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Anti-peptic ulcer activity of TLC separated fractions of root extract of *Astilbe rivularis* in rats

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ABSTRACT

Anti-peptic ulcer activity of TLC separated fractions of root extract of *Astilbe rivularis*, a plant of Eastern Himalaya, was studied in experimental peptic ulcer models. Gastric ulcers were induced in rats by ethanol, HCl indomethacin, swimming stress and pyloric ligation while duodenal ulcers were developed in the animals by cysteamine. Results were compared with omeprazole, a known drug for peptic ulcer. It was found out that one TLC separated fraction of root extract of *Astilbe rivularis* exerted anti peptic ulcer activity against all the models studied. The anti-peptic ulcer activity of the fraction was, however, less than that of omeprazole.

Keywords: *Astilbe rivularis.*, TLC Separated Fraction, Anti-peptic Ulcer Activity.

1. Introduction

Astilbe rivularis (Family: Saxifragaceae) is one of the medicinal plants of Eastern Himalaya specially of Sikkim Himalaya. The plant, known as 'Buriokahti' in Nepali and 'Pango' in Lepcha, is distributed in Common Temperate Himalaya at a range of 5000 – 9000 feet. It is also found on sloppy waste place. The plant has tall herb stem , leaves are covered with hairs. Ethnic use of *Astilbe rivularis*, as reported in literature[1], is in peptic ulcer. Root juice of the plant, two tea spoonful thrice a day, is given to patients suffering from peptic ulcer.

Tempted on this ethnic use it was thought worthwhile to evaluate scientifically the anti-peptic ulcer activity of the root extract of *Astilbe rivularis*. We conducted experiments and found that the root extract of *Astilbe rivularis* has anti-peptic ulcer activity[2]. We tried to purify active constituent from the root extract of *Astilbe rivularis* responsible for anti-peptic ulcer activity. Various chromatographic experiments were done. TLC separated fractions were isolated and anti-peptic ulcer activity of the fractions were separately investigated in various experimental ulcer models. Results are reported herewith.

2. Materials and Methods

2.1 Plant Material

Roots of *Astilbe rivularis* were collected from the local herbal practitioners and were identified by the experts of the department of Botany of the University of North Bengal, Siliguri, Dist. Darjeeling, West Bengal. A voucher specimen of the root was kept in the department for future references. Roots were shade dried until it attained a constant weight. Roots were then powdered. The powdered root was used in isolation work.

2.2 Acute oral toxicity study

Acute toxicity studies were carried out on Swiss albino mice by the method of Ghosh[3]. Root powder of *Astilbe rivularis* was given orally at doses of 100, 200, 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.

2.3 TLC separated fractions in connection with isolation of anti-ulcer compound from roots of Astilbe ***rivularis***

50g of powdered root of Astilbe *rivularis* were extracted with 500 ml ethanol - water mixture (1:1 v/v) for 30 minutes using soxhlet apparatus at room temperature (35±2 ^{0}C). The extract was concentrated under reduced pressure by a rotary evaporator to a volume of about 10 ml. This was refluxed with 100 ml 1 (N) hydrochloric acid for 15 min at 100^{0}C. After centrifugation for 10 min at 3000g the supernatant was made neutral with 1(N) sodium hydroxide. The material was then subjected to column chromatography using alumina as adsorbent. Elution was done by 50% ethanol-chloroform mixture. Eluted materials were evaporated to dryness separately. Third band was found active. The band was extracted with 10 ml ethyl alcohol and subjected to another column chromatography using silica gel as adsorbent. The first fraction obtained after elution with 50% methanol - chloroform mixture was subjected to TLC (silicic acid as adsorbent) using solvent system ethanol: chloroform: water (60: 20: 20, v/v/v). Three spots were identified. They were separately collected and kept for anti-ulcer study.

2.4 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 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 10 animals were used. The animal experiment had approval of the institutional ethics committee.

