EVALUATION OF ANTIULCEROGENIC POTENTIAL OF *PIPER BETEL* LEAVES EXTRACT AS AN ADJUVANT THERAPY WITH OMEPRAZOLE AGAINST STRESS ULCERS IN RATS

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Summary

In Indian traditional system of medicine, the leaves of *Piper betel* are recommended for the management of peptic ulcer. In light of this, the present investigation was carried out to study the antiulcerogenic potential of *Piper betel* leaves extract as an adjuvant therapy with omeprazole against stress ulcers in rats. The rats were divided into five groups with six rats in each group. Group 1 animals were treated with vehicle (distilled water, 1 ml/kg, p.o) for 7 days and served as control group. Rats in group 2, 3 and 4 were treated with *Piper betel* extract (200 mg/kg, p.o) for 7 days along with omeprazole 10, 15 and 20 mg/kg, p.o, respectively, 24 hours prior to the evaluation while rats in group 5 received omeprazole 20 mg/kg, p.o. 24 hours prior to the evaluation . For the induction of ulcers, the rats were fasted for 24 hours, followed by 4 hours of cold stress in deep freeze at 4°C.

The results showed a significant ($p<0.01$) antiulcerogenic potential of *Piper betel* leaves extract (200 mg/kg, p.o) as an adjuvant therapy with omeprazole (15 and 20 mg/kg, p.o). Furthermore, the dose of 15 mg/kg of omeprazole along with 200 mg/kg of *Piper betel* leaves extract showed similar antiulcerogenic level of significance as those produced by omeprazole 20 mg/kg alone. These results support our hypothesis of adjuvant role of *Piper betel* leaves extract with omeprazole for its ulcer protective activity. To conclude, the co-administration of *Piper betel* leaves extract with omeprazole lowers the effective dose of omeprazole and consequently will reduce its dose dependent side effects to achieve better pharmacotharapy.

Keywords: Adjuvant therapy, antiulcerogenic, omeprazole, *Piper betel*, stress ulcer.

Introduction

Peptic ulcer is one of the major gastro-intestinal disorders, which mainly occur due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors (1). It is a lesion of gastric or duodenal mucosa occurring at a site where the mucosal epithelium is exposed to aggressive factors (2). Potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products and drugs have been implicated in the pathogenesis of gastric ulcer, including increased gastric acid and pepsin secretion, decreased gastric blood flow, the suppression of endogenous generation of prostaglandins, inhibition of mucosal growth and alteration of gastric mobility (3).

Various synthetic antiulcer drugs presently available in market includes antacids, proton pump inhibitors, anticholinergics, H_2-receptor antagonists and cytoprotective agents, used to prevent and treat various types of ulcers (4). Reduction of gastric acid production as well as re-inforcement of gastric mucosal production has been the major approaches for current therapy of peptic ulcer disease (2). The high recurrence rate which in turn increases financial burden and mental stress to the patient is major hurdle of aforementioned therapy (5). In addition, these drugs confer simpler to severe side effects ranging from diarrahoea, itching and dizziness to arrhythmia, impotence and gynaecomastia (6-8). These conditions may be more worsen when they exhibit certain drug-drug interactions, which may reduce the overall outcome of the therapy (9).

The role of traditional medicines in the solution of health problems is invaluable on a global level. Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine (10). In recent years, there has been growing interest in alternative therapies and the use of natural products, especially those derived from plants since, medicinal plants are among the most attractive sources of new drugs and have been shown to produce promising results for treatment of various diseases and disorders including gastric ulcer (9). With the associated side effects of the modern medicine, traditional medicines are gaining importance and are now being studied to find the scientific basis of their therapeutic actions (11).

The *Piper betel* Linn. (Piperaceae) is found widely growing in the tropical humid climate of South East Asia, and its leaves, with a strong pungent and aromatic flavor, are widely consumed as a mouth freshner (12). The roots and fruits of Piper betel are also used for different ethnobotanical purposes (13, 14). The leaves are further reported to possess antioxidant (15), antidiabetic (16), nootropic (17), anti microbial, antifungal, wound healing, hepatoprotective, anti-inflammatory, antifertility activity in male rats and anti

motility effects on washed human spermatozoa (15, 18). It had also shown inhibitory activity against photosensitization induced damage to lipids and proteins (4).

