

ANTI-ULCER ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *PIPER BETLE* LEAF ON EXPERIMENTAL ANIMALS

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ABSTRACT

Objective: The main objective of this study is to establish the anti-ulcer activity of hydroalcoholic extract of *Piper betle* leaf on experimental animals based on previously existing aspects such as antioxidant, antihistaminic, and antimicrobial properties of *P. betle* leaf.

Methods: The leaves were collected, shed dried, and extracted by the Soxhlet apparatus using 70% ethanol. The anti-ulcer activity of the extract was evaluated in albino Wistar rats employing pyloric ligation and stress-induced antiulcer models. Ranitidine was served as a standard drug in both the models. The significance of activity was assessed using a one-way analysis of variance followed by Dunnett's post-parametric test.

Results: In the pyloric ligation model, the untreated control has shown 4.3 mEq/l of acidity, whereas the ranitidine-treated standard group shown 2 mEq/l and *P. betle* has shown 2.5 mEq/l acidity, respectively. In the stress-induced antiulcer model, the activity was more prominent, in the untreated control, there was 26 number of sores present, whereas the standard group showed only one number of ulcer sore, and in the *P. betle* treated group, there was four number of ulcer sores present.

Conclusion: In the present study, *P. betle* exhibited potent antiulcer potential while compared with the untreated control and the activity is comparable with standard ranitidine. From the above findings, it can correlate the use of betel leaf as a digesting or gastroprotective agent.

Keywords: Antiulcer, *Piper betle*, Pyloric ligation, Stress-induced model.

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INTRODUCTION

An ulcer is one of the major gastrointestinal disorders, which occurs due to an imbalance between offensive (gastric acid, pepsin, bile, and oxidative stress) and defensive (mucus, prostaglandin, and blood flow) factors [1]. An ulcer in the stomach is known as gastric ulcer and when it appears in the first part of the intestine is called duodenal ulcer [2]. Common causes which are responsible for peptic ulcer are *Helicobacter pylori* [3], NSAIDs [4], tobacco smoking [5], Crohn's disease [6], etc. It shows various symptoms such as upper abdominal pain, belching, and vomiting with some complications such as bleeding, perforation, and blockage of stomach. The medication used to either decrease acid secretion (proton-pump inhibitor, H₂ blocker, and prostaglandin analog) or neutralize the acid secreted in the stomach (antacids). Stop smoking, alcohol, and NSAID consumption help to heal the ulcer rapidly [7,8].

Nowadays, ulceration constitutes a major problem in a vast developing country like India. According to the latest WHO data published in May 2014, 0.96% or 85,487 deaths are occurring by peptic ulcer disease. In the case of ulceration, India gets the 26th rank in the world for the death rate [9].

In recent years, people show interest in using natural products other than conventional drugs, which exert more adverse effects side by its desired effect. *Piper betle* is a heart-shaped leaf belonging to the family Piperaceae. Betel leaf is frequently consumed in Asia with areca nut and/or tobacco [10]. *P. betle* exhibits lots of medicinal activities such as antioxidants [11], antihistaminic [12], and antimicrobial [13] which are scientifically proven. Conventionally, betel leaf also used to treat stomach problems including gastric ulcer, but relevant scientific data are not available. A drug possesses three important activities such as antioxidant, antihistaminic, and the antimicrobial is obvious to have antiulcer activity because oxidative stress is initiate and aggravates

the peptic ulcer [14], and antihistaminic blocks histamine release and prevents ulcer production. In this study, there is an attempt to investigate the reason behind the traditional uses of *P. betle* leaves as gastroprotective agents.

MATERIALS AND METHODS

Animal

Albino Wistar rat weighing 150–200 g either sex is used for the study in different models and placed in polypropylene cages (32 cm×24 cm×16 cm). The animals were purchased from an authorized animal breeder. The animals were kept in CPCSE approved NSCBIP animal house (approval no: 1502/PO/a/11/CPCSEA), well maintained under standard hygienic conditions, at a temperature (22±2°C), 65% relative humidity, and 12-h light and dark cycle. Commercial food pellets and tap water *ad libitum* were provided. All experiments were conducted between 10 am and 6 pm.

Plant extract

Leaves of *P. betle* were collected and the specimen was authenticated by the botanist. The leaves were washed gently with fresh water and shed dried. Leaves were made coarse by hand crush and subjected to Soxhlet extraction using 70% ethanol for consecutive 48 h. The extract was dried using desiccators.

Preparation of drug solution

The extract was dissolved in distilled water to prepare a stock solution of 150mg/kg. Ranitidine was dissolved in distilled water to prepare the solution 20 mg/kg.