2.5 Chemicals

Indomethacin (Torrent Research Centre, Gandhinagar), ethanol (Baroda Chemical industries Ltd., Dabhoi), HCl LR (Thomas baker, Mumbai), omeprazole (Kopran Pharma Ltd. Mumbai), cysteamine (Sigma Chemical Co., USA) were used in the study.

2.6 Test drug

TLC separated compounds were used as test drugs.

2.7 Production of gastric ulcers

2.7.1 Ethanol induced gastric ulcer (Sairam *et al*. 2001) [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 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 general morphology and for the presence of ulcers.

2.7.2 HCl induced gastric ulcer (Parmar and Desai, 1993) [5]

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.

2.7.3 Indomethacin induced gastric ulcer (Parmar and Desai, 1993) [5]

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.

2.7.4 Swimming stress induced gastric ulcer (Alder, 1984) [6]

Rats were fasted for 24h when no food but water was supplied *adlibitum*. 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 25degree centigrade for 3h. Rats were then sacrificed. Rest part was same to that of ethanol induced gastric ulcer group.

2.7.5 Induction of gastric ulcer by pyloric ligation method (Parmar and Desai, 1993) [5]

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.

2.8 Production of duodenal ulcers

2.8.1 Cysteamine induced duodenal ulcer

This was done by the method of Parmar and Desai[5]. 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.

2.9 Anti peptic ulcer study

Rats were divided into 6 groups;

Group 1: Control

Group 2: Ulcerogenic drug or Method (Cysteamine/ Ethanol / HCl / Indomethacin / Swimming stress / pyloric ligation)

Group 3: Ulcerogenic drug or method + TLC fraction 1 (10 mg/kg)

Group 4: Ulcerogenic drug or method + TLC fraction 2 (10 mg/kg)

Group 5: Ulcerogenic drug or method + TLC fraction 3 (10 mg/kg)

(TLC fraction was given orally 30 minutes prior to administration of ulcerogenic drug or method, dose was fixed depending on our earlier experience)

Group 6: 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[7].

2.10 Evaluation of ulcer index (Szelenyi and Thiemer, 1978)[8]

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.

2.11 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 $p < 0.05$.

3. Results and Discussion

3.1 Acute Toxicity Studies

Acute toxicity studies revealed that root powder of *Astilbe rivularis* 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.

Fig 1: *Astilbe rivularis*

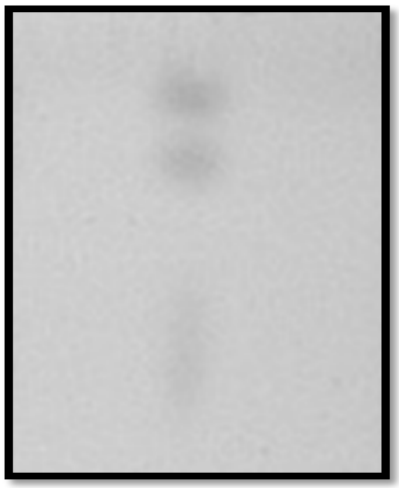

Fig 2: Showing TLC separated fractions.

3.2 Effect of TLC separated fractions of root powder of *Astilbe rivularis* on ethanol induced gastric ulcer in rats

Result is given in Table-1

Ethanol produced massive gastric ulcers in all albino rats. Ulcers were mostly superficial. There was bleeding in the stomach. Adhesion and dilatation were also seen in the stomach. Ulcer index came 30.2 ± 3.0 Pretreatment of rats with TLC fraction - 1 of root powder of *Astilbe rivularis* produced significant protection ($p<0.001$) in rats from ulcer production induced by ethanol. Ulcer index came 15.1 ± 1.1. Protection was thus 50%. Omeprazole gave more protection (66.22%) to the rats from ethanol induced gastric ulcers. Ulcer index was 10.2 ± 1.4. Pretreatment of rats with TLC fraction – 2 and TLC fraction - 3 of root powder of *Astilbe rivularis*, however, did not prevent ulcer formation in rats by ethanol.

