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Original Article

Isolation of antiulcerogenic compound (AR-I) from *Astilbe rivularis* leaves and effect of season on yield of the compound

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ABSTRACT

A compound (AR-I) was isolated from *Astilbe rivularis* leaves. The compound had anti peptic ulcer activity in ethanol induced gastric ulcers and cysteamine induced duodenal ulcers in albino rats. Seasonal variation in concentration of the compound (AR-I) in *A. rivularis* leaves was studied. Results showed that leaves of *A. rivularis* for the months of May and June yielded maximum amount of the compound.

Keywords: *Astilbe rivularis* leaves, peptic ulcer, ethanol, cysteamine, ranitidine, ulcer index

INTRODUCTION

Astilbe rivularis, one of the medicinal plants of Eastern Himalaya specially of Sikkim Himalaya, is known as Buriokahti in Nepali and as Pango in Lepcha [1].The plant 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 [2,3]. Ethnic use of Astilbe *rivularis*, as reported in literature is in peptic ulcer. Root juice of the plant, two tea spoonful thrice a day, is given to patients suffering from peptic ulcer [4]. Tempted on the ethnic use we undertook studies and noted that leaves of *A. rivularis* could exert anti peptic ulcer activity against ethanol induced gastric ulcer as well as cysteamine induced duodenal ulcer models in albino rats [5]. As medicinal values of a plant depend on its chemical compound(s) it was thought worthwhile to isolate the active compound(s) from *A. rivularis* leaves responsible for anti peptic ulcer activity. Further, accumulation of chemical compounds in plants varies with seasons [6]. Therefore, seasonal variation in yield of the active compound in course of isolation from the plant leaves was also studied.

MATERIALS AND METHODS

Plant Material: *Astilbe rivularis* leaves were collected in morning hours (9 - 10 AM) from the medicinal plants garden of the University of North Bengal, Dist. Darjeeling, West Bengal, India randomly and during the months of January – February, March – April, May – June, July – August, September – October and November – December 2012. Leaves were authenticated by the experts of the department of Botany of the said University. A voucher specimen was kept in the department of Biochemistry, North Bengal Medical College, Dist. Darjeeling, West Bengal, India for future reference.

Isolation of the active constituent: Isolation of the active constituent from the leaves of *A. rivularis* collected randomly and during the months of January –February, March – April, May – June, July – August, September – October and November – December were separately processed by the following method to collect active constituent.

***First step*:** Leaves of *A. rivularis* were properly washed, shade dried and powdered. 50g of this powder were extracted with 500 ml of 1:1 (v/v) chloroform – ethyl alcohol mixture for 15 min on a rotary shaker. It was then centrifuged. Supernatant was collected and evaporated to dryness.Dry mass was obtained.

Second step: Dry mass was refluxed with 100 ml of 10% HCL for 1h on a water bath at 100 degree centigrade. It was cooled and centrifuged. Supernatant was evaporated to dryness. .

***Third step*:** Dry mass thus obtained from the supernatant was extracted with 100 ml of petroleum ether on a rotary shaker for 30 min. It

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was then centrifuged. Supernatant was evaporated to dryness.

***Fourth step*:** Dry mass obtained was dissolved in 10 ml ethanol and subjected to column chromatography using alumina as adsorbent. Six bands were separated. Bands were collected in separate beakers. Elution was done by 10% ethanol – chloroform mixture. Third band had antiulcerogenic activity against ethanol induced gastric ulceration in albino rats.

***Fifth step*:** Collection of third band was evaporated to dryness. Dry mass obtained. It was dissolved in 10 ml ethanol and subjected to column chromatography using silica gel G as adsorbent. Five bands were separated. Bands were collected in separate beakers . Elution was done by 50% ethanol – chloroform mixture. Second band had antiulcerogenic activity against ethanol induced gastric ulceration in albino rats.

***Sixth step*:** Eluent of second band was evaporated to dryness. The dry mass was extracted with 15 ml ethyl formate for 10 minutes. It was then filtered. With filtrate polyamide column chromatography was done. Elution was made by ethyl formate : formic acid mixture (10 : 1v/v). Three bands were separated. Second band had antiulcerogenic activity against ethanol induced gastric ulceration in albino rats.

***Seventh step*:** Eluent of second band was evaporated to dryness. Repeated crystallization was done from ethyl acetate–formic acid (50:50, v/v) mixture. Crystals obtained. The compound was given a trivial name (AR-I).
In each case yield of the compound was noted.

