Research Article

Antiulcer Study of *Capparis Zeylanica* Extract on Gastric Secretions and Phenylbutazone Induced Chronic Ulceration in Experimental Animals

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ABSTRACT

Purpose: The objective of the study was to investigate the possible effects of 50% ethanolic extract of *Capparis zeylanica* (CZE) on gastric secretion and Phenylbutazone ulcer in rats. **Approach:** Twenty four adult male rats were used in this study (four groups) and several parameters were determined to assess any degree of protection. The ulceration was induced by Phenylbutazone at a dose of 100 mg/kg b.w.

Findings: Phenylbutazone toxicity induced + CZE treated rats showed a significant decrease ($p < 0.001$) in volume of gastric juice, free and total acidity, pepsin concentration and acid output with a significant increase ($p < 0.001$) in pH compared to Phenylbutazone toxicity induced rats. Phenylbutazone caused a significant decrease ($p < 0.001$) in TC:P with a concomitant increase ($p < 0.001$) in protein and DNA. After treatment of CZE in rats, a significant increase ($p < 0.001$) in the carbohydrate concentration in terms of TC:P ratio and significantly lowered ($p < 0.001$) protein concentration in gastric juice was observed. CZE tended to increase mucin activity in Phenylbutazone + CZE group.

Conclusion: It can be concluded that CZE imparted a protective action against phenylbutazone induced chronic ulcer in rats.

Keywords: *Antiulcer, Capparis zeylanica, Phenylbutazone, Plant extract, Ranitidine.*

INTRODUCTION

Gastric ulcer is an illness that affects a considerable number of people worldwide.[1] The development and progression of gastric ulcer depends to some extent on the type of the food consumed by the patient. It has been shown that spicy food, food with full of fat or foods having caffeine stimulates acid secretion in stomach and increase the risk of ulcer formation.[2] Since herbs are the mines of useful drugs and medicinal plants have always been the principle source of medicine in India since ancient past and presently they are becoming popular throughout the developed countries.

Capparis zeylanica Linn (Family: *Capparidaceae*) is a rigid, climbing, much-branched shrub mainly distributed in deciduous forest of South India along the Eastern Ghats of Andhra Pradesh and Orissa states. Root bark of this plant is used as sedative, cooling, cholagogue, stomachic, antihidrotic and in fever. Leaves are used as a counter irritant and as a cataplasm in boils, swellings, piles and rheumatism. Flowers are used as laxative.[3] The methanolic extract of the leaves was found to possess antidiarrheal activity.[4] Chemically the plant contains a saponin and p-hydroxybenzoic, syringic, vanillic, ferulic and p-coumaric acids. The leaves and seeds contain glucocapparin, alpha-amyrin, n-triacontane,

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betacarotene and fixed oil.[5] A new fatty acid E-octade-7-en-5-ynoic acid has been isolated from chloroform extract of the roots.[6] The bark has mild diuretic property.[7] In Indian traditional system of medicine, the plant parts are the vital ingredients of Rasayana. In ancient time, roots were consumed by ancient Egypt and Arab for treatment of kidney disease, liver disease, stomach problem and scorpion's stings.[8, 9] Recently, it has been reported that the leaf extract also exhibited sig-nificant effect against gastric lesions.[10] In the limelight of its traditional use, the present investigation was carried out to evaluate the anti-ulcer activity of the 50% ethanolic extract of *Capparis zeylanica* Linn. against phenylbutazone chronic ulcer model in rats.

MATERIALS AND METHODS

Collection and authentication of plant material:

The root of *Capparis zeylanica* were collected in March 2012 from district Rewa Madhya Pradesh, India and identified by Dr. P. Arjun Tiwari, Scientist, Botanical Survey of India Allahabad. A voucher specimen TR 097021 is preserved in BSI herbarium for future reference. The roots were dried under shade and powdered with a mechanical grinder. The powder was then passed through sieve no. 40 and stored in an airtight container.

Preparation of the extract:

The root powder (1 kg) of *Capparis zeylanica* was extracted with petroleum ether to remove fatty substances; the marc was further exhaustively extracted with 50% ethanol by hot percolation method. The extract was concentrated by vacuum distillation to reduce the volume to 1/10; the concentrated extracts were transferred to beaker and the remaining solvent was evaporated on a water bath and finally the extract has been lyophilized to get it in powder form. Thus 100 g of solid residue (yield 10% w/w) was obtained. The dried extract was packed in airtight container and used for further studies such as phytochemical screening and pharmacological activities.

