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Evaluation of Antiulcer Activity of *Piper Chaba* Leaves Extract Against Indomethacin Induced Gastric Ulcer In Experimental Animals

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ABSTRACT

The *Piper chaba ethanolic* 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. Indomethacin produces necrotic lesions in the gastric mucosa by reducing the secretion of bicarbonate and production of mucus, increasing vascular permeability and decreasing non-protein sulfhydryl groups of gastric mucosa. Indomethacin rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intracellular membrane permeability to sodium and water and thus causing gastric mucosa damage. Suppression of prostaglandins synthesis by ethanol results in increase susceptibility of the stomach to mucosal injury and gastro duodenal ulceration. The *Piper chaba ethanolic* extract significantly reduced ulcer index. The *Piper chaba ethanolic* 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.

Keywords: Antiulcer, Indomethacin, Wistar Rats, Piper Chaba, Ethanolic Extract.

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INTRODUCTION

Ulcer

An ulcer is erosion in the lining of the stomach or duodenum[1]. It is basically an inflamed break in the skin or the mucus membrane lining the alimentary tract[2]. Ulceration occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance[3]. About 19 out of 20 peptic ulcers are duodenal[4]. Gastric ulcers, found in the stomach wall, are less common.[5] The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products *(Helicobacter pylori)* and drugs.[6] These agents 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 gastricmotility.[7]

Piper chaba

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 other warmer regions of Asia including Malaysia, Indonesia, Singapore and SriLanka.[8]

Figure 1: Piper chaba

Table 1: Taxonomic description of *Piper chaba*

Scientific classification	
Kingdom:	Plantae
Clade:	Angiosperms
Order:	Piperales
Family:	Piperaceae
Genus:	*Piper*
Species:	*P. chaba*
Binomial name	
Piper chaba	

It is a creeper plant that spreads on the ground.[9] It may also grow around large trees. The leaves are oval-shaped and about 2 to 3 inches long. The flowers are monoceous and blossom during the monsoon. The fruit looks similar to other varieties of long pepper, with an elongated shape that can grow up to 3 inches long. The fruit is red when ripe, which turns dark brown or black whendry[10]. Chavak / Chavya is named as *Piper chaba* belongs to Piperaceae. Dalhana quoted Chavya as the root of Raja Pippali. The fruits of *Piper chaba* are considered as Raja pippali or Chavika while its root is considered as Chavya. Both are used in Ayurvedic material medica for a long time. Bhava Mishra quoted the properties of Chavika & Chavika Shoola separately. He quoted the properties of Chavya to those of Pippali Moola.[10]

CHEMICAL CONSTITUENTS[11]

Stem: Piperine, sitosterol, piplatine (alkaloid)

New amides: Retrofractamide A, B, C&D, isolated from aerial parts

Mono terpens (thujene, pinene, camphene, salcenine, careen, myrcene, limonene, phellandrene), to 80% sesquiterpener, 20-30% oxygenated compounds.

MATERIALS AND METHOD:

Plant material and authentication of plant

Collection of plant material: The plant Piper chaba collected from Garhwal District Himachal Pradesh and will identify and authenticated by Dr. P Jayaraman in Plant Anatomy Research Center Chennai.

Chemical Requirements:

Table 2: List of chemicals used

Sr. No.	Chemical	Company
1	Ascorbic acid	Merck
2	ferric chloride	Merck
3	Hydrogen peroxide	Merck
4	Deoxyribose, Phosphate	Merck
5	Thiobarbituric acid	Merck
6	Sodium hydroxide	Merck
7	Omeprazole drug,	Gift sample from Cipla LTD Mumbai
8	Molisch reagent,	Merck
9	Fehling A and B,	Merck
10	Benedict reagent,	Merck
11	Barfoed reagent,	Merck
12	Copper sulphate,	Merck
13	Millons reagent,	Merck
14	Ninhydrin reagent,	Merck
15	Sulphuric acid,	Merck
16	Benzene,	Merck
17	Pyridine,	Merck

18	Indomethacin,	Gift sample from Cipla LTD Mumbai
19	Dragendroffs reagent,	Merck
20	Hager reagent,	Merck
21	Wagner reagent	Merck
22	Ethanol	Merck
23	Lead acetate	Merck
24	Gelatin	Merck
25	Chloroform	Merck
26	Magnesium	Merck
27	Hydrochloric acid	Merck
28	Mayer reagent,	Merck

Method

Antiulcer activity

Indomethacin induced gastric ulcer in rat[21]

Adult albino rats of either sex weighing between 150-250 grams divided into 5 groups consisting of 6 in each. Rats were fasted for 12 hours, and group 2,3,4,5 then treated with two oral doses (2*10 mg/kg) of indomethacin suspension as a standard dose, at an interval of 15 hours, 2 hours after the second dose of indomethacin pylorus ligation was carried out as per the method[21]. 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

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

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.

