

Cytoprotective Activity of *Cissus quadrangularis* Linn Variant I Against Gastric and Duodenal Ulcer in Rat Models

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Abstract: The square stemmed variety of *Cissus quadrangularis* L. variant I is a medicinal plant widely used for peptic ulcer and bone fractures in Indian Systems of Medicine. Though this medicinal plant is abundantly used in India, detailed studies are lacking on the variants and seasonal variations, which are to be addressed. The present study is carried out to evaluate the antiulcerogenic property of aqueous ethanolic extracts of the aerial parts in various ulcer models in animals. The study clearly elucidated that the drug was found to increase the defensive factors *by virtue* of its ulcer score, carbohydrate protein ratio and decrease in aggressive factors like free acidity, pepsin apart from other biochemical parameters. Plants collected during flowering season are more effective than that collected during vegetative seasons. Extract significantly reduced the formation of gastric and duodenal lesions *by virtue* of its cyto-protective and mucin productive activities.

Key Words: *Cissus quadrangularis*, antiulcer activity, vitaceae, peptic ulcer disorders.

INTRODUCTION

Cissus quadrangularis Linn (Family: Vitaceae) is a climber with stout fleshy quadrangular stem found throughout the hotter parts of India [1]. Three morpho-variants of *Cissus quadrangularis* with square-stemmed, round-stemmed and flat-stemmed are available. They are differentiated as variant I, II and III respectively [2, 3]. Variant I is quite common than the other two, which is considered to be a remedy for gastric ulcer [3, 4]. The toxicological [5] and helicobactericidal [6] activities of *C. quadrangularis* variant I have been reported earlier. The antiulcer activity of Variant II has also been reported [7]. In the present study *C. quadrangularis* has been investigated for its antiulcer activities in animal models. Plants were collected during flowering and vegetative seasons and subjected to pharmacological studies to evaluate the seasonal variation with respect to its antiulcer activity in order to prove the effect and influence on seasonal variations.

MATERIALS AND METHODS

Plant Material

Aerial parts of *C. quadrangularis* were collected from Palayankottai, Tirunelveli district, Tamilnadu, India and identified by the co-author. A voucher specimen has been deposited in the department herbarium for future reference (TUH No. 189). Plant samples were collected during two-growth phases viz., flowering (November, 2000) and vegetative season (May, 2000). The plant materials were cleaned,

shade-dried, disintegrated and filtered in a sieve (40 mm) and stored in an airtight container and used for the entire study.

Extraction

Cold, aqueous (50%) ethanolic extract was prepared according to the methodology described in Indian Pharmacopoeia (Yield: 18.64%) [8]. Preliminary phytochemical screening [9] of the aqueous extract gave positive results for alkaloids, saponins, fixed oils, tannins and phenols and negative results for flavonoids. The dried extract was reconstituted with water whenever needed.

Animals

Six male Wistar strain albino rats of weighing 180-220 g were taken from the inbred group maintained at Tamil University animal house, Thanjavur. The animals were fed with standard pelleted diet supplied by Lipton & Co. Ltd., Bangalore. Feed and water were made available to animals *ad libitum*.

Antiulcer Activity

Modified pyloric ligated (Shay) rat, aspirin induced ulcerogenesis in pyloric ligated (Shay) rat and cysteamine induced duodenal ulcer models [10] were used to study the antiulcer activity.

Modified Pyloric Ligated (Shay) Rats

Rats were divided into groups of six animals each and were placed in cages with grating floor to avoid coprophagy and fasted for 48 hours allowing free access to water. One group received water (1 ml/kg) and was served as control.

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Ranitidine (30 mg/kg) and omeprazole (2 mg/kg) were selected as reference drugs and was given to two groups, for comparison. In the test group, the animals were grouped into three, receiving aqueous ethanolic extracts at a dose level of 150, 300 & 450 mg/kg b.w. The test drugs and reference drugs were administered once daily, orally, for two days prior to and one hour before pyloric ligation. The animals were deprived of both food and water during the post-operative period. The animals were anaesthetized with chloroform. Four hours after ligation, animals were sacrificed.