Preliminary phytochemical screening:

The extract was subjected to preliminary phytochemical screening for the identification of various active constituents by using standard procedures.[11]

Drugs and chemicals:

Phenylbutazone was purchased from Wanbury limited, Mumbai. The standard drug Ranitidine was procured from Viva laboratories Pvt. Ltd. Ahmedabad as a gift sample. All other chemicals and reagents used were of analytical grade.

Experimental Animals:

Wistar albino rats (180-250 g) of either sex and of approximate same age, used in the present study, were procured from animal breeding centre, Indian Institute of Toxicology Research, Lucknow. All the animals were housed in polypropylene cages under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for one week before starting the experiment. The animals were fed with standard pellet diet (Amrut, India) and water *ad libitum*. The experimental protocols were approved by Institutional Animal Ethics Committee of United Institute of Pharmacy, Allahabad (UIP/CPCSEA/Nov-2012/03) after scrutinization. The animals received the drug treatment by oral gavages tube.

Oral acute toxicity study:

The lethal median dose (LD50) determination was done in rats by OECD guidelines 423.[12] A single dose of the extracts (5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg) in suitable quantity of water was given orally by gavage to different group of rats (three each). The animals were allowed free access to water and food. However, all the animals were deprived of food for 2 hr before and 4 hr after dosing. The animals were continuously monitored during first 4 hrs and every one-hour during the first 12 hrs for any undesirable effects. Later they were monitored (daily twice) for any abnormal changes throughout the study period of 14 days.

Evaluation of anti-ulcer activity

Phenylbutazone induced ulceration:

Gastric ulceration, according to the method described by Liu et al, 2001 with slight modification[13] was

induced in 36 hour fasted rats by the administration of ulcerogenic drug phenylbutazone 100 mg/kg, *p.o.* Groups of six animals each were pre-treated with 50% ethanolic extract of *Capparis zeylanica* 30 minutes before the ulcerogenic procedure in the following manner.

Group I - Control rats received vehicle solution

Group II - Rats received standard drug ranitidine 50 mg/kg

Group III - Rats treated with extract 200 mg/kg body weight

Group IV - Rats treated with extract 400 mg/kg body weight

Phenylbutazone is suspended in 1% carboxy-methylcellulose in water (20 mg/ml) and administered orally (gavage) in a dose of 100 mg/kg in 36 hours fasted rats. Two doses are given at an interval of 15 hours and 6 hours. Gastro-protective effects have been studied by administering the plant extract 30min before each dose of phenylbutazone. After the dose administration, animals are sacrificed and assessed for the gastric mucosal damage. Gastric juice was collected by holding the stomach over a funnel placed on a graduated centrifuge tube and puncturing it with a scissors. Volume of the juice was noted and then centrifuged at 2500 rpm for 15minutes and the supernatant was used for various biochemical estimations. After juice collection the stomach was opened along the greater curvature, washed under running water and the inner surface was carefully observed with a magnification lens. Number of ulcer in both glandular and fundic region and severity of ulceration was noted. The ulcer index was calculated according to the method.[14] The lesion was counted with the aid of hand lens (10x) and scoring of ulcer will be made as follows:

Normal colored stomach	0
Red coloration	0.5
Spot ulcer	1
Hemorrhagic streak	1.5
Deep Ulcers	2
Perforation	3

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows:

Ulcer index (UI) was measured by using following formula:

$$UI = UN + US + UP \times 10^{-1}$$

Where, UI= Ulcer Index; UN = Average number of ulcers per animal; US = Average number of severity score; UP = Percentage of animals with ulcers

Percentage inhibition of ulceration was calculated as below:

$$\%\ \text{Inhibition of Ulceration} = \frac{(\text{Ulcer index}_{\text{Control}} - \text{Ulcer index}_{\text{Test}}) \times 100}{\text{Ulcer index}_{\text{Control}}}$$

Estimation of Biochemical parameters

Gastric volume:

Gastric volume was measured after centrifugation of the gastric fluid, allowed to stand, decanted and poured into the measuring cylinder of graduation 0.01 ml.

