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Antiulcer Activity of Black Pepper against Absolute Ethanol Induced Gastric Mucosal Damage in Mice

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ABSTRACT

Piper nigrum Linn. (Piperaceae) known as black pepper has long been used in folk medicine as stomachic, aromatic, stimulant, diaphoretic and hepatoprotective. Black pepper stimulates taste buds causing reflex stimulation gastric secretions, improving digestion and thus used in the treatment of gastrointestinal upsets and flatulence. Therefore, present study was designed to investigate the gastroprotective effects of pet ether extract of *Piper nigrum* (PEPN) against absolute ethanol induced gastric mucosal damage in mice. The PEPN 200 mg/kg, orally, once daily, was administered to mice for 14 days. Ranitidine (80 mg/kg, oral.) was used as a standard drug. The severity of gastric mucosal damage induced by absolute ethanol (99%) was analyzed in terms of ulcer index value. The anti-lipid peroxidative activity of PEPN and ranitidine in gastric mucosa was also evaluated. Administration of PEPN for 14 days to mice, significantly decreased the ulcer index value when compared to saline control treated animals in ethanol induced gastric ulcer model. Ranitidine (80 mg/kg, oral) also produced a significant decrease in ulcer index when compared with the saline control treated group. However, the anti ulcer activity of ranitidine was higher than that of PEPN. Also a significant decrease in thiobarbituric acid reacting substances (TBARS) was found following 14 days pretreatment with PEPN. Thus, it can be concluded that *Piper nigrum* has anti ulcer activity, which can be attributed to its anti-oxidant mechanism of action.

KEYWORDS: Anti-ulcer; Anti-oxidant activity; Lipid peroxidation; *Piper nigrum;* TBARS.

INTRODUCTION

Peptic ulcer being one of the most rampant gastrointestinal disorders continues to occupy the key position in concern of both, clinical practitioners and researchers. As a consequence, many drugs are being searched for, offering newer and better options for treatment of peptic ulcer. However, the type of drug differs from being a H_2 receptor antagonists, proton pump inhibitors or cytoprotective agents such as sucralfate. Unfortunately, most of these drugs confer simpler to severe side effects like arrythmias, gynaecomastia, enterochromaffin like cell (ECL) hyperplasia and hematopoietic changes (1). Thus, there is an urgent need to search an indigenous drug with fewer side effects to have a better and safer alternative for the treatment of peptic ulcer. In this context, extensive studies and research has been undertaken which mainly focuses on search of anti-ulcer agents of plant origin. The participation of reactive oxygen species in the etiology and pathologies of human diseases, such as neurodegenerative disorders, inflammation, viral infections, autoimmune pathologies and digestive system disorders such as gastrointestinal inflammation and gastric ulcer has been reported earlier (2). Several studies have shown alterations in the anti-oxidant status following ulceration, implying that free radicals may be associated with ethanol induced gastric mucosal damage in rats (3-4). Drugs with multiple mechanisms of protective action, including antioxidant properties, may be beneficial in minimizing tissue injury in human disease (5). Black pepper (*Piper nigrum* Linn.), the king of spice, is one of the oldest and most popular spice in the world. It belongs to family Piperaceae and used in many Asian countries as a stimulant, for the treatment of colic, rheumatism, headache, diarrhoea, dysentery, cholera, menstrual pain, removing excessive gas from gastrointestinal tract and increasing flow of urine. It is used in folk medicine for stomach disorders, digestive problems, neuralgia and scabies. Its active constituent, piperine has been investigated to reduce liver damage in rats (6). The methanolic extract of *P. nigrum* fruits has hepatoprotective and antioxidant effects in rats (7). Additionally its pet ether extract has been investigated for *in vitro* antioxidant activity (8). Hence, present study was aimed to evaluate the anti ulcer activity of pet ether

extract of *Piper nigrum* (PEPN).

MATERIALS & METHODS

Plant material

Piper nigrum Linn. fruits (1 Kg) were collected in the month of August, 2007 from Kottayam, Kerala, India. The plant materials were identified and authenticated by Dr. N N Sharma, taxonomist, Sri Sai Institute of Pharmaceutical Education and Research (SSIPER), Badhani, Pathankot, Punjab, India. A voucher specimen (SAI-77) of the collected plant material was also deposited in the Department of Pharmacognosy, SSIPER for future reference. The collected materials were washed thoroughly in water, air dried for a week at 35-40^{o}C and pulverized in electric grinder. The powder obtained was extracted in pet ether by cold extraction at room temperature for 72 hours.