Aspirin-Induced Ulcerogenesis in Pyloric Ligated Rats

In aspirin-induced ulcerogenesis in pyloric ligated rats, aspirin is administered at a dose of 200 mg/kg orally in a suspension prepared in 1% Carboxy Methyl Cellulose (CMC) with water 1 hour prior to pyloric ligation (Time interval between reference drugs and Aspirin should be one hour) and the process described above was followed.

Biochemical Parameters

The stomach was excised carefully keeping the oesophagus closed, opened along the greater curvature and the luminal contents were removed. The gastric contents were collected in a beaker and centrifuged at 1000 rpm for 10 minutes. The samples were analysed for gastric volume [11], pH, free and total acidity [12] and sodium and potassium ion concentration [13]. Bio-chemical estimations like total proteins [14], total carbohydrate [15], total hexoses [16], hexosamine [17], fucose [18], sialic acid [19] and pepsin [20] were also made. The mucosa was flushed with saline and stomach pinned on a frog board and scored.

Collection of Gastric Juice

Gastric juice was collected from the pyloric-ligated rats [11]. The gastric juice thus collected was centrifuged and the volume of gastric juice as well as pH of gastric juice was measured. Then the gastric juice was subjected to biochemical estimation as follows.

Determination of Free and Total Acidity in Gastric Juice

1 ml of gastric juice was pipetted into a 100 ml conical flask, added 2 to 3 drops of Topfer's reagent and titrated with 0.01 N NaOH (which was previously standardized with 0.01 N of oxalic acid) until all traces of the red colour disappears and the colour of solution was yellowish orange. The volume of alkali added was noted. The volume corresponds to free acidity. Then 2 to 3 drops of phenolphthalein solution were added and titration was continued until a definite red tinge reappears. Again the total volume of alkali added was noted. The volume corresponds to total acidity.

Acidity was calculated by using the formula

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{0.1} \text{ meq/l/100g}$$

Sodium and Potassium Ion Concentration in Gastric Juice

This was carried out in Systronics mediflame 127- flame photometer. Stock solution was prepared. Sodium stock solution was prepared by dissolving 2.542 g NaCl in 1 litre of

distilled water. It contains 1 mg Na per ml (ie., 1000 ppm). Stock solution was diluted to give four solutions containing 10, 5, 2.5 and 1 ppm of sodium ions. Then potassium stock solution was prepared by dissolving 1.909 g KCl in 1 litre of distilled water. It contains 1 mg potassium per ml (ie., 1000 ppm). Stock solution was diluted to give four solutions containing 20, 10, 5 and 2 ppm of potassium ions.

For sodium and potassium, the flame intensity corresponding to the concentration of stock solution was noted by using appropriate filters. The results were plotted in a graph. The flame intensity of the gastric juice was noted. The concentration of sodium and potassium ions was calculated from the graph. The results are expressed in terms of mg/l.

Total Proteins

The dissolved protein in gastric juice was estimated in the alcoholic precipitate obtained by adding 90% alcohol with gastric juice in 9:1 ratio. Then 0.1 ml of alcoholic precipitate of gastric juice was dissolved in 1 ml of 0.1 N NaOH and from this 0.05 ml was taken in another test tube, to this 4 ml of alkaline mixture was added and kept for 10 min. Then 0.4 ml of phenol reagent was added and again 10 min was allowed for colour development. Reading was taken against blank prepared with distilled water at 610 nm in Hitachi 15-20 spectrophotometer. The protein content was calculated from standard curve prepared with bovine albumin and was expressed in terms of µg/ml of gastric juice.

Total Carbohydrates

The dissolved mucosubstances in gastric juice were estimated in the alcoholic precipitate obtained by adding 90% alcohol with gastric juice in 9:1 ratio. Briefly the method consists of taking two aliquots of gastric juice and treated as described. To 1 ml of gastric juice, 9 ml of 90% alcohol was added. The mixture was kept for 10 minutes before it was centrifuged. The supernatant was discarded. The precipitate was dissolved in 0.5 ml of 0.1 N NaOH. To this 1.8 ml of 6 N HCl was added. The mixture was hydrolysed in water bath at 100°C for 2 hours. The hydrolysate was neutralised by 5 N NaOH using phenolphthalein as indicator and the volume was made upto 4.5 ml with distilled water and used for the estimation of total hexoses, hexosamine and fucose as described. To the other aliquot of 0.5 ml of gastric juice, 4.5 ml of alcohol was added. The mixture was shaken for 10 minutes and centrifuged to obtain precipitate. The precipitate was dissolved in 0.5 ml of 0.1 N H₂SO₄. This reconstituted solution was transferred to glass-stoppered tubes and then hydrolysed in a water bath at 100°C for 1 hour. After hydrolysis, the volume restored to 0.5 ml; 0.2 ml of this hydrolysate was used for the estimation of sialic acid.

