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RESEARCH PAPER

Evaluation of Antiulcer Activity of *Piper chaba* leaf extract against indomethacin induced gastric ulcer in rats

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ABSTRACT

The anti-ulcer activity of ethanolic extract of *Piper chaba* leaves was investigated using indomethacin induced ulcer models in albino rats. The parameters determined were ulcer index, free acidity and total acidity. Ethanolic extract of *Piper chaba* leaves at doses of 200 mg/kg and 400 mg/kg p.o produced significant inhibition of the gastric lesions in indomethacin induced gastric ulcer. The extracts (200 mg/kg & 400 mg/kg) showed significant ($P < 0.05$) reduction in gastric volume, pH of gastric juice, total Acidity free acidity, ulcer index as compared to control. Thus present study indicates that *Piper chaba* leaves extract have potential anti ulcer activity and these results may further suggest that ethanolic extract was found to possess antiulcerogenic as well as ulcer healing properties, which might be due to its antisecretory activity.

Keywords: - *Piper chaba*, gastric ulcer, Indomethacin, ulcer index.

INTRODUCTIO

One of the widespread diseases, gastric ulcer, is supposed to be due to an imbalance between aggressive and protective factors [1]. Some potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility [2, 3]. Drug treatment of peptic ulcers is targeted at either

counteracting aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor "PAF", leukotrienes, endothelins, bile or exogenous factors including NSAIDs) or stimulating the mucosal defences (mucus, bicarbonate, normal blood flow, prostaglandins(PG), nitric oxide) [4]. The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Currently the treatment of peptic ulcer which can meets all these objectives is costly. Hence, efforts are on to find a suitable treatment from natural product sources.

Piper chaba is a flowering vine in the family Piperaceae that is native to South and Southeast Asia. *P. chaba* is found throughout India, and

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other warmer regions of Asia including Malaysia, Indonesia, Singapore and Sri Lanka [5]. It is well known for the treatments of poor circulation, arthritis and rheumatism, sprains, stiffness, also used in cold, flu, diarrhea, flatulence, nausea, catarrh, and cough. In Greece, it was used for fever and stomach problems. Pepper grains were sometimes swallowed whole as a general body tonic. The plant contains Piperine, sitosterol, pipalotine (alkaloid), aromatic oils that are about 0.7%, piperine 4-5% and an alkaloid and pipalotine. Besides this it contains sesamin and pipalsterol. The root contains piperine 0.55-0.18% pipalotin (0.13-0.20%), piper ceaguminin, sterol and glycoside [6]. *Piper chaba* has shown several therapeutic activities like antioxidant, cytotoxic, anti-inflammatory and hypolipidemic effects [7]. However there are no reports on the antiulcer activity of the plant hence the present study was designed to verify the claims of the native practitioners.

MATERIALS AND METHODS

Plant material

The plant *Piper chaba* was collected from area near by Udaipur, Rajasthan and identified and authenticated by Dr. P Jayaraman in Plant Anatomy Research Center, Chennai.

Preparation of extract

The leaves of *Piper chaba* were shade dried and reduced to coarse powder in a mechanical grinder. The powdered material obtained was then subjected to successive extraction by using petroleum ether, benzene, chloroform, ethanol

and water as solvents in a Soxhlet extractor. The different extracts obtained were evaporated at 45°C to get a semisolid mass. The extracts thus obtained were subjected to phytochemical analysis. Ethanolic extract was used for further studies.

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out by using standard procedures described by Kokate [8] and Harborne [9].

Animals used

Albino rats of either sex weighing between 150-250 gm were used. Institutional Animal Ethics Committee approved the experimental protocol; animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The animals were housed in Polypropylene cages and maintained at 24°C ± 2°C under 12h light/ dark cycle and were fed ad libitum with standard pellet diet and had free access to water. The animals were given standard diet.