Leaves of *Piper betel* are claimed to possess antiulcer activity according to the Indian traditional system of medicine, though it has not been scientifically documented. Moreover, there is evidence concerning the participation of reactive oxygen species in the etiology and pathologenesis of digestive system disorders such as gastrointestinal inflammation and gastric ulcer (19). Since *Piper betel* has been documented for its nootropic (17) and antioxidant activities (15), it could be considered as effective and better agent for antiulcer activity, as nootropics sometimes act by their ability to scavenge the free radicals, hence supporting their use for ulcerprotection. Current drugs for antiulcer therapy like omeprazole are symptomatic and confer various side effects, suggesting a need of alternative therapy. Consequently the aim of the present study was to evaluate the antiulcerogenic potential of *Piper betel* leaves extract as an adjuvant therapy with omeprazole against stress ulcers in rats.

Materials and Methods

Plant material:
The plant material collected by Professor John and authenticated by society health environment research on biodiversity at Pondecheri was used. The hydroalcoholic extract of *Piper betel* leaves prepared by the following procedure was received as gift sample (PBT-4031) from Green Chem., Bangalore, India.

Preparation of extract:
Leaves were extracted with 50% aqueous alcohol and concentrated. The concentrated mass was washed with petroleum ether several times to remove the resinous matter. Then the mass was diluted with 25% aqueous alcohol, filtered and concentrated, dried to get the powdered form of the extract.

Preparation of drug solution:
Accurately weighed quantity of powdered extract was dissolved in the distilled water to prepare the appropriate solution of the drug i.e. 200 mg/ml. The doses were administered orally. Omeparazole was dissolved in distilled water to prepare the solution of 10, 15 and 20 mg/ml.

Chemicals and drugs:
Omeparazole (Omez, Dr. Reddy's Labs), was purchased from local market.

Animals:
Healthy male and female wistar rats (120-150g) and male swiss albino mice (18-22 g) were obtained from Yash Farms, Pune and were housed in CPCSEA approved animal house of AISSMS COP, Pune in groups of six in polypropylene cages. They were maintained at 25 ± 2°C, relative humidity of 45 to 55% and under standard environmental conditions (12 hrs light 12 hrs dark cycle). All the animals were acclimatised for 10 days to the animal house conditions prior to the start of experimental protocol with free access to food (Amrut feed, Chakan Oil Mills, Pune, India) and water *ad libitum*. They were fasted for 24 hours as per protocol with water *ad libitum* prior to experiment. All the procedures were performed in accordance with the Institutional Animal Ethical Committee (IAEC) constituted as per the directions of the CPCSEA. All experiments were carried out between 12:00-16:00 hours.

Acute toxicity test:
Acute toxicity study was performed in healthy adult male albino mice (18-22 g) as per guidelines (AOT 425) suggested by the Organisation for Economical Co-operation and Development (OECD). The mice were observed continuously for two hour for behavioral and autonomic profiles and for any other sign of toxicity or mortality up to a period of seven days.

Ulcer Index:
Ulcer index was determined by following the scoring method of Suzuki et al (20).
Score 1: maximal diameter of 1mm.
Score 2: maximal diameter of 1-2 mm.
Score 3: maximal diameter of 2-3 mm.
Score 4: maximal diameter of 3-4 mm.
Score 5: maximal diameter of 4-5 mm.
Score 10: an ulcer over 5mm in diameter.
Score 25: a perforated ulcer.

Experimental procedures:
The rats of either sex were divided into five groups with six rats in each group. Group 1 animals were treated with vehicle (distilled water, 1 ml/kg, p.o) for 7 days and served as control group. Rats in group 2, 3 and 4 were treated with *Piper betel* extract (200 mg/kg, p.o) for 7 days along with omeprazole 10, 15 and 20 mg/kg, p.o, respectively, 24 hours prior to the testing while rats in group 5 were treated with omeprazole 20 mg/kg, p.o. 24 hours prior to the evaluation. Rats were deprived of food, but not water, for 24 hours prior to the experiment. For the induction of ulcers, on 7^{th} day, 1 hour after the respective treatments, all the animals were subjected to cold stress by keeping them in a deep freeze at 4 °C for 4 hours. After 4 hours, animals were sacrificed by cervical dislocation and their stomachs were dissected out and cut open along the greater curvature. The length in mm of each lesion was measured and the ulcer index was calculated using aforementioned method (20, 21).