After obtaining the concentration (µg/ml) of individual carbohydrates namely hexoses, hexosamine, fucose and sialic acid, the total carbohydrate content was calculated by adding the concentration of individual carbohydrates. Mucosubstances activity has been expressed as ratio of total carbohydrates to total proteins.

Total Hexoses

To 0.4 ml of hydrolysate, 3.4 ml of orcinol reagent was added. The mixture was then heated in the boiling waterbath

at 60°C for 15 minutes. This was then cooled under running tap water and intensity of the colour was read in Hitachi 15-20 spectrophotometer at 540 nm against the blank by using distilled water instead of hydrolysate. Total hexoses content was determined from the standard curve of D(+)-galactose-mannose and has been expressed in µg/ml of gastric juice.

Hexosamine

0.5 ml of the hydrolysate fraction was taken. To this 0.5 ml of acetyl-acetone reagent was added. The mixture was heated in boiling waterbath at 60°C for 20 minutes, then cooled under running tap water. 1.5 ml of 90% alcohol was added and allowed for 30 minutes. The colour intensity was measured in Hitachi 15-20 spectrophotometer at 530 nm against blank prepared by using distilled water instead of hydrolysate. Hexosamine content was determined from the standard curve prepared by using D (+)-glucosamine hydrochloride and concentration has been expressed in µg/ml of gastric juice.

Fucose

In this method, three test tubes were taken. In one tube 0.4 ml of distilled water was taken to serve as control and in each of the other two tubes 0.4 ml of hydrolysates were taken. To all three tubes 1.8 ml of H₂SO₄: water (6:1) was added by keeping the test tubes in ice-cold waterbath to prevent breakage due to strong exothermic reaction. The mixture was then heated in boiling waterbath for exactly 3 min. The tubes were taken out and cooled. To the blank and to one of the hydrolysate containing tube (unknown), 0.1 ml of cysteine reagent was added while cysteine reagent was not added to the last test tube containing the hydrolysate (unknown blank). It is then allowed for 90 min to complete the reaction. The reading was taken in Hitachi 15-20 spectrophotometer at 396 and 430 nm setting zero with the distilled water. The optical density for the fucose in the hydrolysate was calculated from the differences in the reading obtained at 396 and 430 nm and subtracting the values without cysteine. This was read against standard curve prepared with D(+) – fucose. The fucose content was expressed in terms of µg/ml of gastric juice.

$$\text{True optical density} = \frac{(\text{OD}_{396} - \text{OD}_{430})^{\text{Unknown}} - (\text{OD}_{396} - \text{OD}_{430})^{\text{Unknown blank}}}{(\text{OD}_{396} - \text{OD}_{430})^{\text{water blank}}}$$

Sialic Acid

To 0.5 ml of the hydrolysate in 0.1 N H₂SO₄, 0.2 ml of sodium periodate was added and mixed thoroughly by shaking. A time of 20 min was allowed to elapse before addition of 1 ml of sodium arsenite solution to this mixture. The brown colour produced disappeared after shaking. Then 3 ml of thiobarbituric acid was added and the mixture was heated in boiling waterbath for 15 minutes. After cooling the tubes, 4.5 ml of cyclohexanone was added and thorough shaking was done for 15 sec till all the colour was taken up by the cyclohexanone supernatant. The mixture was centrifuged to get a clear pink layer of cyclohexanone. This supernatant was pipetted out and intensity of colour was measured in Hitachi 15-20 spectrophotometer at 550 nm. The sialic acid content of the sample was determined from the standard

curve of sialic acid and has been expressed in terms of µg/ml of gastric juice.

