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Inhibition of Gastric Mucosal Damage by Piper Nigrum (Black pepper) Pretreatment in Wistar Albino Rats

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Inhibition of Gastric Mucosal Damage by Piper Nigrum (Black pepper) Pretreatment in Wistar Albino Rats

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Abstract. The fruits of Piper nigrum Linn (Black pepper) are used as a spice in the intercontinental food preparation and have many therapeutic applications in the folk medicine. The present study on the ulcerogenic and/or anti-gastric ulcer activity of pepper was undertaken in view its large scale uses and a paucity of any systematic study. The investigation included (i) the effect of pepper (250 and 500 mg/kg) on normal gastric mucosa and (ii) The effect of Black pepper pretreatment on gastric mucosal injuries caused by different necrotizing agents (ethanol, NaCl, NaOH and indomethacin), pylorus ligation-accumulated gastric acid secretions, ethanol-induced changes in gastric mucus secretions, levels of non-protein sulfhydryl groups (NP-SH) and histopathological changes was investigated in rats. The results of preliminary experiments on the effect of Black pepper (alone) revealed lack of any effect. Pretreatment with Black pepper (250 and 500 mg/kg) was found to inhibit the ulcers induced by different necrotizing agents. It prevented the increase of gastric acid secretions, depletion of stomach wall mucus and prevented the histological changes caused by ethanol, however, there was no effect on the concentration of NP-SH in the gastric mucosa. The exact mechanism of protection is not clear, nevertheless, it might be related to the stimulation of bioenergetic processes in the gastric epithelium under the influence of piperine (the active constituent of Black pepper) on coenzyme Q10.

Key words: Black pepper, Necrotizing agents, Gastric mucosal injuries, Protection,

Introduction

The fruits of Piper nigrum Linn., family Piperaceae, constitute the Black pepper, which is a popular spice. Black pepper is commonly used as a spice in preparation of intercontinental food and is used in the folk medicine as a carminative, stimulant and stomachic in dyspepsia and flatulence. The herbal physicians prescribe Black pepper against the treatment of cholera, malaria, bacterial infection, paraplegia and arthritic diseases. As a tonic, it is used to treat weakness following fevers, vertigo and coma. Externally, it is used as a rubifacient and as a local application for treatment of sore-throat, piles and skin diseases (1-3).

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In view of the folkloric importance, Black pepper became the focus of a wide range of experimental research. It is found to possess diaphoretic, diuretic and lipolytic properties (4). In mice, Black pepper was reported to protect against the audiogenic seizures and convulsions produced by N-methyl-DL-aspartate and maximal electroshock (5). Black pepper and its volatile oils are demonstrated to possess antioxidant potentials (6,7).

Clinical studies on Black pepper revealed significant increases in parietal secretion, pepsin secretion and potassium loss (4). These reports are contrary to the reported folkloric use of Black pepper as a carminative, stimulant and stomachic (1-3). Nevertheless, our preliminary experiments on the ulcerogenic activity of Black pepper failed to demonstrate any drastic changes in the gastric mucosa or body weight in rats, even upto a dose of 10 gm/kg. The present investigation on the ulcerogenic and the antigastric ulcer activity was undertaken in view of: (i) the world-wide consumption of Black pepper as a spice (ii) its importance in the folk medicine (iii) reported discrepancies in the literature and (iv) our preliminary experiments on the ulcerogenic activity.

Materials and methods

Plant material

Dried fruits of Black pepper were purchased from local market in Riyadh, identified by our taxonomist Dr. Atiqur Rahman (College of pharmacy, King Saud University). A voucher specimen was preserved at the herbarium of College of Pharmacy for future reference. The dried fruits were ground to a very fine powder in a Sanyo electric grinder and the suspension was made with water. The aqueous suspension was used for treatment in different experiments.

Animals

Male Wistar albino rats, aged 8-10 weeks, weighing about 150-200 g. were obtained from the Experimental Animal Care Centre, King Saud University, Saudi Arabia. The animals were maintained under standard conditions of temperature (24 ± 2), humidity (60%) and light (12 hr dark, 12 hr light). They were provided with Purina chow and free access to water. Before testing, the animals were fasted for 36 hours with access to water ad libitum. The experimental protocols were approved by the Ethics Committee of the Experimental Animal Care Society, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia, in accordance with the guide to the care and use of experimental animals (Canadian Council) (8).

