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Screening of *Piper cubeba* (Linn) Fruits for Anti-Ulcer Activity

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ABSTRACT: The study was designed to investigate the antiulcer activity of methanolic extract of the fruits of *Piper cubeba Linn* (Piperaceae) using model of gastric in rats which were induced by pyloric ligation. The extract was administered at a dose of 100, 200 and 400 mg/kg orally 30 min prior to ulcer induction i.e., pyloric ligation. Omeprazole (8mg/kg) was used as a reference standard. The antiulcer activity was accessed by determining and comparing the ulcer index in the test group with that of the control group. Gastric volume, total acid and free acid were estimated in the pylorus-ligated rats. *Piper cubeba* (400mg/kg) showed maximum inhibition of gastric acid, free acid and total acid to 23.61%, 66.94 and 56.71% respectively. The ulcer index in the *Piper cubeba* treated animals was found to be significantly less in all the models compared to control and standard drug treated cases. The antiulcer activity of *Piper cubeba* was, however, less than that of Omeprazole. The results suggest that *Piper cubeba* possesses significant antiulcer property which could be due to cytoprotective action of the drug or strengthening of gastric mucosa with the enhancement of mucosal defence.

Keywords: *Piper cubeba,* Omeprazole, Pyloric ligation, Antiulcer.

INTRODUCTION

Peptic ulcer therapy has undergone many studies over past few years and a number of synthetic drugs are now available for treatment. Reports on clinical evaluation of these drugs show that there are incidences of relapses and several adverse effects and danger of drug interaction during therapy [1&2].

The development of new antiulcer drug from medicinal plants is an attractive proposition because diverse chemical compounds have been isolated from medicinal plants with antiulcer activity[3] and have been shown to produce promising results in the treatment of gastric ulcers[4].

The bioactive molecules (generally alkaloids, glycosides, essential oils etc) are isolated/extracted from crude drugs may be used directly as therapeutic agents or as starting materials for the synthesis of useful drugs or serve as a model for pharmacologically active compounds in the period of drugs in synthesis [5].

An extensive literature survey reveals no pharmacological validation of antiulcer activity of this plant's fruits. This made us to screen the fruits of *Piper cubeba* for antiulcer activity in a scientific manner.

Therefore based on the above facts, the present study has been under taken with the main objective of evaluating the methanolic extract of fruits of *Piper cubeba* linn. For antiulcer activity using Albino Wistar Strain Rats as experimental animal model .

MATERIALS AND METHODS

APPARATUS

Soxhlet apparatus, P^H meter, Microscope

DRUGS AND MATERIALS

The following drugs and chemicals were used for the experimental study.

Anaesthetic Ether was obtained from S.D.Fine Chemicals Ltd., Mumbai. Omeprazole was obtained

from Dr.Reddy's Laboratory, Hyderabad. Topfer's Reagent and NaOH was obtained from Nice chemicals Pvt. Ltd., Cochin

PLANT COLLECTION AND IDENTIFICATION

The basic plant material of *Piper cubeba* linn. Fruits used for the investigation was purchased from the JOTHI MALIGAI-HERBALS, Baazar street CHIDAMBARAM-608002 in the month of November 2006. The plant material was identified and authenticated by Department of Botany, Research officer (Botanist), Annamalai Nagar, Annamalai University, Chidambaram – 608 002.

PREPARATION OF EXTRACT

The shade dried coarsely powdered Fruits of *Piper cubeba* linn. (400gms) was extracted using methanol as solvent by continuous hot extraction process using Soxhlet apparatus. The extraction was continued till the extraction completion. After completion of extraction the extract was concentrated under reduced pressure. That extract was stored in an airtight container in a refrigerator below 10°C.

EXPERIMENTAL ANIMALS

Colony inbred albino wistar strain rats (either sex) weighing (130-180gms) were used. The animals were maintained in well-ventilated room temperature with natural day-night cycle, in polypropylene cages. They were fed balanced rodent pellet diet obtained from central animal house RMMC, Annamalai University Chidambaram-608 002 and tap water *ad libitium* through out the experimental period. The animals were housed for one week prior to the experiments to acclimatize to laboratory conditions. The animals were randomly distributed into five different groups with six animals in each group. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC).Reference No.: Central animal house registration number 160/1999/CPCSEA Proposal No.:399, Approved Date: 16.11.2006.