Pepsin

For each determination four tubes (1) and (2) containing 5 ml of substrate, (3) and (4) containing 10 ml of TCA was placed in the waterbath at 37°C. The gastric juice was mixed with an equal volume of HCl at pH 2.1, warmed to 37°C and added 1 ml of mixture to each tubes (1) and (4), incubated for 15 minutes and at the end mixed the contents of tube (1) with tube (3) and allowed to stand in the bath for about 4 minutes. Contents of tube (1) and tube (3) gives test and contents of tube (2) and tube (4) gives blank. Both the contents were filtered after 25-30 minutes, 2 ml of filtrate was pipetted into 10 ml of NaOH, mixed by gentle rotation, then 1 ml of phenol was added and again mixed by gentle rotation. After 30 min, the intensity of colour was measured at 680 nm in Hitachi 15-20 spectrophotometer.

The difference between test and blank gives a measure of peptic activity. As standard, mixed 2 ml of freshly prepared phenol solution containing 50 µg/ml with 10 ml of NaOH and 1 ml of phenol reagent was added. After 5 – 10 minutes, the colour intensity was measured at 680 nm.

Ulcer Score

The scoring is done as described by Laurence and Bacharach [21]. 0 corresponds to normal rugal pattern, 1 for alteration in normal rugal pattern, 2 for scattered haemorrhage lesions, 3 for haemorrhage lesions and ulcers and 4 for penetrating and perforating ulcers

Cysteamine-Induced Duodenal Ulcer Model

In cysteamine-induced duodenal ulcer model [10], ulcers were induced by the administration of cysteamine hydrochloride (Sigma Chemicals) 400 mg/kg (10% solution in normal saline) orally, two times, at an interval of four hours and the animals were fed with food and water *ad libitum* throughout the experiment and plant extract was administered 30 minutes before each dose of cysteamine hydrochloride. After 24 hours from the first dose the animals were sacrificed and the duodenum was exposed and scored according to Selye and Szabo, 1973. 0 corresponds to normal mucosa, 2 for superficial mucosal erosion, 3 for deep ulcer or transmural ulcer and 4 for perforated ulcer respectively.

Statistics

Data were expressed as the mean ± standard error of the means (S.E.M.) and statistical analysis was carried out using student's *t*-test.

RESULTS

Extracts of *C. quadrangularis* possessed anti ulcer activities comparable with standard drugs in animal models well accepted in gastric and duodenal ulcers. The study clearly elucidated that the drug increased the defensive factors and decreased the offensive factors studied. Observations on pyloric ligated (Shay) rat model treated with extract are given in Tables 1 and 2. Increase in defensive factors (ulcer score, carbohydrate protein ratio) and decrease in aggressive factors

Table 1. Effect of *C. quadrangularis* Variant I on Ulcer and Acidity in Modified Pyloric Ligated (Shay) Rats

Parameter	Control 1ml/kg	Standards (mg/kg)		Vegetative Period (mg/kg)			Flowering Period (mg/kg)		
		Ranitidine	Omeprazole	150	300	450	150	300	450
Gastric volume (ml)	7.10 ± 0.52	6.48 ± 0.99	4.73 ± 1.28	5.73 ± 0.16	5.03 ± 0.12***	4.13 ± 0.08***	5.77 ± 0.51	4.68 ± 0.20**	4.53 ± 0.13***
Ulcer score	3.00 ± 0.26	1.00 ± 0.26*	0.33 ± 0.21***	2.33 ± 0.21**	1.67 ± 0.21***	0.67 ± 0.21***	1.33 ± 0.21	0.33 ± 0.21***	0.33 ± 0.21***
pH	3.10 ± 0.20	2.83 ± 0.09	2.69 ± 0.09	2.97 ± 0.08***	2.74 ± 0.11***	2.63 ± 0.09***	2.95 ± 0.08	2.92 ± 0.08	2.81 ± 0.11
Na ⁺ (mg/l)	1.83 ± 0.21	1.47 ± 0.14	1.50 ± 0.13	2.08 ± 0.28***	1.96 ± 0.04***	1.49 ± 0.03***	1.62 ± 0.03	1.62 ± 0.05	1.80 ± 0.06
K ⁺ (mg/l)	0.45±0.02	0.34±0.04*	0.36±0.06	0.47±0.01***	0.39 ± 0.01***	0.38 ± 0.004***	0.55 ± 0.03	0.51 ± 0.03	0.43 ± 0.04
Free acidity (meq/l)	41.94±0.63	32.81±0.68***	28.36±0.67***	37.58 ± 0.34***	38.52 ± 0.30***	35.33 ± 0.61***	33.82 ± 0.8***	33.68 ± 0.34***	29.00 ± 0.40***
Total acidity (meq/l)	79.38±0.83	49.84 ± 1.42***	42.48 ± 0.57***	54.29 ± 0.41***	60.70 ± 0.43***	59.18 ± 0.54***	51.57 ± 0.73***	49.02 ± 0.55***	49.19 ± 0.66***

Values are expressed as Mean ± SEM, N = 6. *P<0.05; **P<0.01; ***P<0.001 compared with control group.