Homogeneity of the active compound: This was ascertained by silica gel- G thin layer chromatography by using the following solvent systems: Chloroform : acetone - 50 : 50;n-butanol : acetic acid : water - 60 : 20 : 20; Chloroform: methanol : water - 80 : 10 : 10

Experimental animals: Wistar strain albino rats (180 - 200 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: Ethanol (Baroda Chemical Industries Ltd., Dabhoi) and cysteamine(Sigma Chemical Co., USA) were used in the study, ranitidine (Cipla pharmaceuticals)

Acute toxicity study: Acute toxicity studies were carried out on albino rats by the method of Ghosh [7]. Compound (AR-I) isolated from the leaves of *A. rivularis* collected randomly was given at doses of 1, 2, 5, 10 and 30 mg/kg to different groups of mice each group containing six animals. Watery suspension of the test drug was given to the animals orally through a feeding tube. 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.

Production of peptic ulcer:
Ethanol induced gastric ulcer: This was done by the method of Sairam *et al*.[8]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 [9]. 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 anti mesenteric side. Duodenum was then examined for the presence of ulcers.

Antiulcer Study:
Rats were divided into six groups.
1. Control: Rats took normal diet, water and vehicle of the drug.
2. Drug treated : Rats were treated with drug either with ethanol or cysteamine.
3. Drug + AR-I (100 mg/kg) : AR- I in the dose of 100 mg/kg in watery suspension was given to the rats orally through feeding tube 30 minutes prior to administration of drug.
4. Drug + AR-I (200 mg/kg) : AR-I in the dose of 200 mg/kg in watery suspension was given to the

rats orally through feeding tube 30 minutes prior to administration of drug.

5. Drug + AR-I (300 mg/kg) : AR-I in the dose of 300 mg/kg in watery suspension was given tothe rats orally through feeding tube 30 minutes prior to administration of drug.

6. 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*. [10].

Evaluation of ulcer index: Evaluation of ulcer index was done by the method of Szelenyi and Thiemer [11].Gastric /duodenal 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.

Statistical analysis: The values were expressed as mean ± SEM and were analyzed using one-way analyses 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

Acute toxicity studies: Acute toxicity studies revealed that the isolated compound (AR-I) from the leaves of *A. rivularis* did not produce any toxic symptoms when administered orally to rats in doses of 1, 2, 5, 10 and 30 mg/kg. Animals were healthy, cheerful and behaved normal throughout the experimental period. No death of animal was recorded during seven days of experiment.

Homogeneity of the isolated compound: This was ascertained by silica gel- G thin layer chromatography by using three solvent systems as mentioned earlier. In each case single spot was obtained. The isolated compound(AR-I) was thus pure.

Anti gastric ulcer activity of the isolated compound AR-1: Anti gastric ulcer activity of the compound (AR-I) isolated from the leaves of *Astilbe rivularis* in ethanol induced gastric ulcer in albino rats was shown in Table - 1. Ethanol produced massive gastric ulcers in all rats. Ulcers were mostly superficial. There was bleeding in the stomach which was associated with adhesion and dilatation. Ulcer index came 31.3 ± 1.13. AR-I reduced ulcer index in dose dependent manner. Maximum anti gastric ulcer activity was noted with the dose of 300 mg/kg of AR-I. Ulcer index came down to 10.3 ± 1.11 with ulcer protection 67.09% . This was comparable to that of ranitidine (50 mg/kg) Ulcer index in this group was 8.5 ± 1.01 with ulcer protection 72.84% .

Anti duodenal ulcer activity of the isolated compound AR-1: The result was given in Table – 2. Results showed that cyateamine produced massive duodenal ulcers in all rats. Ulcers were mostly superficial. There was bleeding in the duodenum which was associated with adhesion and dilatation. Ulcer index came 25.1 ± 1.12. AR-I reduced ulcer index in dose dependent manner. Maximum anti duodenal ulcer activity was noted with the dose of 300 mg/kg of AR-I. Ulcer index came down to 10.1 ± 1.11 with ulcer protection 59.76% . This was comparable to that of ranitidine (50 mg/kg) group where ulcer index came 8.0 ± 1.14 with ulcer protection 68.12% .