Percentage protection = 100- $U_t/U_c \times 100$

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

Statistical Analysis

The values were expressed as mean ± SEM and analyzed using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) 20th version.

RESULTS AND DISCUSSION

Table 3: Effect of *Piper chaba* Leaves extract on Gastric ulcer in rats

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- *Piper chaba ethanolic* extract(200 mg/kg)	44.81 ± 2.00*	63.74 %
Group V-*Piper chaba ethanolic* extract (400mg/kg)	34.91 ± 1.54*	71.84 %

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

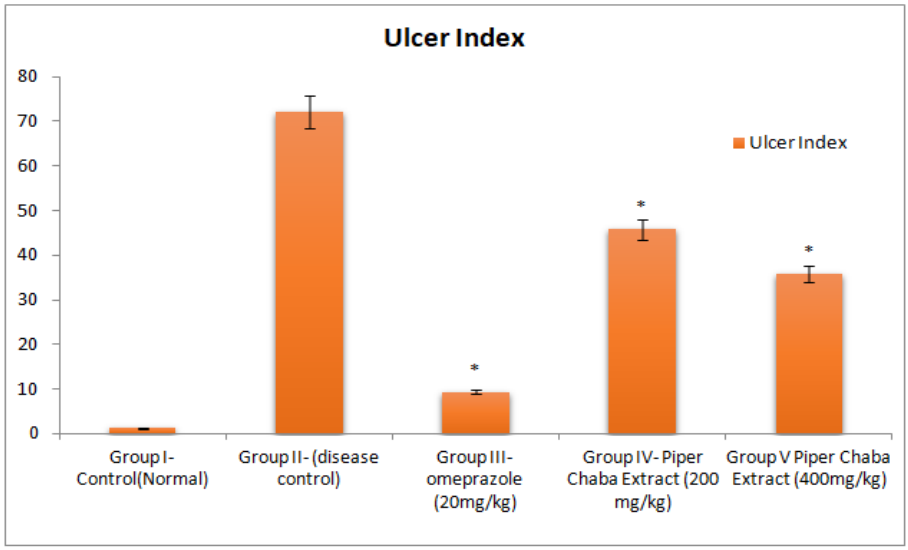

Figure 1: Ulcer index

Data was expressed as mean ± SEM, compared with normal control by unpaired t-test (#p< 0.05) by one-way ANOVA.