Table 2. Effect of *C. quadrangularis* Variant I on Protein & Metabolic Status in Modified Pyloric Ligated (Shay) Rats

Parameter	Control 1ml/kg	Standards (mg/kg Rat)		Vegetative Period (mg/kg Rat)			Flowering Period (mg/kg Rat)		
		Ranitidine	Omeprazole	150	300	450	150	300	450
Pepsin (µg/ml)	9.56 ± 0.27	4.39 ± 0.16***	3.57 ± 0.13***	5.17 ± 0.09***	4.99 ± 0.08***	4.58 ± 0.14***	5.10 ± 0.29***	4.21 ± 0.05***	4.71 ± 0.12***
Total proteins (µg/ml)	544.92±12.37	220.81±6.38***	278.23±4.47***	304.68 ± 4.02***	303.68 ± 2.99***	303.22 ± 3.00***	270.01 ± 5.99***	219.71 ± 0.68***	262.72 ± 4.65***
Total hexoses (µg/ml)	199.18 ± 0.66	309.01 ± 7.67***	385.69 ± 4.01***	221.90 ± 1.66***	327.83 ± 3.91***	323.67 ± 2.66***	251.51 ± 1.61***	345.33 ± 3.88***	315.40 ± 3.17***
Hexosamine (µg/ml)	218.28 ± 5.85	306.45 ± 4.12***	492.53 ± 9.03***	292.81 ± 6.96***	334.87 ± 2.49***	342.30 ± 6.57***	295.01 ± 2.13***	437.17 ± 2.68***	540.44 ± 4.94***
Fucose (µg/ml)	41.54 ± 0.62	25.33 ± 1.33***	23.10 ± 1.21***	27.98 ± 0.95***	30.35 ± 0.18***	24.19 ± 1.23***	31.28 ± 0.21***	31.24 ± 0.74***	30.03 ± 0.55***
Sialic acid (µg/ml)	22.24 ± 0.61	31.70 ± 0.83***	32.72 ± 0.69***	28.17 ± 1.01***	30.98 ± 0.54***	26.79 ± 0.39***	29.17 ± 0.49***	31.42 ± 0.24***	30.04 ± 0.50***
Carbohydrate protein ratio	0.89 ± 0.02	3.05 ± 0.07***	3.36 ± 0.06***	1.87 ± 0.01***	2.39 ± 0.03***	2.37 ± 0.04***	2.25 ± 0.04***	3.85 ± 0.06***	3.49 ± 0.04***

Values are expressed as Mean ± SEM, N = 6. ***P<0.001 compared with control group.

(free acidity, pepsin) were observed. Aspirin induced ulcerogenesis in pyloric ligated (Shay) rats model results are given in Tables 3 and 4. Ulcer score was decreased in a dose dependent manner. Free acidity, pepsin content and carbohydrate / protein ratio were also altered. The activity of flowering sample is found to be active than vegetative sample.

In duodenal ulcer model, extract of flowering period showed a significant reduction in ulcer score from 300 mg/kg onwards. Vegetative period also showed a reduction in ulcer score at the same dose levels (Fig. 1) but with less significance.

DISCUSSION

The extracts of *C. quadrangularis* significantly reduced the formation of gastric and duodenal lesions in rats induced by various ulcerogenic procedures and cyto-destructing agent. A dose dependent ulcer protective response was noted. Dose of 300 mg/kg was significant in reducing the ulcer in modified pyloric ligated (Shay) rat model. A corresponding dose in aspirin induced ulcerogenesis in pyloric ligated (Shay) rat model, 450 mg, which is due to the degeneration in the gastric mucosa. In duodenal ulcer model both the extracts were almost effective in ulcer protection.