ACUTE TOXICITY STUDIES

The procedure was followed by using OECD guidelines-423 (Acute Toxic Class Method).

The method uses defined doses (5, 50, 300, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the globally Harmonized System (GHS) for the classification of chemical which causes acute toxicity.

The Methanolic extract of *Piper cubeba* linn. fruits starting dose 2000 mg/kg body weight p.o. was used as most of the crude extracts posses LD50 value more than 2000 mg/kg p.o.

Body weight of the rats before and after termination were noted and any changes in skin and fur, eyes and mucous membrane and also respiratory, circulatory, autonomic and central nervous system and somatomotor activity and behavior pattern were observed, and also sign of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma were noted. The onset of toxicity and sings of toxicity were also noted, if any.

EVALUATION OF ANTIULCER ACTIVITY (PYLORUS LIGATED ULCER)[6-9]

Five Groups of each six numbers

Group	**Descriptions**
Group I	Control (vehicle)
Group II	Standard (omeprazole 8mg/kg p.o)
Group III (Test-I)	Methanolic extract (100mg/kg p.o)
Group IV (Test-II)	Methanolic extract (200mg/kg p.o)
Group V (Test-III)	Methanolic extract (400mg/kg p.o)

Albino wistar strain rats of either sex weighing between (130-180gms) were divided into five groups of six animals in each.

In this method albino rats were fasted in individual cages for 24 hrs. Care is taken to avoid coprophagy. In this methods albino rats were fasted in individual cages for 24 hrs. Care is taken to avoid coprophagy. Control vehicle , Standard drug Omeprazole 8mg/kg p.o Methanolic extract of *Piper Cubeba* (Linn.) 100 mg/kg , 200mg/kg p.o , 400mg/kg p.o is administered to group I to group V respectively 30 minutes prior to pyloric ligation.. Under light ether anesthesia, the abdomen was opened and the pylorus was ligated. The abdomen was then sutured, at the end of 4hrs of ligation the animals were sacrificed by cervical decapitation or deep anaesthesia and the abdomen is opened and ligature is placed around oesophagus, close to diaphragm and stomach was dissected out, gastric juice was collected in graduated tubes and then centrifuged at 1000 rpm for 10 minutes and the volume is noted. The pH of the gastric juice was recorded by pH meter. Then the contents were subjected for analysis free and total acidity. The stomachs were then washed with running water to see for ulcers in glandular portion of the stomach. The number of ulcers per stomach was noted and severity of the ulcers scored microscopically with the help of hand lens (10 x) and scoring is done [7&8].

0 = Normal stomach
0.5 = Red coloration
1 = Spot ulcers
1.5 = Hemorrhagic streaks
2 = ulcers > 3 mm but < 5mm,
3 = ulcers > 5mm

$$\text{Percentage protection} = \left[100 - \frac{ut}{uc}\right] x100$$

Mean ulcer score for each animal is expressed as ulcer index. The percentage protection is calculated using the above formula.
Where, Ut = ulcer index of treated group and Uc = ulcer index of control group

DETERMINATION OF FREE ACIDITY AND TOTAL ACIDITY

1ml of gastric juice is pipette out in 100ml conical flask, 2-3 drop of topfer's reagent is then added and titrated with 0.01 N sodium hydroxide until all traces of pink colour disappears and the colour of the solution turns to yellowish orange. The volume of alkali added was noted. This volume corresponds to free acidity. Titration is continued until pink color of solution reappears. Again the total volume of alkali added is noted, this volume corresponds to total acidity. Acidity (MEq/l/100g) can be calculated by using the formula.

Acidity =

$$\frac{volume\ of\ NaoH\ x\ Normality\ of\ NaOH\ x 100}{01}\ \text{MEq/l/100gm}$$

STATISTICAL ANALYSIS

The data were expressed as mean+SEM.Results were analyzed statistically by One-way ANOVA followed by DUNNETT's TEST using standard statisticcal software package of social science (SPSS) . The difference was considered significant if $P<0.05$.

