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ORIGINAL ARTICLE

A preclinical study on the gastric ulcer protective activity of the world's hottest chilli, *Capsicum frutescenes*

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ABSTRACT

Objective: To evaluate the ulcer protective activity of the ethanolic extract of Capsicum Frutescenes (EECF) and Ranitidine on aspirin induced gastric ulcer on albino rats.

Materials and Methods: Four groups of albino rats were taken for the study (n=6): Group I was taken as normal control (3% gum acacia orally for 7 days),Group II as experimental control (3% gum acacia orally for 7 days),Group III (EECF 100mg/kg for 7 days),Group IV (150 mg/kg ranitidine for 7 days).On the 7th day, the Groups II,III and IV received aspirin (400mg/kg) as a single dose . After 24 hours of aspirin administration; pyloric ligation was done in all animal Groups and they were kept for 4 hours .Thereafter, the rats were sacrificed and their stomachs were removed to measure the(i) ulcer index (ii)pepsin activity (iii)gastric mucus (iv) free acidity (v) total acidity and (vi)gastric volume

Results : One way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test, were used for statistical analysis of the results. Values of $p < 0.05$ were considered significant. The ulcer index, pepsin activity, and free and total acidity showed a significant decrease ($p<.05$), whereas, there was increase in gastric mucus secretion in Groups III and IV . The reverse was seen in group II.

Conclusion : The study showed that *capsicum frutescenes* has *an* ulcer protective effect similar to that of Ranitidine.

Key Words : aspirin ,gastric ulcer ,ranitidine

Key Message: Gastric ulcer is a very common problem . Indians have the habit of using more chilli in their food than their western counterparts. The Guinness World Record Book had recorded *Capsicum frutescenes* as the world's hottest chilli which is found in Assam , India. Chilli was found to show gastric ulcer protection in experimental animals.

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Inroduction

An ulcer is defined as disruption of the mucosal integrity of the stomach and/or duodenum leading to a local defect or excavation due to active inflammation. The life time prevalence of peptic ulcer disease (PUD) in United States is approximately 12% in men, and 10% in women[1]. In India, peptic ulcer is more prevalent in Jammu and Kasmir, followed by Southern India. North India comes next, and East and North East have comparatively lower prevalence[2]. Indians have the habit of eating spicy food. One major ingredient of spicy food is chilli powder. A majority of people believe that chilli causes gastric ulcer, and advise those having gastric ulcer not to use chilli. On the contrary, there is a small number of people who believe that chilli helps in curing peptic ulcer.The present study was aimed to study whether chilli has ulcerogenic or ulcer protective effects.In the present study, we evaluated the ulcer protective effect of the ethanolic extract of *Capsicum frutescenes* on aspirin

induced gastric ulcer in albino rats, and compared it with the standard drug, ranitidine. *Capsicum frutescenes* is cultivated (grown) in the hills near the central Assamese town of Tezpur. This variety of chilli is dubbed as "nagajolokia" or "bhootjolokia" by the people of Assam.

Materials and Methods

Experimental animals : The study was carried out on twenty four healthy albino rats (Rattus norvegicus) of either sex, and weighing 100-200gms. The rats were obtained from Animal House, Assam Medical College, Dibrugarh. All the animal procedures were performed according to CPSEA (Chennai ,India) norms .The institutional animal ethics committee approved the experimental procedure. The standard animal diet was maintained with Bengal gram, wheat, maize and carrot in sufficient quantity, daily. Water was given *ad libitum*.

Plant Materials

Materials required for the extraction of plant products were ,Fruit of *Capsicum Frutescenes* collected from Dibrugarh district, Assam. Fresh ripe *Capsicum frutescenes,* approximately one and a half kilogram, was cut into small pieces and was air dried at room temperature. The dried chilli were ground to fine powder and soaked in 95% ethanol for 24 hours in a percolator. After 24 hours, it was allowed to percolate slowly and the ethanolic extract of *Capsicum Frutescenes* (EECF) was collected in Petri dishes[3].

Experimental procedure

The acute toxicity of EECF was determined on albino rats. After administration with different doses of these extracts, the mortality with each dose was noted at 48 hours (acute) and 14 days (chronic). $LD_{50}$was calculated as per OECD guidelines 425, and by using AOT 425 software. As per guidelines, five rats were chosen and the plant extract 2000mg/kg was orally given to the animals[4].

For the experiment, four groups, each containing six animals, were chosen: Group I was taken as the normal control (3% gum acasia orally for 7 days), Group II was the experimental control (3% gum acasia orally for 7 days), Group III (EECF 100mg/kg for 7 days), and Group IV (150 mg/kg ranitidine for 7 days). On the 7th day, the Groups II,III and IV received aspirin (400mg/kg) as a single dose. After 24 hours of aspirin administration, pyloric ligation was done in all animal groups and they were kept for 4 hours.Thereafter, the rats were sacrificed and their stomachs were removed to measure the (i) ulcer index, (ii)pepsin activity, (iii)gastric mucus, (iv) free acidity, (v) total acidity and (vi)gastric volume

Biochemical assessment

Pepsin activity

Pepsin activity was measured by the methods of Debnath PK et al (1974)[5]. Briefly, one ml of diluted gastric juice was mixed with 2% haemoglobin solution in 0.06M HCl and incubated for 20 mins. 0.6 M ice cold Trichloroacetic acid was then added to it. Later, the solution was centrifuged and the supernatant fluid was mixed with reagent C and Reagent E, and the optical density was measured at 610 nm against a blank of distilled water .