Table 3. Effect of *C. quadrangularis* Variant I on Protein & Carbohydrate Status in Aspirin Induced Ulcerogenesis

Parameter	Control 1ml/kg	Standards (mg/kg)		Vegetative Period (mg/kg)			Flowering Period (mg/kg)		
		Ranitidine	Omeprazole	150	300	450	150	300	450
Gastric volume (ml)	7.80 ± 0.22	4.24 ± 0.17***	3.60 ± 0.17***	5.13 ± 0.56**	4.07 ± 0.06***	3.77 ± 0.08***	5.28 ± 0.20**	4.23 ± 0.11***	4.57 ± 0.17***
Ulcer score	3.33 ± 0.21	1.67 ± 0.21***	0.67 ± 0.21***	2.33 ± 0.21***	1.33 ± 0.21***	0.67 ± 0.21***	1.33 ± 0.21	0.67 ± 0.21	0.50 ± 0.22**
pH	1.74 ± 0.04	3.94 ± 0.12***	3.73 ± 0.03***	3.47 ± 0.12***	3.65 ± 0.15**	3.78 ± 0.12***	3.26 ± 0.07	3.42 ± 0.06	3.61 ± 0.04
Na ⁺ (mg/l)	0.60 ± 0.02	0.27 ± 0.06***	0.22 ± 0.02***	1.81 ± 0.01***	1.69 ± 0.01***	1.70 ± 0.02***	1.66 ± 0.15	1.41 ± 0.01	1.43 ± 0.01
K ⁺ (mg/l)	0.28 ± 0.02	0.38 ± 0.01***	0.65 ± 0.09***	0.39 ± 0.02**	0.44 ± 0.02***	0.49 ± 0.01***	0.50 ± 0.01*	0.38 ± 0.01**	0.35 ± 0.01***
Free acidity (meq/l)	52.81±0.32	41.89 ± 0.54***	36.52± 0.47***	39.61 ± 0.29***	36.52 ± 0.34***	31.20 ± 0.52***	37.87 ± 0.37***	36.88 ± 0.53***	33.06 ± 0.23***
Total acidity (meq/l)	87.15±0.14	59.22 ± 0.08***	51.95 ± 0.59***	65.93 ± 0.58***	50.74 ± 0.63***	49.68 ± 0.40***	65.4 ± 0.57***	59.44 ± 0.51***	57.62 ± 0.95***

Values are expressed as Mean ± SEM, N = 6. *P<0.05; **P<0.01; ***P<0.001 compared with control group.

Table 4. Effect of *C. quadrangularis* Variant I on Protein and Carbohydrate Status in Aspirin Induced Ulcerogenesis

Parameter	Control 1ml/kg	Standards (mg/kg)		Vegetative Period (mg/kg)			Flowering Period (mg/kg)		
		Ranitidine	Omeprazole	150	300	450	150	300	450
Pepsin (µg/ml)	10.13 ± 0.09	4.85 ± 0.04***	4.10 ± 0.07***	5.86 ± 0.04***	4.95 ± 0.07***	5.13 ± 0.05***	6.10 ± 0.07***	5.18 ± 0.07***	4.77 ± 0.11***
Total proteins (µg/ml)	709.75 ± 2.17	305.66 ± 3.38***	245.50 ± 2.75***	337.46 ± 4.41***	291.51 ± 4.51***	286.80 ± 3.28***	300.30 ± 0.76***	282.94 ± 2.58***	271.1 ± 11.58***
Total hexoses (µg/ml)	156.99 ± 2.51	287.36 ± 4.91	275.01 ± 2.48***	280.67 ± 2.38***	354.31 ± 4.17***	327.89 ± 2.61***	267.58 ± 2.19***	302.46 ± 1.49***	301.79 ± 0.85***
Hexosamine (µg/ml)	250.77 ± 3.03	342.61±0.21***	397.58±7.55***	297.30±3.37***	354.16 ± 3.60***	384.44 ±4.60***	292.51 ± 1.70***	305.06 ± 2.57***	333.31± 10.26***
Fucose (µg/ml)	65.97 ± 2.01	27.89 ± 0.39***	24.40 ± 1.32***	28.54 ± 0.65***	29.14 ± 0.38***	31.78 ± 0.64***	30.76 ± 0.51***	32.76 ± 0.33***	30.32 ± 0.58***
Sialic acid (µg/ml)	39.64 ± 0.75	32.29 ± 0.98***	33.13 ± 0.53***	26.38 ± 0.47***	28.36 ± 0.76***	28.95 ± 0.31***	28.73 ± 0.30***	30.18 ± 0.61***	28.23 ± 0.94***
Carbohydrate protein ratio	0.72 ± 0.002	2.26 ± 0.02***	2.97 ± 0.04***	1.88 ± 0.01***	2.64 ± 0.06***	2.69 ± 0.03***	2.06 ± 0.01***	2.37 ± 0.01***	2.43 ± 0.07***