RESULTS

The extract of dried fruits of *Piper cubeba* linn was prepared using Continuous hot extraction and the yield obtained was 12%. which was subjected to preliminary chemical screening for their presence or absence of active phytochemical constituents by following methods and resulted positively for the presence of alkaloids, terpenes and saponins as shown in **Table-1**
The extract did not produce any toxic symptoms of mortality up to the dose level of 2000 mg/kg body weight in rats, and hence the drugs were considered safe for further pharmacological screening. According to the OECD-423 guidelines for acute oral toxicity, the LD50 dose of 2000 mg/kg and above is categorized as unclassified.
Effect of Standard drug Omeprazole and test extract of *Piper cubeba* on gastric volume, free acid, total acid, pH and ulcer index in pylorus ligated rats were studied.
Omeprazole (8 mg/kg). The standard drug inhibited the volume of gastric juice secreted by the control rats by 46.39 %. The free acid and the total acid were reduced by Omeprazole to 89.69 % and 88.07 % .
*Piper cubeba (*100, 200 and 400 mg/kg) inhibited the volume of gastric juice secreted by the control rats by 18.05%, 22.22% and 23.61% respectively. The free acid and the total acid were reduced by the extract to 41.73 and 39.66%, 54.54 and 47.76%, 66.94 and 56.71% respectively for the 100, 200 and 400mg/kg. *Piper cubeba administered* in doses 100, 200 and 400 mg/kg orally cause a dose dependent decrease in ulcer index in pylorus ligated rats. The dose of 400mg/kg showed maximum ulcer protection of 52.53 %. The values are shown in **Table-2**
The ulcer index in the *Piper cubeba* treated animals was found to be significantly less in all the models compared to control and standard drug treated cases. The antiulcer activity of *Piper cubeba* was, however, less than that of Omeprazole.

Table 1. Preliminary phytochemical test of *Piper cubeba* fruit methanolic extract

Sl. No	Phytochemical Tests	Results
1.	Test for Alkaloids	+
2.	Test for Carbohydrates	-
3.	Test for Steroids	-
4.	Test for Flavanoids	-
5.	Test for Glycosides	+
6.	Test for Saponins	+
7.	Test for Terpenes	+

+ Indicates the presence of compounds.
- Indicates the absence of compounds

Table-2 Effect of *Piper cubeba* fruits ethyl acetate extract on gastric volume, free acid, total acid and ulcer index in pylorus ligated rats.

Parameter	Groups								
	GroupI	GroupII	% inhibit-ion	GroupIII	% inhibit-ion	GroupIV	% inhibit-ion	GroupV	% inhibit-ion
Gastric Vol.(m1)	3.60±0.29	1.93±0.13	46.39	2.95±0.12	18.05	2.80 ±0.07	22.22	2.75 ±0.18	23.61
Free acidity (Eq/I) 100gm	40.33±4.60	4.16±0.40	89.69	23.5 ±2.26	41.73	18.33±2.90	54.54	13.33±1.62	66.94
Total acidity (Eq/I) 100gm	78.16±8.55	9.33 ±0.66	88.07	47.16±3.73	39.66	40.83±6.80	47.76	33.83±3.64	56.71
Ulcer Index	3.16±0.45	1.08 ± 0.27	65.82	2.58± 0.30	18.35	2.16±0.42	31.64	1.5 ± 0.38	52.53

Values are mean ± SEM of 6 animals in each group; **P*<0.05 compared with respective control group

DISCUSSION

Peptic ulcer disease (PUD) encompassing gastric and duodenal ulcers is the most prevalent GIT disorder that affects a considerable number of people in the world 10 and some authors consider gastric ulcer a as the new "plague" of 21st century[11]. The defense potential of mucus perimeter of gastric mucosa depends upon a delicate balance between the processes affecting the synthesis and secretion of its mucin constituents. To regain the balance, different therapeutic agents including plant extracts are used to inhibit the gastric acid secretion or to encourage the mucosal defense mechanisms by increasing mucus production, stabilizing the surface epithelial cells, or interfering with the prostaglandin synthesis thus the primary therapeutic approach of an antiulcer agent involves maintenance of a delicate balance of factors controlling the synthesis, secretion and breakdown of its proteins, glycoproteins, and lipid components, so as to strengthen the mucosal integrity [12].

In the present study the reduction in ulcer index shows the ability of the extract either to protect the gastric mucosal (cytoprotective) against ulceration or may be suppression of already established ulcers.

CONCLUSION

In the present study *Piper cubeba* showed prevention of gastric lesions was evaluated in pyloric-ligated rats. *Piper cubeba* was found to increase the mucous and decrease the acid volume, free and total acid contents in rats. Piper cubeba treatment affects the parameters that influence the initiation and perpetuation of ulceration.

Thus the results suggest that the methanolic extract of *Piper cubeba* has a potential antiulcer effect.

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