Chemicals and drugs

Formalin, ethanol, sodium hydroxide (NaOH), and hydrochloric acid were obtained from Loba Chemie Pvt., Ltd., while ranitidine (Rantac (R)

150 mg by JB Chemicals and Pharmaceutical Ltd.), diethyl ether was purchased from Merck Specialities Pvt. Ltd., Mumbai.

Acute oral toxicity studies

The acute toxicity study was performed as per the OPPTS guideline applying up and downhill methods [15].

Preliminary phytochemical investigations of the extracts

Hydroalcoholic extracts of *P. betle* leaf were evaluated for the phytochemical investigation to check the presence of various phytoconstituents such as alkaloid, carbohydrates, saponin, flavonoids, glycosides, tannins, and proteins [16].

Experimental procedures

Antiulcer activity by pyloric ligation

Albino Wistar rats were divided into three groups, each group contains six animals. Animals fasted for 24 h. The 1st group received normal saline 2 ml/kg (control), the 2nd group received ranitidine 20 mg/kg by oral route (standard), and the third group received a hydroalcoholic extract of *P. betle* (150 mg/kg) by the oral route, 30 min before to pyloric ligation. Animals were sacrificed 4 h later and the stomach was opened to collect the gastric content. The total volume of gastric content was measured [17]. Centrifugation of gastric content was done at 1000 rpm for 10 min. About 1 ml of the supernatant liquid was pipette out and diluted to 10 ml with distilled water. The solution was titrated against 0.01 N NaOH using phenolphthalein as an indicator, to the endpoint when the solution turned an orange color. The volume of NaOH needed was taken according to the free acidity [18,19]. Titration was further continued until the solution regained a pink color. The volume of NaOH required was noted and was taken as corresponding to the total acidity. Acidity was expressed as follows [20]:

$$\text{Acidity} = \frac{\text{Volume of NaOH normality } 100\text{mEq/L}}{0.1}$$

Stress - induces antiulcer activity by water immersion model

The procedure for inducing ulcers with the water immersion stress-induced ulcer model includes animals being fasted for a period of 24 h before the experiment. The animals were divided into three groups each containing six number of animals. The 1st group received normal saline 2 ml/kg (control), the 2nd group received ranitidine 20 mg/kg, and the 3rd group received hydroalcoholic extract of *P. betle* (150 mg/kg) by oral route, after 30 min of the treatment, the animals were placed individually in a restricted cage and immersed them in water tank (20°C–23°C) for 4 h. After 4 h, the animals were taken out from the water immersion cage and sacrificed. The stomach of each animal was isolated, mounted and the number of ulcer formation was counted for evaluation [21-23].

Statistical analysis

The experimental data were expressed as mean ± SEM for each treatment group. The significance of activity was assessed using a one-way analysis of variance followed by Dunnett's post-parametric test between the data of control and treated groups. *p<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Extract preparation

The leaves of *P. betle* were subjected to Soxhlet extraction using 70% ethanol for consecutive 48 h and 12% yield was obtained.

Acute oral toxicity studies

The acute toxicity study of the extracts obtained from the leaves of *P. betle* was performed as per the OPPTS guideline and found that 2000 mg/kg dose was safe.

Preliminary phytochemical investigation

The extracts obtained from the leaves of *P. betle* were subjected for preliminary investigation and followed that observations were found Table 1.

Antiulcer activity by pyloric ligation

The anti-ulcer activity of hydroalcoholic extract of *P. betle* was evaluated in albino Wistar rats employing pyloric ligation [Table 2]. In the pyloric ligation model, the untreated control has shown 4.3 mEq/l of acidity, whereas the ranitidine-treated standard group shown 2 mEq/l and *P. betle* has shown 2.5 mEq/l acidity, respectively [Fig. 1].

Stress-induced antiulcer activity by water immersion model

In the stress-induced antiulcer model, the activity was more prominent; in the untreated control, there was 26 number of pores present, whereas the standard group showed only one number of ulcer pore, and in the *P. betle* treated group, there was four number of ulcer pores present [Table 3 and Figs. 2 and 3].

Oxidation is a chemical reaction by which free radicals produces; these free radicals are dangerous for the living cells and tissues in our body.