Indomethacin induced ulcer model [10, 11]

Adult albino rats of either sex weighing between 150-250 grams divided into 5 groups consisting of 6 in each. Rats in group 3, 4 and 5 were pretreated with Omeprazole (reference drug) at a dose of 20 mg/kg body weight, and test drugs at 200 and 400 mg/kg body weight respectively, once daily for 7 days prior to ulcer induction. On 7th day, ulceration was induced by oral administration of indomethacin (10 mg/kg body

weight) to group 2, 3, 4, 5 two times at an interval of 8 hours. 2 hours after the second dose of indomethacin, pylorus ligation was carried out. After 4 hours of ligation, the rats sacrificed with an overdose of anesthetic ether. The stomach dissected out by its greater curvature and contents drain into a sterile tube. The inner surface of the empty stomach examined for gastric lesions.

Group 1: Normal Rats

Group 2: Control Rats (Indomethacin 2x10 mg/kg)

Group 3: Standard rats- Omeprazole (20mg/Kg) + Indomethacin 2x10 mg/kg

Group 4: Test rats I- Test drug (200 mg/Kg) + Indomethacin 2x10 mg/kg

Group 5: Test rats II- Test drug (400 mg/ Kg) + Indomethacin 2x10 mg/kg

Ulcer Index [12, 13]

After the incision of the stomach at the greater curvature the ulcers were observed and the number of ulcers was counted using a magnifying glass and the diameter of the ulcers were measured using vernier calipers. The following arbitrary scoring system was used to grade the incidence and severity of lesions.

- Normal coloration – 0
- Red coloration – 0.5
- Spot ulcer – 1
- Hemorrhagic streaks – 1.5
- Ulcers >3mm but <5mm
- Ulcer >5mm

The ulcer index was determined using the formula

$$\text{Ulcer index} = 10/X$$

Where X = Total mucosal area/Total ulcerated area.

Based on their intensity, the ulcers were given scores as follow

0 = no ulcer, 1 = superficial mucosal erosion,

2 = deep ulcer or transmural necrosis,

3 = perforated or penetrated ulcer

Percentage protection was calculated using the formula.

$$\text{Percentage protection} = 100 - \frac{U_t}{U_c} \times 100$$

U_t = Ulcer index of treated group U_c = Ulcer index of control group

Determination of free acidity and total acidity

1 ml of gastric juice was pipette into 100 ml conical flask, added 2-3 drops of topfer reagent and titrated with 0.01 N sodium hydroxide until all traces of red color disappears and the color of the solution turns to yellowish orange. The volume of alkali added was noted. This volume corresponded to free acidity. Then 2-3 drops of phenolphthalein solution was 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

$$\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100$$

acidity = 0.1 meq/L/100gm.

Statistical Analysis

The values were expressed as mean \pm SEM and analyzed using one-way analysis of variance

(ANOVA) using Statistical Package for Social Sciences (SPSS) 20th version.

RESULTS

Preliminary phytochemical screening

The phytochemical analysis of *Piper chaba* revealed the presence of various chemical constituents such as carbohydrate, alkaloids, flavonoids, glycosides. (Table-I)

Physicochemical constants

Physicochemical analysis of *Piper chaba* leaves were determined by standard method and the results are shown in Table II.

Foaming index

Foaming index of leaf powder: NIL

Swelling index

Swelling index of leaf powder: 1.5

Indomethacin induced gastric ulcer

In control animal, oral administration of indomethacin produced characteristic lesions in the glandular portion of rat stomach which appeared as elongated bands of thick, black & dark red lesions. Ethanolic extracts of *Piper chaba* leaves have shown significant protection index of 63.74 % and 71.84 % with the dose of 200 and 400 mg/kg respectively in comparison to control, Omeprazole as reference standard drug was reduction of ulcer 93.29 %. (Results are tabulated in Table-III)

Gastric volume, Free Acidity and Total Acidity

The results of various acid secretary parameters such as gastric volume, pH, free acidity and total

acidity of ethanolic extract of *Piper chaba* on gastric ulcer in rats are summarized in Table IV. Estimation of acid secretary parameters was increased significantly in the control group. Administration of *Piper chaba* ethanolic extract exhibited a significant ($p < 0.05$) reduction in all the parameters and the results were comparable with the standard drug Omeprazole 20mg/kg.