Statistical analysis:
The results are expressed as mean ±SEM. The statistical analysis of all the results was carried out using one way ANOVA followed by Dunnetts 't' test using graph pad instat 3.

Results

Acute toxicity test:

All mice were free of any toxicity as per acceptable range given by the OECD guidelines up to the dose of 2000 mg/kg. From these data and pilot study reports; 200 mg/kg was selected for further study.

Stress induced ulcers:

Subjecting the animals to 4 hours of cold stress in deep freeze at 4 °C was sufficient to induce the ulcers. The ulcer index of vehicle control group was found to be 48.33 ± 1.978. The ulcer index of group 3 and group 4, treated with *Piper betel* 200 mg/kg, in adjuvant therapy with omeprazole 15 and 20 mg/kg respectively, was significantly ($p<0.01$) reduced when compared against vehicle treated control group. Omeprazole at 20 mg/kg dose produced highest inhibition of ulcers. The ulcer inhibiting capacity of *Piper betel* (200 mg/kg) as an adjuvant therapy with omeprazole 15 mg/kg was found to be similar as that of omeprazole (20 mg/kg) alone (Table 1).

Table 1: Effect of co-administration of hydroalcoholic extract of *Piper betel* (PB) and omeprazole (Omez) on cold stress induced ulcers

Sr. No	Treatment (p.o.)	Ulcer index (mm^2)	% ulcer inhibition
1	Vehicle control	48.33 ± 1.978	-----
2	Omez 10 mg/kg + PB 200 mg/kg	42.16 ± 1.078 *	12.67%
3	Omez 15 mg/kg + PB 200 mg/kg	21.83 ± 0.600 **	54.84%
4	Omez 20 mg/kg + PB 200 mg/kg	21.33 ± 1.606 **	55.87%
5	Omez 20 mg/kg	19.00 ± 1.483 **	60.69%

Values are expressed as mean±SEM, n=6, Data was analysed by one way analysis of variance (ANOVA) followed by Dunnetts 't' test. *P<0.05, **P<0.01.

Discussion

The present investigation revealed a significant antiulcerogenic effect of hydroalcoholic extract of leaves of *Piper betel* as an adjuvant therapy with omeprazole against stress ulcers in rats.

Ulcer mainly results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defense mechanism (22). To regain the balance, different therapeutic agents including herbal preparations are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanism by increasing mucus production (23).

Stress plays an important role in aetiopathology of gastroduodenal ulceration. Stress induced ulcers are probably mediated by histamine release with enhancement in acid secretion and a reduction in mucus production (1). Increase in gastric motility, vagal overactivity (24), mast cell degranulation (25), decreased gastric mucosal blood flow (26) and decreased prostaglandin syntheses (27) are involved in genesis of stress-induced ulcers (28). It is also hypothesized that free radicals may play a major role in stress involved gastrointestinal injury. The dose 200 mg/kg of *Piper betel* has been selected since it was found to be most effective in nootropic studies of this drug (17), which are further linked with stress and its management.

Stress is the body's physical, mental or chemical reaction mainly observed during excitation, confusion or in unsafe condition. A large proportion of all illness is believed to occur due to stress that is closely associated with modernization of life. This increasing stress ultimately leads to stress ulcers. Also, the high recurrence rate even after complete healing of the ulcer is major hurdle of current therapy (29, 30). In addition, these drugs confer simpler to severe side effects ranging from diarrahoea, itching and dizziness to arrhythmia, impotence and gynaecomastia (31-35). The condition may be more worsen when it exhibit certain drug-drug interactions that may reduce the overall outcome of the therapy.

In our study, the dose of 15 mg/kg of omeprazole along with 200 mg/kg of *Piper betel* leaves extract showed similar antiulcerogenic level of significance as those produced by omeprazole 20 mg/kg alone. These results support our hypothesis of adjuvant role of *Piper betel* leaves extract with omeprazole for its ulcer protective activity. Accordingly, the protective action of *Piper betel* leaves extract against stress induced ulceration could be due to its histamine antagonistic and free radical scavenging effects, suggesting its ability to inhibit the gastric prostaglandin depletion and/or decrease of the acid secretion in the mucosa.

Conclusion

To conclude, the co-administration of *Piper betel* leaves extract with omeprazole lowers the effective dose of omeprazole and consequently will reduce its dose dependent side effects to achieve better pharmacotharapy. Hence *Piper betel* leaves extract could be used as an adjuvant therapy with omeprazole against stress ulcers.

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