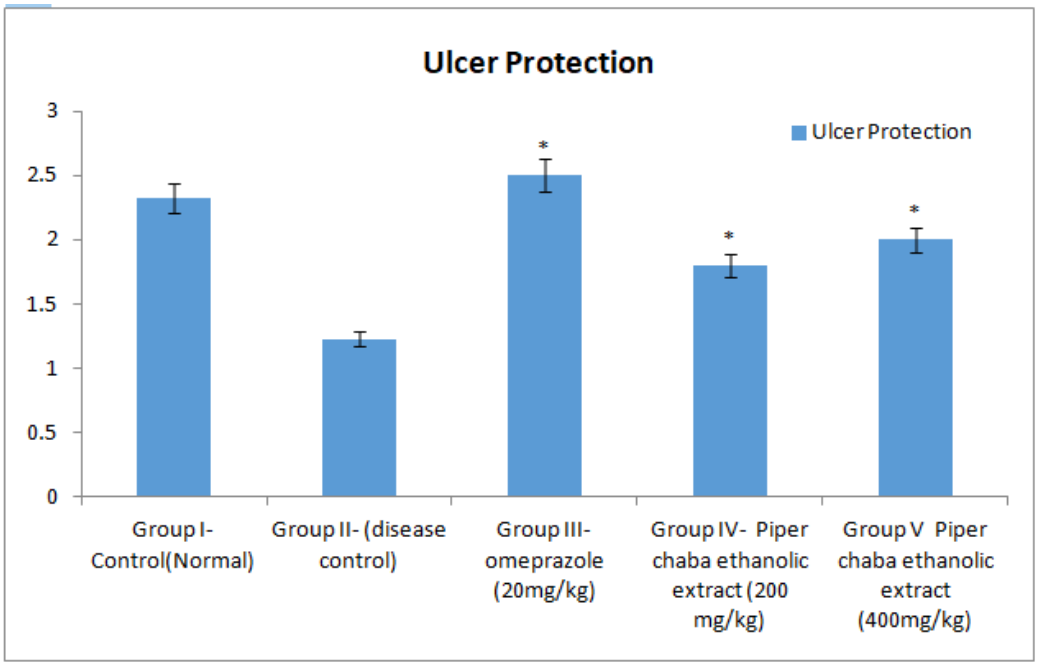

Figure 2: Percent ulcer protection

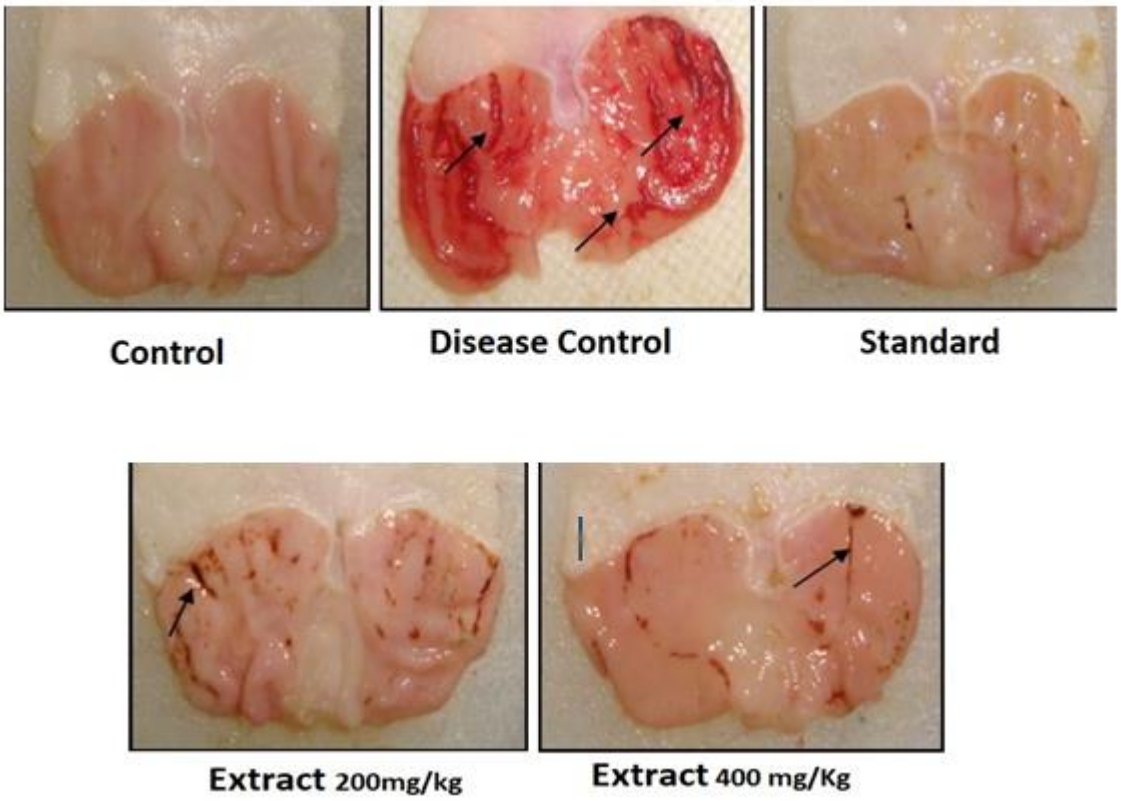

Figure 3: Representative stomach of rats after Indomethacin induced gastric ulcer

In present study, observed that the *Piper chaba ethanolic* extract significantly reduced ulcer index. The *Piper chaba ethanolic* 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. Indomethacin produces necrotic lesions in the gastric mucosa by reducing the secretion of bicarbonate and production of mucus, increasing vascular permeability and decreasing non-protein sulfhydryl groups of gastric mucosa. Indomethacin rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intracellular membrane permeability to sodium and water and thus causing gastric mucosa damage. Suppression of prostaglandins synthesis by ethanol results in increase susceptibility of the stomach to mucosal injury and gastro duodenal ulceration. The *Piper chaba ethanolic* extract significantly reduced ulcer index.

CONCLUSION

The *Piper chaba ethanolic* extract showed protection against characteristic lesions produced by ulcer due to Indomethacin administration. This gastroprotective effect of *Piper chaba ethanolic* extract due to both reductions in gastric acid secretion and gastric cytoprotection which further contribute in the treatment of peptic ulcers. The results obtained in the present investigation concluded that the ethanolic extract of *Piper chaba* has shown gastroprotective effect.

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