Animals and drug administration

Healthy Swiss albino mice of either sex weighing 20-30g were selected for the study. The animals were housed and fed with standard diet and water *ad libitum*. The animals were acclimatized for 5 days before starting the experiment. Procedures involving animals and their care were conducted in conformity with Committee for the Purpose of Control and Supervision of Experiments on Animals (Regd. No.911/ac/95/CPCSEA). Animals were deprived of food at least 24 hr before start of the experiment but were allowed free access to water. Coprophagy was prevented by keeping the animals in cages with grating as the floor. The mice were divided into three groups with 7 mice each. Group 1 served as saline control and was given saline (0.5 ml/kg/day oral.) for 14 days. Group 2 served as reference standard drug group and received Ranitidine (80 mg/kg/day oral.) for 14 days. Group 3 served as test drug group and received PEPN (200 mg/kg/day oral.) for 14 days.

Acute Toxicity test

The acute toxicity (LD_{50}) of the PEPN was determined in albino mice by the method of Lorke (9) using the oral route.

Anti-ulcer activity

Ethanol-induced gastric ulcer model

On day 14, absolute ethanol (0.2 ml, oral) was administered 1 hr after respective treatments. After 2 hr of absolute ethanol administration, the animals were sacrificed. Stomachs were removed, opened along the greater curvature and examined for lesion severity (10). Lesion severity was determined by measuring ulcer index. It was calculated as follows:

$$\text{Ulcer index} = \frac{10}{x}$$

Where x is total mucosal area/ total ulcerated area.

Biochemical estimation

Lipid peroxidase (LPO) activity

The extent of lipid peroxidation is estimated according to the method of Yang et al. (11), by measuring the secondary lipid peroxidation products–thiobarbituric acid-reacting substances (TBARS) formed in the lipid peroxidation processes. The glandular portion of the gastric mucosa was homogenized with cold 50mM phosphate buffer (pH 7.4) to give a 10% w/v homogenate. After 10 min, 10% Sodium dodecyl sulphate (0.1 ml), 0.1 N HCl (2 ml) and 1% Thiobarbituric acid (TBA) (1 ml) were added to 0.1 ml of the homogenate. The mixture was heated for 45 minutes on a boiling water bath. After cooling, 4 ml of butanol was added and mixed vigorously. The butanol phase was separated by centrifugation and the absorbance was measured using UV/VIS spectrophotometer (DU 640i, Beckman) at 532 nm. The lipid peroxidation is expressed as nanomoles of thiobarbituric acid reacting substances per 100 mg of wet tissue. TBARS values were calculated using a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ at 532 nm.

Statistical analysis

Results are represented as mean $\pm$ standard error of mean. Statistical difference between the means of the various groups was analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Data were considered statistically significant at $P<0.001$.

RESULTS

Ethanol induced gastric ulcer

The acute toxicity test for PEPN in mice was conducted in oral dose range of 100 mg/kg to 5000 mg/kg and and LD_{50} of 4012 mg/kg was established. In the present study, PEPN was evaluated for its anti-ulcer activity against ethanol-induced gastric ulcer in mice; the results are shown in Figure 1. Oral administration of ethanol produced severe ulceration. PEPN as well as standard drug ranitidine significantly reduced the incidence and severity of ulceration in ethanol induced ulcer model.

Anti oxidant activity by Lipid peroxidase (LPO) estimation method.

The extent of lipid peroxidation as evidenced by the formation of TBARS in the gastric mucosa of animals in each group is represented in Figure 2. Significantly lower ($p<0.001$) levels of TBARS was observed in PEPN and ranitidine group compared to saline treated control group.