Table 1: A preliminary phytochemical investigation of *Piper betle* leaves extract

S. No.	Test names	Results
1	Test for alkaloid	Positive
2	Test for carbohydrates	Positive
3	Test for steroids	Positive
4	Test for saponins	Positive
5	Test for tannins	Positive
6	Cardiac glycoside	Positive
7	Flavonoids	Positive

Table 2: Acidity index (mEq/l) in the pyloric ligation model

Groups	Acidity index (mEq/l)
Control	4.3±0.33
Standard	2±0.16**
<i>Piper betle</i>	2.5±0.31**

All values are mean±SEM, n=6, *p<0.05, **p<0.01, ***p<0.001, versus vehicle control

Table 3: Ulcer index (number of pores in the stomach) in water immersion model

Groups	Number of pores in stomach
Control	26±1.26
Standard	1±0.21***
<i>Piper betle</i>	4±0.36***

All values are mean±SEM, n=6, *p<0.05, **p<0.01, ***p<0.001, versus vehicle control

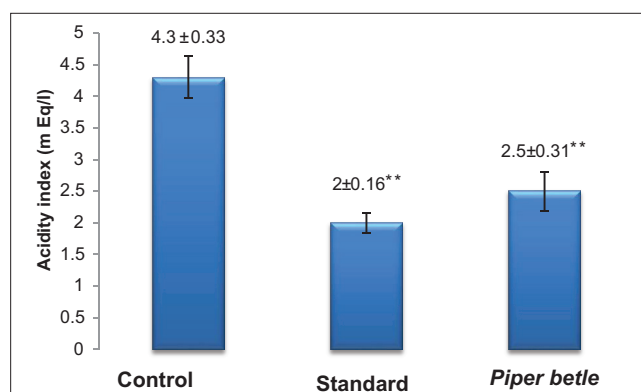


Fig. 1: Acidity index (mEq/l) in pyloric ligation model. All values are mean±SEM, n=6, *p<0.05, **p<0.01, *p<0.001, versus vehicle control**

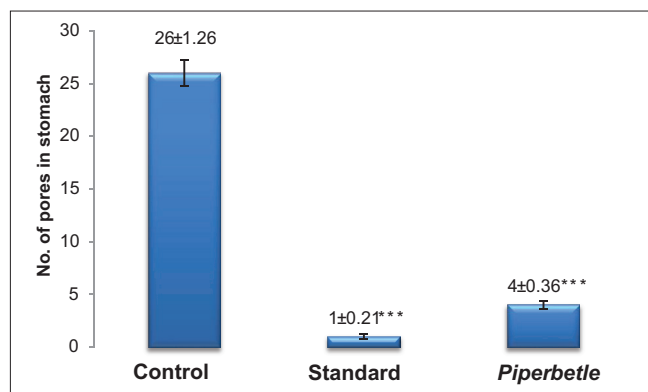


Fig. 2: Ulcer index (number of pores in the stomach) in water immersion model. All values are mean ± SEM, n=6, *p<0.05, **p<0.01, *p<0.001, versus vehicle control**

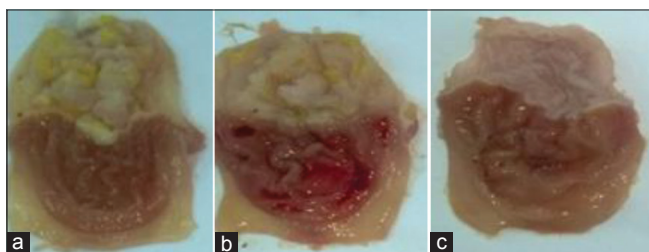


Fig. 3: Sample of dissected stomach and formation of an ulcer in the stress-induced antiulcer model. (a) Control, (b) standard (ranitidine), (c) Piper betle

A number of conditions such as ulcer, cancer, erosion, and aging are produced due to overloading of free radical, any compound, or drug which can scavenge these free radicals are called as an antioxidant and use to treat the number of ailments.

Any inflammatory condition uses to get worse in the presence of microbial load. *H. pylori*, Gram-negative bacteria came to the focus in 1982 and found responsible for worsening of the ulcerative condition.

Physiologically, histamine plays a major role in acid secretion in the parietal cell of the stomach. In any pathological condition, where histamine gets overactive, threats to create an ulcer.

The antioxidant, antimicrobial, and antihistaminic activities of *P. betle* are already scientifically established. The drug which shows antioxidant activity may also produce healing activity in the majority of the cases irrespective of open wound or ulcerative wound [24].

Antihistaminic is the type of drug that most responsible for ulcer protective action by inhibiting the histamine secretion from the parietal cell of the stomach. Antimicrobials are a very good choice of coprescription in the treatment of ulcers, which acts by its cidal or static action on microbes. Hence, a drug that already has the above said three major activities obvious to show its antiulcer or gastroprotective activity.

CONCLUSION

P. betle leaves were collected and extracted with 70% hydroalcoholic mixture and subjected for antiulcer activity using two experimental models, namely pyloric ligation model and stress-induced antiulcer model. The extract of *P. betle* has shown significant antiulcer activity in both the models. Its protective mechanism of the antiulcer effect probably due to numerous phytochemicals presents in the extract. This leaf can be encouraged for further evaluation which may expect

a new promising herbal drug in the treatment of ulcer and related disorders.

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AUTHORS' CONTRIBUTIONS

All authors are equally contributed.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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