Table 1: Effect of TLC separated fractions of root powder of *Astilbe rivularis* on ethanol induced gastric ulcers

Group	Ulcer index (mean ± SEM)
Control	Nil
Ethanol	30.2 ± 3.0
Ethanol + TLC fraction 1	15.1 ± 1.1**
Ethanol + TLC fraction 2	28.5 ± 2.8
Ethanol + TLC fraction 3	25.6 ± 2.3
Ethanol + Omeprazole (8 mg/kg)	10.2 ± 1.4**

3.3 Effect of TLC Separated Fractions of Root Powder of *Astilbe rivularis* on Hydrochloric acid Induced Gastric Ulcer in rats

0.6M hydrochloric acid (HCl) when administered to rats orally produced massive ulcers in stomach of all rats. Adhesion and dilatation of the stomach were seen. Ulcer index was 29.5 ± 3.1. Pretreatment of rats with TLC fraction - 1 of root powder of *Astilbe rivularis* produced significant protection ($p<0.001$) in rats from ulcer production induced by HCl. Ulcer index came, 13.2 ± 1.0 Omeprazole gave more protection to the rats from HCl induced gastric ulcers. Ulcer index was 11.1 ± 1.2. Pretreatment of rats with TLC fraction – 2 and TLC fraction - 3 of root powder of *Astilbe rivularis*, however, did not prevent ulcer formation in rats by HCl. With TLC fraction – 2 ulcer index was 28.8 ± 3.9 and with TLC fraction – 3 ulcer index came 27.7 ± 3.5. Results are given in Table – 2.

3.4 Effect of TLC separated fractions of root powder of *Astilbe rivularis* on indomethacin induced gastric ulcer in rats

Table – 3 shows effect of TLC separated fractions of root powder of *Astilbe rivularis* on indomethacin induced gastric ulcer in rats. Indomethacin produced gastric ulcers in all albino rats. Ulcers were superficial in nature. There were adhesion, dilatation and bleeding in the stomach. Ulcer index came 32.3 ± 3.4. Pretreatment of rats with all three TLC separated fractions of root powder of *Astilbe rivularis* showed that only fraction – 1 produced 54.79% protection in rats from ulcer production induced by indomethacin. Protection was statistically significant up to the level of $p<0.001$. Ulcer index was 14.6 ± 1.1 Omeprazole gave more protection (62.53%) to the rats from indomethacin induced gastric ulcers. Ulcer index came 12.1 ± 1.1. Pretreatment of rats with TLC fraction – 2 and TLC fraction - 3 of root powder of *Astilbe rivularis*, however, did not prevent ulcer formation in rats by indomethacin.

Table 2: Effect of TLC separated fractions of root powder of *Astilbe rivularis* on HCl induced gastric ulcer in rats

Group	Ulcer index (mean ± SEM)
Control	Nil
HCl	29.5 ± 3.1
HCl + TLC fraction 1	13.2 ± 1.0*
HCl + TLC fraction 2	28.8 ± 3.9
HCl + TLC fraction 3	27.7 ± 3.5
HCl + Omeprazole (8 mg/kg)	11.1 ± 1.2*

Each group had ten rats, ** p<0.001

Table3: Effect of TLC separated fractions of root powder of *Astilbe rivularis* on indomethacin induced gastric ulcer in rats

Group	Ulcer index (mean ± SEM)
Control	Nil
Indomethacin	32.3 ± 3.4
Indomethacin + TLC fraction 1	14.6 ± 1.1**
Indomethacin + TLC fraction 2	30.9 ± 3.8
Indomethacin + TLC fraction 3	29.5 ± 3.6
Indomethacin + Omeprazole (8 mg/kg)	12.1 ± 1.1**