Free acidity and Total acidity

Free acidity and total acidity was measured by the method of Kulkarni SK (1999)[6]. Briefly, 2 drops of Topfer's reagent was added in a diluted supernatant of gastric juice in a conical flask . 0.01N NaoH was taken in a burette, and was allowed to titrate till the contents of the flask changed to yellow colour.Then two drops of phenolphthalein was added, and the titration continued till the solution reached orange colour .

Gastric mucus

Gastric mucus was measured by the method described by Crone SJ et al (1974)[7]. Briefly excised glandular portions of

stomach was soaked in 0.1% alcian blue solution buffered with 0.05M sodium acetate and HCl. The uncomplexed dye adhered to the tissue, which was washed with 0.025M sucrose and again soaked in $MgCl_2$. The resulting blue solution was mixed with ether and its optical density was measured against 605nm.

Volume of Gastric Juice

The volume of gastric juice was measured by the method described by Deshpande SS et al (2003)[8]. The resected stomach content was centrifuged and the supernatant fluid was taken.

Ulcer index

Ulcer index was measured by the method described by Goyal RK (2002)[9]. Briefly, the ulcerated lesion of the opened stomach was measured with the help of a magnifying glass, and the ulcer index was calculated .

Results

In the acute oral toxicity test, no mortality was seen up to the dose of 2000mg/kg Therefore ,1/20 of the dose was selected for the study .

Group III and Group IV showed a lesser ulcer index than the Group II experimental control . [Table/fig 1].

Group III and Group IV showed a lesser pepsin activity than the Group II experimental control. [Table/fig 1] .

Group III and Group IV showed approximately equal level of free acidity, which was significantly lesser than the Group II experimental control [Table /Fig1].

Group III and Group IV showed significant decrease in total acidity in comparision to the Group II experimental control. Group III(EECF)showed the highest reduction in total acidity, which was even better than the standard drug ranitidine. [Table /Fig 1].

Gastric mucus secretion was increased in Group III (EECF) and Group IV(ranitidine) as compared to the Group II experimental control [Table /Fig 1].

Gastric volume was decreased in Group III (EECF) and Group IV(ranitidine) as compared to the Group II experimental control[Table/Fig1].

[Table/ Fig 1]THE ULCERPROTECTIVE EFFECTS OF CAPSICUM FRUTESCENES AND RANITIDINE IN ASPIRIN INDUCED ULCERS ON ALBINO RATS (Mean ± SEM)

Groups	Dose P.O.	Ulcer Index	Pepsin Activity (µmol tyrosine /ml)	Free Acidity (mEq/l)	Total Acidity (mEq/l)	Gastric Mucus (mg alcian blue/gm glandular tissue)	Volume of Gastric Juice (ml/ 4 hrs)
Group–I (Normal control)	3% gum acacia 5 ml/kg	0.02 ± 0.01	30.67 ±8.41	91.66 ±2.47	103.33 ±3.3	25.83 ±1.5	3.25 ±0.36
Group–II (experimental control)	400 mg/kg	19.24 ±0.95*	180.65 ± 8.18*	127.50 ±2.7*	157.5 ± 4.60*	11.66 ±1.05*	4.85 ± 0.28*
Group–III (EECF)	100 mg/kg	6.4 ± 0.03**	48.46 ±6.14**	99.16 ±7.12**	105 ±3.16**	31.66 ±2.78**	1.51 ± 0.10**
Group– IV (Ranitidine)	150 mg/kg	3.86 ± 0.01**	27.42 ±6.8**	100 ±3.16**	138.33 ±1.6**	30.83 ±3.00**	1.66 ± 0.24**
ANOVA	p	p<.05	p<.05	p<.05	p<.05	p<.05	p<.05

n = 6 in each group; *p < 0.05 when compared to normal control:
** p <0.05when compared to experimental control; ANOVA followed by Dunnet's Multiple Comparison Test .

Discussion

Peptic ulcer therapy has undergone many strides over the past few years, and a number of drugs are now available for treatment. These drugs are broadly classified into two, those that decrease or counter acid pepsin secretion, and those that provide cytoprotection by virtue of their effects on mucosal defensive factors (Goel RK and Bhattacharya SK, 1991)[10]. A Chinese study published in 1992 stated that "our data supports the hypothesis that the chilli used has a protective effect against peptic ulcer disease".(1992)[11] . Another study of 1995 found that Capsicum can even protect the stomach lining from aspirin induced ulcers.(K.G Yeoh et al ,1995)[12]. Yasutada Akiba et al (2001)[13] found out the mechanism of action of capsaicin (Vanillyl amide Isodecenoic acid) which is active component of capsicum.They studied the rat stomach, duodenum, ileum and colon using western blot analysis .The VR-1 receptor was detected at 95 kda in the dorsal root of

ganglia (DRG) and throughout the GI tract at ~95 and~100 kDa, suggesting expression of different types of VR in GI. Vanillyl Receptors(VR) are responsible for acid sensing and hyperaemic response in rat GI mucosa .Increased blood flow into the gastric mucosa stimulates the mucosal defence mechanism .

The present study showed that ethanolic extract of Capsicum Frutescenes lowered gastric ulcer by decreasing acid pepsin and increasing mucus secretion .

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