results were shown in Table – 4. Indomethacin significantly reduced gastric protein and mucin content. In control rats values of gastric protein and mucin were 37.11 ± 1.09 and 6.87 ± 0.25 respectively. Indomethacin lowered the value to 15.22 ± 1.63 and 1.67 ± 0.14 . Pretreatment of rats with AS-1 increased significantly ($p<0.001$) gastric protein and mucin content. Values were comparable to that of ranitidine.

Table 1. Effect of AS-1 on Indomethacin (IDM) induced gastric ulcer in albino rats.

Group	Ulcer index (mean ± SEM)	% Ulcer protection
Control	Nil	
IDM	31.7 ± 2.12	
IDM+ AS-1 (100mg/kg)	23.2 ± 1.12*	26.8
IDM+ AS-1 (200mg/kg)	12.8 ± 1.23**	59.6
IDM+ Ranitidine (50mg/kg)	11.3 ± 1.10**	64.3

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

Table 2. Effect of AS-1 on volume and pH of gastric juice during Indomethacin (IDM) induced gastric ulcer in albino rats.

Group	Volume of gastric juice (ml)	pH of gastric juice
Control	1.13 ± 0.04	2.88 ± 0.05
IDM	3.88 ± 0.08**	1.73 ± 0.06**
IDM + AS-1 (200 mg/kg)	1.45 ± 0.07**	2.62 ± 0.05**
IDM + Ranitidine (50mg/kg)	1.13 ± 0.05**	2.88 ± 0.06**

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

Table 3. Effect of AS-1 on free and total gastric acidity during Indomethacin (IDM) induced gastric ulcer in albino rats.

Group	Free acidity (mEq/l/100g)	Total acidity (mEq/l/100g)
Control	10.31 ± 0.54	33.25 ± 0.48
IDM	22.17 ± 1.41**	79.21 ± 1.61**
IDM + AS-1 (200 mg/kg)	14.78 ± 0.55**	40.23 ± 1.32**
IDM + Ranitidine (50mg/kg)	11.54 ± 0.51**	32.31 ± 1.59**

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

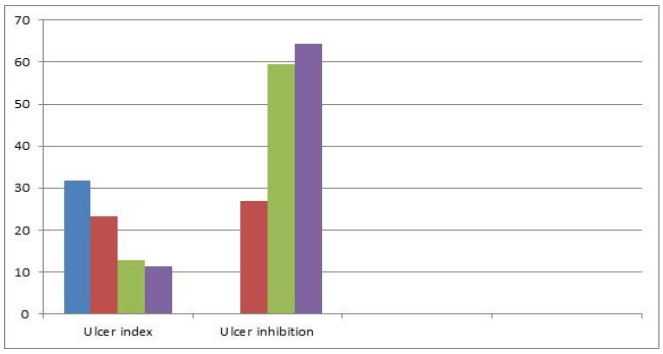
Table 4. Effect of AS-1 on gastric protein and mucin content during Indomethacin (IDM) induced gastric ulcer in albino rats.

Group	Protein (mg/ml)	Mucin (microgram/g)
Control	37.11 ± 1.09	6.87 ± 0.25
IDM	15.22 ± 1.63**	1.67 ± 0.14**
IDM + AS-1 (200mg/kg)	31.27 ± 1.24**	4.99 ± 0.22**
IDM + Ranitidine (50mg/kg)	32.58 ± 1.30**	5.54 ± 0.21**

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

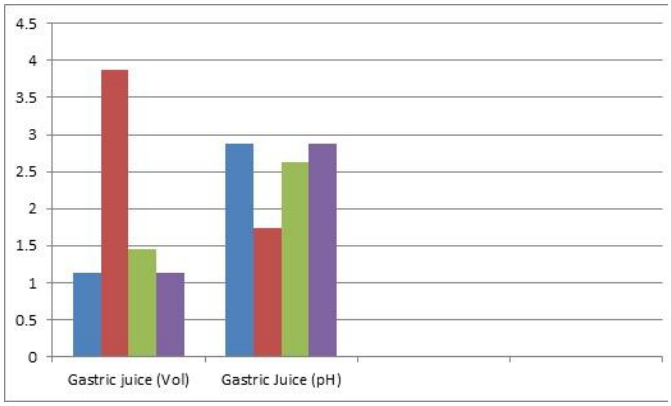
Figure 1. *Amaranthus spinosus* L.