Determination of pH:

The pH of the gastric juice was measured using the pH meter (Fisher scientific, India)

Estimation of total acid:

The total acid content of the gastric juice was determined by titrating it with 0.01 N NaOH, using phenolphthalein as indicator, and was expressed as mEq/4 h/100 g.[15] The acidity of gastric juice was calculated as total acid content/gastric juice volume in mEq/mL.[16]

Estimation of pepsin:

Estimation of pepsin has performed according to method described by Wolker et al with slight modification.[17] For each determination placed four tubes, first and second containing 5 ml of substrate, third and fourth containing 10 ml of trichloro acetic acid. The gastric juice was mixed with an equal volume of HCl for maintain the pH 2.1 warmed at 37° C and

added 1ml of mixture to the tube first and fourth, incubated for 15 minutes. After the incubation tubes were allowed to stand for 4 min. First and third gives test and second and fourth gives blank. Filtered 25 minutes after the beginning of the filtration, 2 ml of filtrate was pipette into 10 ml of sodium hydroxide. Mixed by gentle rotation. After 30 min, the intensity of colour was measured at 680 nm in Systronic spectrophotometer. The difference between test and blank gives a measure of peptic activity. As standard mixed 2ml of freshly prepared phenol solution containing 50 µg/ml /ml with 10 ml of sodium hydroxide and 1ml of phenol reagent was added. After 5 to 10 minutes, the colour intensity was measured at 680 nm.

Estimation of DNA content:

DNA content was estimated and expressed as µg/ml gastric juice 100 g weight of rats according to the previously reported process with slight modification.[18] Briefly, Superficial layers of the mucosa were taken, wiped with a piece of Whatman filter paper and weighed quickly on a digital balance. The tissues were then transferred into chilled Warburg vessels containing 3 ml of incubation fluid (141 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1.3 mM MgSO4, 1.3 mM KH_2PO_4, 10 mM glucose and 10 mM phosphate buffer, pH 7.4) with 15 µCi of [^{3}H]thymidine (BARC, Bombay). Incubation was carried out for 1 h at 37°C. After the incubation period, the mucosal slices were recovered, wiped and homogenized in 2.5 ml ice-cold 0.6 N perchloric acid (PCA). The precipitate was allowed to flocculate at 0°C for 10 min and then centrifuged at 2000 rpm for 5 min at 2°C. The precipitate was obtained by discarding the supernatant and was washed twice with 5 ml of 0.2 N ice-cold PCA. To the precipitate was added 4 ml of 0.3N KOH and it was then incubated at 37°C for 1h. After the incubation, the tubes were ice-cooled and to each tube 2.5 ml of ice-cooled 1.2N PCA was added. The precipitate and supernatant were separated by centrifugation and used respectively for the estimation of DNA. The precipitate was hydrolyzed by 2 ml of 0.5N PCA at 80°C for 30 min and the supernatant was used for DNA estimation.

Estimation of total proteins:

The gastric juice was precipitated by using 90% alcohol (1:9 ratios). Then 0.1 ml of alcoholic precipitate of gastric juice was dissolved in 1ml of 0.1N 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 minutes then 0.4 ml phenol reagent was added and again 10 minutes was allowed for colour development. Reading was taken against blank prepared with distilled water at 610nm in Systronic spectrophotometer. The protein content calculated from standard curve prepared with bovine albumin and was expressed in term of µg/ml of gastric juice.

Estimation of total hexose:

Taken 0.4 ml of hydrolysate fraction in a test tube and 3.4 ml of orcinol reagent was added. The mixture was heated with the help of boiling water bath for 15 mins. This was cooled under running tap water. After cooling, the colour intensity was measured by Systronic spectrophotometer at 540nm against the blank prepared by using water. The total hexose content was determined from the standard curve of D (+)-galactose-mannose and concentration has been expressed that the µg//ml of gastric juice.

***Estimation of hexoseamine*:**

0.5 ml of the hydrolysate fraction was taken in a test tube and added 0.5 ml of acetyl-acetone reagent. The mixture was heated with the help of boiling water bath for 20 minutes and cooling under running tap water. After the cooling mixed with 1.5 ml of 90 % alcohol and followed by addition of 0.5 ml of Ehrlich's reagent. The reaction was allowed for 30 minutes. The colour intensity was measured by Systronic spectrophotometer at 530 nm against the blank prepared by using hydrolysate fraction. The 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.