Each group had ten rats, ** p<0.001

3.5 Effect of TLC Separated Fractions of Root Powder of *Astilbe rivularis* on Swimming Stress Induced Gastric Ulcer in rats: Continuous swimming for a period of 3 hours produced massive ulcers in stomach of all rats. Adhesion and dilatation of the stomach were seen. Bleeding was also there. Ulcer index was 33.6 ± 3.3. Pretreatment of rats with of rats with TLC fraction - 1 of root powder of *Astilbe rivularis* gave 61.01% protection (p<0.001) in rats from ulcer production induced by swimming stress. Ulcer index came, 13.1 ± 1.2. Omeprazole gave more protection (70.23%) to the rats from swimming stress induced gastric ulcers. Ulcer index was 10.0 ± 1.0.

Pretreatment of rats with TLC fraction – 2 and TLC fraction - 3 of root powder of *Astilbe rivularis*, however, did not prevent ulcer formation in rats by swimming stress. With TLC fraction – 2 ulcer index was 30.8 ± 3.5 and with TLC fraction – 3 ulcer index came 28.7 ± 3.3. Results are given in Table – 4.

Table 4: Effect of TLC separated fractions of root powder of *Astilbe rivularis* on swimming stress induced gastric ulcer in rats

Group	Ulcer index (mean ± SEM)
Control	Nil
Swimming stress	33.6 ± 3.3
Swimming stress + TLC fraction 1	13.1 ± 1.2**
Swimming stress + TLC fraction 2	30.8 ± 3.5
Swimming stress + TLC fraction 3	28.7 ± 3.3
Swimming stress + Omeprazole (8 mg/kg)	10.0 ± 1.0**

Each group had ten rats, ** p<0.001

3.6 Effect of TLC Separated Fractions of Root Powder of *Astilbe rivularis* on Pyloric Ligation Induced Gastric Ulcer in rats

Result is given in Table-5.

Pyloric ligation produced gastric ulcers in all albino rats. Ulcers were superficial. There were adhesion, dilatation and bleeding in the stomach. Ulcer index came 27.2 ± 2.6. Pretreatment of rats with TLC fraction - 1 of root powder of *Astilbe rivularis* produced significant protection (p<0.001) in rats from ulcer production induced by pyloric ligation. Ulcer index came, 12.0 ± 1.0 Omeprazole gave more protection to the rats from pyloric ligation induced gastric ulcers. Ulcer index was 10.5 ± 1.3. Pretreatment of rats with TLC fraction – 2 and TLC fraction - 3 of root powder of *Astilbe rivularis*, however, did not prevent ulcer formation in rats by pyloric ligation. With TLC fraction – 2 ulcer index was 26.7 ± 3.1 and with TLC fraction – 3 ulcer index came 25.9 ± 3.2.

Table 5: Effect of TLC separated fractions of root powder of *Astilbe rivularis* on pyloric ligation induced gastric ulcer in rats

Group	Ulcer index (mean ± SEM)
Control	Nil
Pyloric ligation	27.2 ± 2.6
Pyloric ligation + TLC fraction 1	12.0 ± 1.0**
Pyloric ligation + TLC fraction 2	26.7 ± 3.1
Pyloric ligation + TLC fraction 3	25.9 ± 3.2
Pyloric ligation + Omeprazole (8 mg/kg)	10.5 ± 1.3**

Each group had ten rats, ** p<0.001

3.7 Effect of TLC separated fractions of root powder of *Astilbe rivularis* on cysteamine induced duodenal ulcer in rats

Cysteamine hydrochloride (400 mg/kg, p.o. in 10% aqueous solution) when administered in two doses at an interval of 4 h produced massive duodenal ulcers in rats. Ulcers were mainly superficial although few were penetrating. Ulcers were associated with bleeding. Ulcer index in this group was 24.8 ± 2.0.