Figure 2. Effect of AS-1, a compound isolated from the leaves of *Amaranthus spinosus* L., on ulcer index, ulcer inhibition (%) in indomethacin induced gastric ulcer in rats.



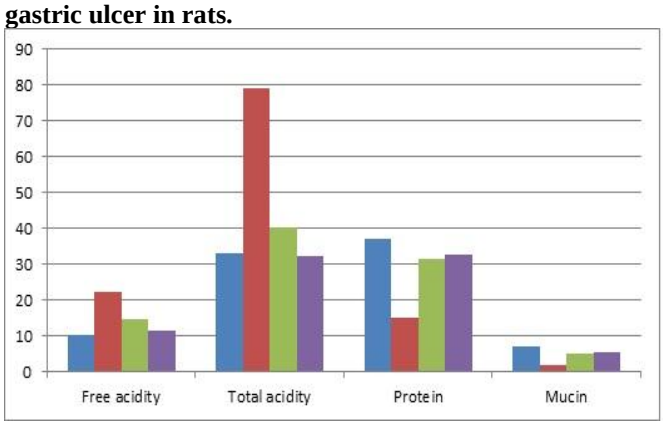
Indomethacin AS – 1 (100 mg/kg) AS – 1 (200 mg/kg) Ranitidine (50 mg/kg)

Figure 3. Effect of AS-1, a compound isolated from the leaves of *Amaranthus spinosus* L., on volume (ml) and pH of gastric juice in indomethacin induced gastric ulcer in rats.



Control Indomethacin AS – 1 (200 mg/kg) Ranitidine (50 mg/kg)

Figure 4. Effect of AS-1, a compound isolated from the leaves of *Amaranthus spinosus* L., on free and total acidity (mEq/l/100g), protein (mg/ml) and mucin content (microgram/g) of gastric juice in indomethacin induced gastric ulcer in rats.



Control Indomethacin AS – 1 (200 mg/kg) Ranitidine (50 mg/kg)

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 *A. 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 *A. spinosus* L. as mentioned in Ayurvedic text [26, 27] are: Leaf infusion is diuretic and used in anemia. Root paste is used in gonorrhea, eczema, menorrhea etc.

Besides, *A. 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 [28-32]. Recent studies showed antidiabetic property of *A. spinosus* Linn [33,34].

Ethnic use of *A. spinosus* L. 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 [35]. Hussain *et al* [36] showed that ethanol extract of whole plant of *A. spinosus* Linn. has anti-diarrheal and anti-ulcer activity in experimental animals.

Recently we observed anti ulcer activity of the leaves of *Amaranthus spinosus* L. against ethanol and cysteamine induced peptic ulcer in albino rats [18]. 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. AS-1 was found effective against ethanol induced gastric ulceration and cysteamine induced duodenal ulceration in albino rats [19].

In present study effect of AS-1 was studied in indomethacin induced gastric ulcer model in rats. Results showed that AS-1 could decrease significantly ulcer index produced by indomethacin. Effect was dose dependent. In dose 200 mg/kg, AS-1 exerted maximum effect which was comparable to that of ranitidine (Table – 1, Figure – 2). AS-1 decreased the elevated level of gastric volume, free and total acidity and increased the decreased level of gastric pH, protein and mucin by indomethacin.

The values were comparable to that of ranitidine, a standard anti gastric ulcer drug (Tables – 2,3,4; Figures – 3,4). The present study thus indicated that AS-1, isolated from the leaves of *Amaranthus spinosus* L., exerted its anti-gastric ulcer effect in indomethacin induced gastric ulcer model in rats through its anti-secretary property and cyto protective activity.

CONCLUSION

Anti-gastric ulcer activity of AS-1, a compound isolated from the leaves of *Amaranthus spinosus* L., was studied in indomethacin induced gastric ulcer model in rats. Result showed that AS-1 exerted anti gastric ulcer activity through its anti-secretary property and cyto protective effect. Results were comparable to that of standard anti-ulcer drug ranitidine.

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