Estimation of 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

hydrolysate were taken. To all three tubes 1.8 ml of sulphuric acid: water (6:1) was added. The test tubes were kept under the ice cold water bath for prevent breakage of glass wares due to strong exothermic reaction. This mixture was heated with help of boiling water bath for exactly 3 minutes. The test tubes were cooled with running tab water. 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 was stand and allowed for 90 minutes. After the 90 mins, the colour intensity was measured by Systronic spectrophotometer at 396nm and 430nm against the blank prepared by using water. The optical density was calculated and standard curve was prepared with D (+) fructose. The fucose content was expressed in terms of µg/ml of gastric juice.

Estimation of sialic acid:

Taken 0.5 ml of the hydrolysate fraction in test tube and added few drorps of 0.1 N H_2SO_4 and 0.2 ml of sodium periodate was allowed and stand for 20 minutes. Twenty minutes later 1 ml of sodium arsenate solution added to this mixture. The brown coloured mixture was disappeared after shaking. The disappeared mixture was treated with 3 ml of thiobarbituric acid and heated with the help of boiling water bath for 15mins. After the heating, the test tube was cooled with the help of running tap water and added 4.5ml of cyclohexanone allowed and stand for 15seconds.

The mixture was centrifuged to get a clear pink layer of cyclohexanone. This supernatant was pipetted out and the intensity of colour was measured by Hitachi-spectrophotometer at 550nm. The sialic acid content was determined from the standard curve of sialic acid and has been expressed in the terms of µg/ml of gastric juice.

Estimation of total carbohydrates:

The dissolved muco substances in gastric juice were estimated in the alcoholic precipitate obtained by adding 1ml of gastric juice to 9ml of 90 % of alcohol in the test tube and mixture was kept for 10mins in the test tube stand and obtained supernatant solution was discarded. The precipitate was taken in a test tube and added 0.5ml of 0.1N sodium hydroxide and 1.8ml of 6 N HCl. This mixture was kept in the boiling water bath for 2hrs. After the boiling, mixture was neutralized by adding 5N sodium hydroxide using phenolphthalein as indicator and the volume was made up to required quantity with distilled water to form hydrolystate fraction.

Statistical analysis

The statistical analysis of the pharmacological analysis was carried out using GraphPad Prism version 5.04 for windows. The values are represented as mean ± S. D. for six rats data were analyzed by Student *t* test and ANOVA with post-hoc difference was analyzed using Newman-keuls method.

RESULTS & DISCUSSION

Preliminary phytochemical screening:

Preliminary phytochemical screening revealed the presence of carbohydrate, phenolic compounds, saponins and flavonoids in 50% ethanolic extract of roots. These secondary metabolites are scientifically supported for the antiulcer activity.

Acute toxicity study:

The LD_{50} determination was done by OECD guidelines 423. The LD_{50} of the 50% ethanolic extract of roots of *Capparis zeylanica* was found to be 2000mg/kg. One-tenth of the maximum dose of the extract tested was selected for the study, i.e. 200mg/kg and double strength 400 mg/kg.

Antiulcer activity:

Table 1 shows the dose dependent changes in lesion index of experimental group of rats. Ulcer inhibition was evident in all treatments of 50% ethanolic extract of *Capparis zeylanica* compare to the control group. However statistically significant ulcer inhibition (24.21% and 45.92%) (p<0.001) could be seen only at dose of 200 and 400mg/kg both of CZE. The volume of gastric juice, free and total acidity, pepsin concentration and acid output of gastric juice were decreased significantly ($p < 0.001$) in 400mg/kg extract treated rats. Results are mentioned in table 2.

Table 1: Effect of 50% ethanolic extract of roots of C. zeylanica on pH and ulcer index

S/No	Treatment/ Dose (mg/kg)	Ulcer Index (mm^2/rat)	Percent Protection	pH
1.	Control	22.12±0.66	-	2.11±.481
2.	Ranitidine(50)	4.25±0.71[c]	76.49[c]	5.43±0.067
3.	CZE (200)	15.91±1.31	24.21[b]	2.78±.08
4.	CZE (400)	11.41+.37[c]	45.92[c]	3.73±.069

The value represents the mean ± S.D. for 6 rats per group. [a]p <0.05, [b]p<0.01; [c]p<0.001 compared to standard group.

In Phenylbutazone + CZE treated rats, a significant increase ($p < 0.001$) in the carbohydrate concentration in terms of TC:P ratio and significantly lowered ($p < 0.001$) protein concentration in gastric juice was observed. The concentration of DNA in gastric juice was significantly decreased ($p < 0.001$) in Phenylbutazone + CZE treated rats. The results are mentioned in Table 2 & 3.