Values are expressed as Mean ± SEM, N = 6. **P<0.01; ***P<0.001 compared with control group.

Apart from antiulcer activity *C. quadrangularis* extract demonstrated other properties such as antisecretory activity. It reduced aggressive factors like pepsin, proteins and acidity, thereby enhancing the cytoprotective effect. Increase in potassium content is significant because it alters the hydrogen ion concentration thereby increasing the level of protection [22]. Carbohydrate/protein ratio also supports the result.

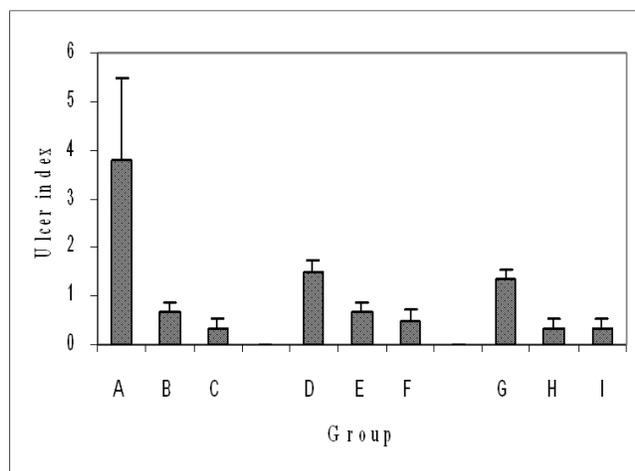
Variation between the two seasons may be due to the change in the concentration of the biological compounds. The mucosal defense mechanism may be due to the epithelial cells of the gastric mucosa, which are impermeable to hydrogen ions thereby forming a physical barrier [22]. The

increase in potassium ion return reflects in increase in hydrogen ion and bicarbonate ion concentrations [23]. The increase in bicarbonate ion concentration plays an important role in protecting the gastric and duodenal mucosa against hydrochloric acid [23].

Extract increased hexosamine and carbohydrate/protein ratio and decreasing pepsin content, which increase mucous secretion. Protective role of mucous on gastric mucosa and is suggested as the defense mechanism [24].

The increased carbohydrate/protein ratio suggests an increase in dissolved mucosubstances. The presence of dis-

solved mucosubstances in the gastric juice is a reliable index for an effective mucosal barrier [25]. This is an direct reflection of mucin activity [26] and suggests an increase in glycoprotein content of the gastric mucosa. Decrease in protein content of gastric juice suggests the decrease of leakage of plasma protein into gastric juice [27].



A - Control; B - Ranitidine (30 mg/kg); C - Omeprazole (2 mg/kg); D, E, F -Vegetative period samples at a dose of 150, 300 and 450 mg/kg dosage respectively; G, H, I - Flowering period samples at a dose of 150, 300 and 450 mg/kg dosage respectively.

Fig. (1). Ulcer index for duodenal ulcer model in *C. quadrangularis* variant I.

The extract prevented duodenal ulcers induced by cysteamine, which has many similarities with duodenal ulcers in man [25]. Cysteamine induced ulcers are considered to be due to continuous hypersecretion of gastric acid.

Variant I collected during flowering period were more effective than that collected during vegetative period. During shift from vegetative to flowering phase there is a shift from primary biochemical pathways to secondary biochemical pathways thereby leading to synthesis of a number of new biological active compounds.

In conclusion, the present study confirms a slightly superior antiulcer activity of *C. quadrangularis* collected during flowering period. More studies are required to identify the active principle present in *C. quadrangularis* responsible for potent antiulcer activity.

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