Pretreatment of rats with all three TLC separated fractions of root powder of *Astilbe rivularis* showed that only fraction – 1 produced 54.83% protection ($p<0.001$) in rats from ulcer production by cysteamine. Ulcer index was 11.2 ± 1.1 Omeprazole gave more protection (64.51%) to the rats from cysteamiine induced gastric ulcers. Ulcer index came 8.8 ± 1.2. Pretreatment of rats with TLC fraction – 2 and TLC fraction - 3 of root powder of *Astilbe rivularis*, however, did not prevent duodenal ulcer formation in rats by cysteamine. Resultts are given in Table - 6.

Table 6: Effect of TLC separated fractions of root powder of *Astilbe rivularis* on cysteamine induced duodenal ulcers in rats

Group	Ulcer index (mean ± SEM)
Control	Nil
Cysteamine	24.8 ± 2.0
Cysteamine + TLC fraction 1	11.2 ± 1.1**
Cysteamine + TLC fraction 2	23.7 ± 1.4
Cysteamine + TLC fraction 2	22.5 ± 1.7
Cysteamine + Omeprazole (8 mg/kg)	8.8 ± 1.2**

Each group had ten rats, ** $p<0.001$

Peptic ulcer disease refers to painful sores or ulcers in the lining of the stomach or first part of the small intestine, called the duodenum. Quincke [9] 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[10]. They are H_2 blockers like ranitidine, famotidine etc., M_1 blockers viz. pirenzepine, telenzepine etc. and the proton pump inhibitors like omeprazole, lansaprazole etc. All these drugs could decrease acid-peptic activity. Sucralfate, carbenoxolone etc. are also used as drug for peptic ulcer. They affect cytoprotection by virtue of their effects in mucosal defense factors [11].

No doubt the above said drugs have brought about remarkable changes in peptic ulcer therapy, 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 [12,13]. 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.

Due to this search numerous medicinal plants were identified having anti peptic ulcer activity. Sanyal *et al.* 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[14]. Akah *et al.* demonstrated anti-gastric ulcer activity of the herb *Cassampelos mucronata*[5]. Likewise, Shetty *et al.*[16] , Sairam *et al.*[4], Maity *et al.*[17,18] and Dharmani and Palit[19] confirmed anti gastric ulcer activities of *Ginkgo biloba*, *Convolvulus pluricaulis Chois*, tea root extract and *Vernonia lasiopus* respectively. We also reported anti gastric ulcer activitiy of few medicinal plants in different experimental ulcer models[20-27]. One such plant is *Astilbe rivularis*. We have shown that root of *Astilbe rivularis* could prevent peptic ulcer in albino rats[2].

Studies were thus undertaken to isolate the active constituent present in *Astilbe rivularis* responsible for anti-peptic ulcer activity. Root powder of *Astilbe rivularis* was subjected to solvent extraction, acid hydrolysis, column chromatography using different adsorbents followed by thin layer chromatography (TLC). TLC gave three fractions. Anti-ulcer activity of these fractions was studied in various peptic ulcer models. Results showed that the TLC separated first fraction had anti-ulcer activity against ethanol, hydrochloric acid, indomethacin, swimming stress and pyloric ligation induced gastric ulcer and cysteamine induced duodenal ulcers in rats. Anti-ulcer activity was comparable with that of omeprazole.

TLC separated first fraction thus contained the active constituent responsible for anti-peptic ulcer activity. Efforts are now being made to crystalize the active constituent and to characterize it.

4. Conclusion

Anti-peptic ulcer activity of TLC separated fractions of root extract of *Astilbe rivularis*, a plant of Eastern Himalaya, was studied in experimental peptic ulcer models. It was found out that one TLC separated fraction exerted anti-peptic ulcer activity against ethanol, hydrochloric acid, indomethacin, swimming stress and pyloric ligation induced gastric ulcer and cysteamine induced duodenal ulcers in rats. Anti-peptic ulcer activity of the TLC fraction was, however, less than that of omeprazole.

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