Results were in mean \pm SEM, each group had six rats, * $p < 0.05$

Data was expressed as mean \pm SEM, compared with normal control ($\#p < 0.05$) and ulcerated control by one-way ANOVA.

CONCLUSION

In present study, it was observed that *Piper chaba* ethanolic extract significantly reduced ulcer index. The extract showed protection against characteristic lesions produced by ulcer due to Indomethacin administration. This gastroprotective effect of *Piper chaba* ethanolic extract may be due to both reductions in gastric acid secretion and gastric cytoprotection which may further contribute in the treatment of peptic ulcers.

The *Piper chaba* ethanolic extract showed protection against characteristic lesions produced by ulcer due to Indomethacin administration. This gastroprotective effect may be due to antisecretory, cytoprotective antioxidant activities and also by the presence of flavonoids, saponins, alkaloids and tannins cause both reductions in gastric acid secretion and gastric cytoprotection which may further contribute in the treatment of peptic ulcers.

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Table-I. Preliminary phytochemical screening of leaf powder of *Piper chaba*

<i>Phytoconstituents</i>	<i>Pet.Ether</i>	<i>Benzene</i>	<i>Chloroform</i>	<i>Ethanol</i>	<i>Water</i>
Test for Alkaloids					
Mayer's Test	+	-	+	++	+
Wagner's Test	+	-	-	++	+
Dragendroff's Test	-	-	-	+	-
Hagers test	-	-	-	+	-
Test for Carbohydrates					
Molisch's Test	+	+	+	+	+
Benedict's test	-	-	-	-	-
Fehling's Test	-	-	-	-	-
Test for Flavonoids					
Alkaline Reagent Test	-	-	-	++	-
Lead acetate Test	-	-	+	++	-
Test for glycosides					
Modified Borntrager's Test	-	-	-	+	-
Legal's Test	-	-	-	-	-
Test for Saponins					
Froth Test	-	-	-	+	-
Foam Test	-	-	-	+	+

Table-II. Ash values and extractive values of *Piper chaba* leaf.

	Parameters	% yield
1	Total ash	4.4 % w/w
2	Acid insoluble ash	0.15 % w/w
3	Water soluble ash	2.077% w/w
4	Loss on drying	4.8 % w/w
EXTRACTIVE VALUES		
5	Water soluble	21.73 % w/w
6	Alcohol soluble	9.14% w/w

Table III. Ulcer index and Ulcer protection (%)

Group	Ulcer index	% Ulcer protection
Group I- Control(Normal)	1.24 ± 2.22	91 %
Group II- Disease control	71.04 ± 1.54	-
Group III- Omeprazole (20mg/kg)	8.32 ± 2.10*	93.29 %
Group IV- <i>Piper chaba</i> ethanolic extract(200 mg/kg)	44.81 ± 2.00*	63.74 %
Group V-<i>Piper chaba</i> ethanolic extract (400mg/kg)	34.91 ± 1.54*	71.84 %

Table-IV. Effect of *Piper chaba* leaf extract on gastric volume, pH and free or total acidity of gastric juice in Indomethacin induced gastric ulcer in rats

Group	Gastric volume(ml)	pH of gastric juice	Free Acidity (mEq/1/100g)	TotalAcidity (mEq/1/100g)
Group I Control (Normal)	1.16± 0.07	2.32 ± 0.09	11.30 ± 0.48	31.27 ± 0.54
Group II (Disease control)	4.78 ± 0.02	1.23 ± 0.07	21.58±1.26	75.24± 2.91
Goup III Omeprazole (20mg/kg)	1.40 ± 0.03*	2.50 ± 0.12*	13.92 ± 0.39*	33.32 ± 1.95*
Group IV Ethanolic extract of <i>Piper chaba</i> (200mg/kg)	1.90 ± 0.04*	1.8 ± 0.06*	14.41± 0.27*	44.78 ± 0.95*
Group V Ethanolic extract of <i>Piper chaba</i> (400mg/kg)	1.60± 0.05*	2.00 ± 0.04*	13.33±0.52*	41.79 ± 0.51*