Seasonal effect in yield of AR-I: Seasonal effect in yield of AR-I isolated from the leaves of *Astilbe rivularis* was shown in Table – 3. Table showed that leaves of *A.rivularis* during the months of May and June yielded maximum amount AR-I. The value was 10.1 ± 0.15 mg/100g of *A.rivularis* leave powder which was statistically significant up to the level of $p<0.001$ when compared to other values of yield during different seasons. Ethnic use of *Astilbe rivularis*is on peptic ulcer [4]. We have noted and reported elsewhere [5] that leaves of *A. rivularis* could exert anti peptic ulcer activity against ethanol induced gastric ulcer as well as cysteamine induced duodenal ulcer models in albino rats. We were interested to isolate the active compound responsible for anti peptic ulcer activity and by solvent extraction, acid hydrolysis, chromatography followed by crystallization, we have isolated a compound (AR-I) from the leaves of *A. rivularis*. It was found out that the compound could exert anti peptic ulcer activity in rats as induced by the above said drugs. Since medicinal values of plants vary with season [12-15], we were also interested to note the seasonal variation, if any, on concentration of the active compound (AR-I) in leaves of *A. rivularis*. Results showed that leaves of *A. rivularis* during the months of May and June yielded maximum amount of (AR-I). This is in confirmation of our earlier findings where we have shown that leaves of *A. rivularis* during the months of May and June had maximum anti peptic ulcer activity in albino rats [16]. We are now interested to characterize the compound (AR-I) isolated from *A. rivularis* leaves and to see the underlying

mechanism of anti peptic ulcer activity of it. Experiments are in progress in this direction.

CONCLUSION

A compound (AR-I) was isolated from the leaves of *Astilbe rivularis.* The compound had anti peptic ulcer activity against ethanol induced gastric ulceration and cysteamine induced duodenal ulcerations in rats. The compound was accumulated in maximum quantity in the leaves of *A.rivularis* during the months of May and June.

Table - 1: Showing anti gastric ulcer activity of the compound (AR-I) isolated from the leaves of *Astilbe rivularis* (randomly collected) in ethanol induced gastric ulcer in rats.

Group	Ulcer index (mean ± SEM)	% Ulcer protection
Control	Nil	--
Ethanol	31.3 ± 1.13	--
Ethanol + (AR-I) (100mg/kg)	22.8 ± 1.21*	27.16
Ethanol + (AR-I) (200mg/kg)	12.2± 1.13**	61.02
Ethanol + (AR-I) (300mg/kg)	10.3 ± 1.11**	67.09
Ethanol + Ranitidine(50mg/kg)	8.5 ± 1.01**	72.84

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

Table - 2 : Showing anti peptic ulcer activity of the compound (AR-I) isolated from the leaves of *Astilbe rivularis* (randomly collected) in cysteamine induced duodenal ulcers in rats.

Group	Ulcer index (mean ± SEM)	% Ulcer protection
Control	Nil	--
Cysteamine	25.1 ± 1.12	--
Cysteamine + (AR-I) (100mg/kg)	20.3 ± 1.02*	19.12
Cysteamine + (AR-I) (200mg/kg)	12.2± 1.12**	51.39
Cysteamine + (AR-I) (300mg/kg)	10.1 ± 1.11**	59.76
Cysteamine + Ranitidine(50mg/kg)	8.0 ± 1.14**	68.12

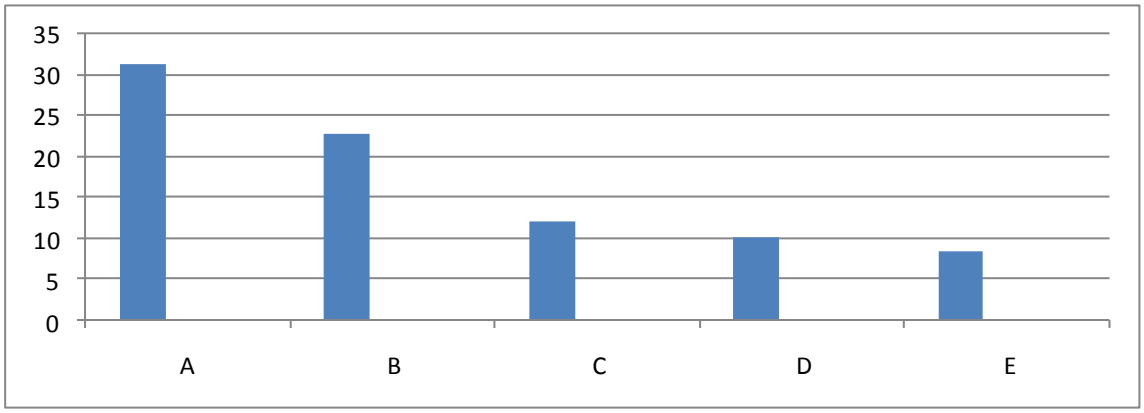
Results were in mean ± SEM, Each group had eight rats,*p<0.05, ** p<0.001