In the present study, increase in pH and decline in acidity, acid output and pepsin concentration were confirmed in ulcerated animals treated with plant extract. The gastroprotective effect of plant extract may be due to the direct action on the acid producing cells. Moreover, one of the essential criteria to determine the status of mucus resistance/barrier is the state of mucus secretion. This mucus consists of mucin type of glycoproteins, which can be estimated by the ratio of total carbohydrates to protein. These high molecular weight glycoproteins are primarily responsible for viscous and gel forming features of the mucus. Increased mucus secretion by the gastric mucosal cells can put off gastric ulceration by a number of mechanisms, including lessening of stomach wall friction during peristalsis and gastric contractions, improving the buffering of acid gastric juice and by acting as an effective barrier to back diffusion of H^+ ions 18. 50% ethanolic extract of plant exhibited ulcer healing activity by increasing hexosamine and carbohydrate protein ratio and adherent mucus content against phenylbutazone induced ulcer. This results in the increase in mucus secretion. The importance of mucus secretion as a response to gastric mucosal trauma has long been recognized.[19] Mucosal barriers are the most significant factors for gastric protection.[20] More production of mucous is responsible for the less degree of ulceration. Mucus also defends the mucosa and sub-mucosa from inflammatory reaction. The higher the mucin contents the lower is the free acidity. Mucosal defense agents are a new dimension in the treatment of gastro-duodenal diseases.[21] In the present study we can conclude that the biochemical parameters reduced after the treatment of extract might be a preventive measure of ulceration.

CONCLUSION

Through the pharmacological activity and statistical analysis, it was confirmed that the 50% ethanolic extract of roots of *Capparis zeylanica* plant is the most effective in prevention of ulceration in experimental animal model. The beneficial multiple properties

Table 2: Effect of 50% ethanolic extract of roots of *C. zeylanica* on gastric secretions

Treatment/ Dose (mg/kg)	Volume (ml/100 gm)	Acid		Pepsin		DNA (µg/ml)
		Conc. (µEq/ml)	Output (µEq/4h)	Conc. (µmol/ml)	Output (µmol/4 h)	
Control	1.80±.1.08	98.35±0.899	142.78±2.49	496.245±9.766	779.63±5.693	118.55±3.955
Ranitidine(50)	1.57±0.122	74.411±1.77	116.405±1.405	295.646±7.250	427.366±5.1808	74.468±1.455
CZE (200)	1.74.075[b]	91.91±1.01	136.27±2.96	274.87±7.337	708.375±7.938[b]	100.93±4.24[c]
CZE (400)	1.67±0.064[c]	84.49±1.25[c]	135.30±2.90[c]	432.51±4.871[c]	570.03±7.047[c]	89.788±2.467[c]

The value represents the mean ± S.D. for 6 rats per group. ap <0.05, bp<0.01; cp<0.001 compared to standard group.

Table 3: Effect of 50% ethanolic extract of roots of *C. zeylanica* on different ratio of carbohydrates and proteins

Treatment/ Dose (mg/kg)	Protein (µg/ml)	Total hexose (µg/ml)	Hexosamine (µg/ml)	Fucose (µg/ml)	Sialic acid (µg/ml)	TC (µg/ml)	TC:P
Control	4382.814±8.955	2475.672±258.53	1164.48±18.329	231.96±2.058	58.028±1.044 393	0.14±279.961	0.8967±31.263
Ranitidine (50)	4013.844±7.958	2930.377±84.86	1328.963±6.819	267.211±6.319	98.17±0.960	4610.565±98.633	1.152±12.436
CZE (200)	4256.144±5.349	2577.133±63.269[b]	1203.963±10.380	240.518±1.768[b]	67.133±1.268b	4088.747±76.685	0.955±12.078
CZE (400)	4190.524±8.077[c]	2752.318±103.2314[c]	1276.313±6.248[c]	251.218±3.365[c]	81.308±1.785[c]	4361.157±114.629[c]	1.040±14.192

The value represents the mean ± S.D. for 6 rats per group. ap <0.05, bp<0.01; cp<0.001 compared to standard group.

present in medicinal plant offer exciting opportunity to develop them into novel therapeutics for ulcer.

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