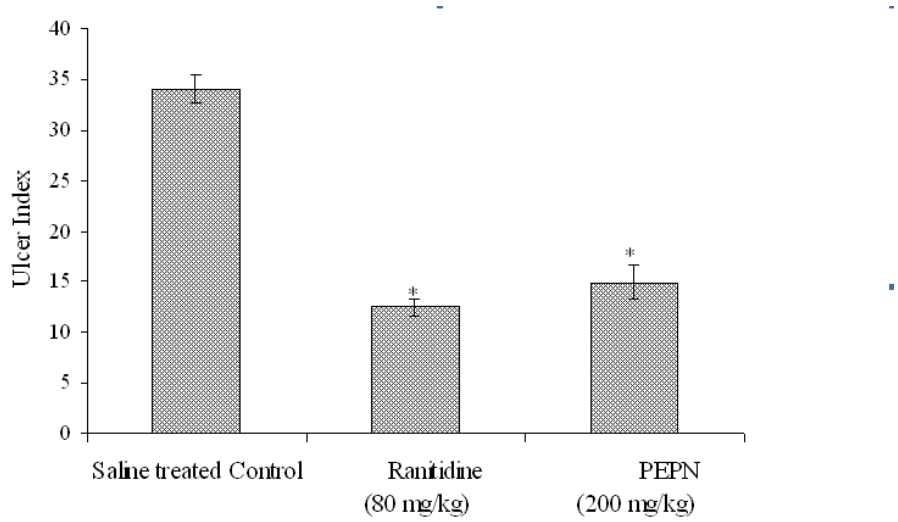

*Figure 1. Effect of pet ether extract of Piper nigrum (PEPN) (200mg/kg, oral/14 days) and ranitidine (80 mg/kg, oral/14 days) in ethanol-induced gastric mucosal damage in mice. Results represent mean ± S.E.M., n=21. Statistical analysis was done by one-way ANOVA followed by Dunnett's test. * Significantly different from saline treated control group (p<0.001).*

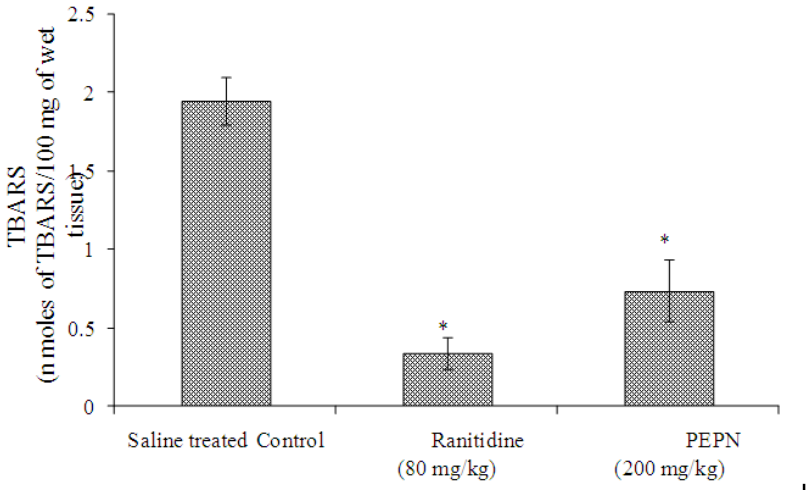

*Figure 2. Effect of pet ether extract of Piper nigrum (PEPN) (200 mg/ kg, oral/14 days) and ranitidine (80 mg/ kg, oral/14 days) on the level of TBARS in ethanol-induced gastric mucosal damage in mice. Result are mean ±S.E.M., n= 21. Statistical analysis was done by one-way ANOVA followed by Dunnett's test. * Significantly different from saline treated control group (p<0.001).*

DISCUSSION

The present study discussed that *Piper nigrum* exhibits both gastroprotective and ulcer healing properties, attributed to the anti-oxidant effect of the drug. Although in most of the cases, the etiology of ulcer is unknown, it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defense mechanism (12). To regain the balance, different therapeutic agents including herbal preparations are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucus production. *Piper nigrum* is an herbal plant, which is mentioned in Indian system of Medicine (Ayurveda) for its remedial properties. The anti-ulcer effect of *Piper nigrum* was tested against gastric lesions induced by ethanol, the experimental model related to lesion pathogenesis with production

of reactive oxygen species. Reactive oxygen species are involved in the pathogenesis of ethanol-induced gastric mucosal injury *in vivo* (3). *Piper nigrum* prevented the mucosal lesions induced by ethanol. Results in the present study also indicate similar alterations in the anti-oxidant status after ethanol-induced ulcers. Lipid peroxidation is a free radical mediated process, which has been implicated in the variety of disease states. It involves the formation and propagation of free radicals, the uptake of oxygen and rearrangement of double bonds in unsaturated lipids, which eventually results in destruction of membrane lipids. Biological membranes are often rich in unsaturated fatty acids and bathed in oxygen-rich metal containing fluid. Therefore it is not surprising that membrane lipids are susceptible to proxidative attack (13). This study has revealed a significant decrease in lipid peroxidation by *Piper nigrum* in the experimental model, which suggests its gastro-protective effect. Thus *Piper nigrum* is an effective antiulcer agent. Further, this study also demonstrates that the anti-ulcer activity of *Piper nigrum* is attributed to its anti-oxidant property. However, further studies employing other experimental models eg: gastric cytoprotection studies, gastric acid secretion studies etc. are under investigation, which would throw more light on the exact mechanism of anti-ulcer effect of *Piper nigrum*.

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