Table - 3: Seasonal variations in the yield of the isolated compound (AR-I) from the leaves of *Astilbe rivularis*

Season	Yield of the compound (CA-I) (mg/100g of *Astilberivularis* leave powder)
January –February	1.3 ± 0.01
March – April	3.9 ± 0.04
May – June	10.1 ± 0.15**
July – August	5.8± 0.06
September – October	4.7 ± 0.05
November - December	2.9 ± 0.02

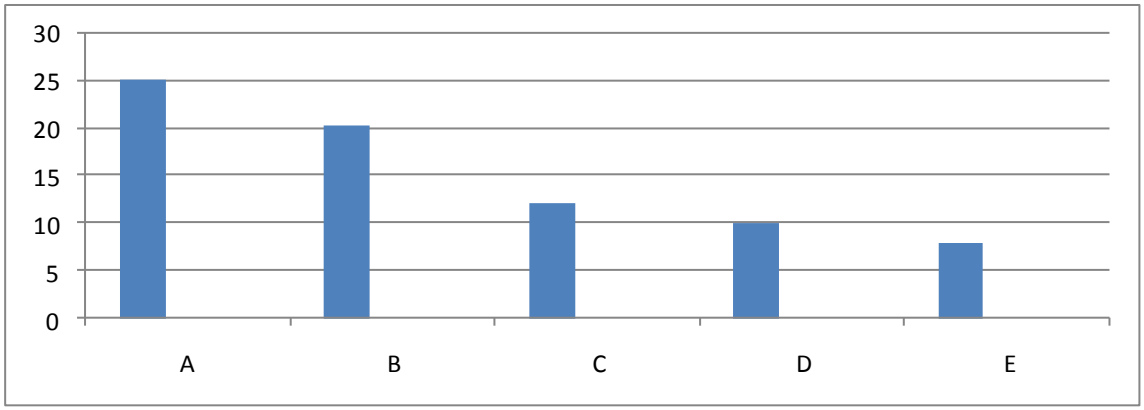
Results are mean of six sets of experiments. ** p<0.001

Fig. 1: *Astilbe rivularis* Buch. – Ham. Ex D. Don



■Ulcer index A : Ethanol B : Ethanol + AR-I (100mg/kg) C : Ethanol + AR-I (200mg/kg) D :Ethanol + AR-I (300mg/kg) E : Ethanol + Ranitidine (50mg/kg)

Fig 2 :: Effect of (AR-I) isolated from leaves of *Astilbe rivularis* (randomly collected) on ulcer index during ethanol induced gastric ulcer in rats



■Ulcer index A : Cysteamine B : Cysteamine + AR-I (100mg/kg) C : Cysteamine + AR-I (200mg/kg) D : Cysteamine + AR-I (300mg/kg) E : Cysteamine + Ranitidine (50mg/kg)

Fig 3: Effect of (AR-I) isolated from leaves of *Astilbe rivularis* (randomly collected) on cysteamine induced duodenal ulcer in rats

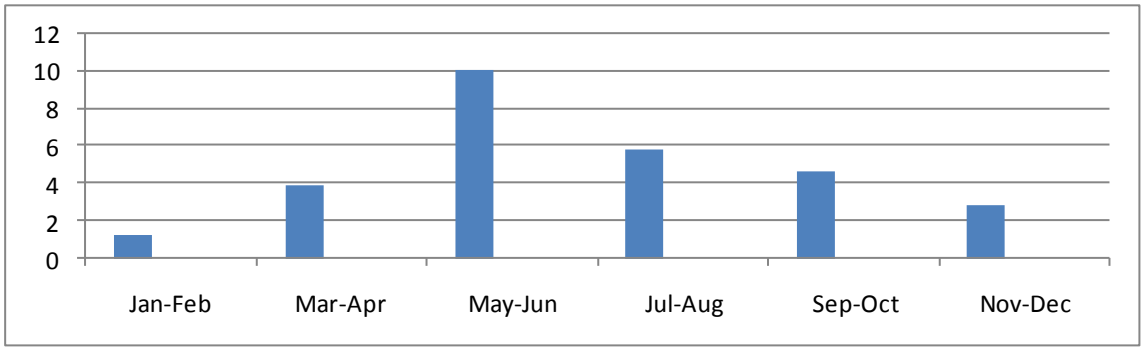


Fig 4 : Seasonal variation in the yield of (AR-I) isolated from *Astilbe rivularis* leaves (The amount was in terms of mg/100g of *A. rivularis* leave powder)

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