Dose selection and route of administration

The doses (250 and 500 mg/kg) selected for the conduct of the experiments were based on preliminary experiments conducted on the pharmacological activity of Black pepper. The route of administration of the aqueous (water) suspension was oral (gastric intubation) in all the experiments.

Gastric lesions induced by necrotizing agents

The animals in the test groups were orally administered 1 ml per rat of different necrotizing agents (80% ethanol, 0.2 M NaOH and 25% NaCl), which are known to produce gastric lesions (9,10). NaCl (25%) and NaOH (0.2 M) were used only in cytoprotection studies. Based on the gastric emptying in fasted rats, Black pepper was given 30 min before the necrotizing agents. Animals were sacrificed under ether anaesthesia 1 hr after treatment with ulcerogenic agents. The stomach was excised and opened along the greater curvature. After washing with normal saline, the gastric lesions were quantified using a binocular magnifier. The ulcers were scored according to the method of Valcavi et al., (11) and assessed on the basis of their circumference: Deep circular more than 8 mm = 9-10; 7-8 mm = 8; 6-7 mm = 7; (if a ulcer is 6 mm then it get a score of 7 or 6) 5-6 mm = 6; 4-5 mm = 5; 3-4 mm = 4; 2-3 mm = 3; 1-2 mm = 2 and 0-1 mm = 1. The deep linear ulcer more than 10 mm in length = 6 and linear ulcer less than 10 mm in length = 3. The score for each single lesion were than summed up for the determination of ulcer index.

Gastric wall mucus determination

The modified procedure of Corne et al. (12) was used to determine gastric-wall mucus. The glandular segments from the stomach was removed and weighed. Each segment was transferred immediately to 1% Alcian blue solution (in 0.25 M sucrose solution), buffered with sodium acetate pH 5), and the excess dye was removed by rinsing with sucrose solution. The dye complexed with the gastric wall mucus was extracted with magnesium chloride solution (0.5 M). A 4-ml aliquot of blue extract was then shaken with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 3600 RPM for 10 minutes and the absorbance of the aqueous layer was recorded at 580 nm. The quantity of Alcian blue extracted/g (net) of glandular tissue was then calculated.

Estimation of Nonprotein Sulfhydryl groups

Gastric mucosal (NP-SH) was measured according to the method of Sedlak and Lindsay, (13) to analyze the oxidant/antioxidant balance. The glandular stomach was removed and homogenized in ice-cold 0.02M ethylenediaminetetraacetic acid. The homogenate was mixed with distilled water and 50% (w/v) aqueous TCA and centrifuged at 3000 RPM for 15 minutes; the supernatant was mixed with 0.1 ml of 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) (pH 8) was added and the sample was vortexed (speed of 2) at room temperature. The absorbance was read within 5 min of addition of DTNB, at 412 nm, against a reagent blank with no homogenates.

Histopathological assessment

The gastric tissue samples were fixed in neutral buffered formalin. The fixed tissues were subjected to treatment in a VIP automatic processing machine. This processing includes (i) fixation in 10% neutral buffered formalin (ii) dehydratiuon through graded alcohol (70%, 95% and absolute) (iii) clearing through xylene (iv) impregnation in paraffin wax and finally (v) embedding in paraffin blocks. After these procedures are completed in the VIP processor, the tissues in wax blocks are taken for section cutting in an American optical rotary microtome. The chosen section size was of thickness 5 µm. These sections were stained with haematoxylin and eosin using standard procedures (14). The slides were then examined under a microscope for morphological changes, such as congestion, hemorrhage, edema and erosions using an arbitrary scale (- = Normal; + = Moderate effect; ++ = Severe effect; +++ = Intensely

severe effect) for the assessment of severity of these changes (15).

Determination of Anti-secretary activity
The method of Shay et al. (16) was used to determine the anti-secretary activity. The animals were fasted for 36 hr with free access to water. Ligation of the pylorus was done under light ether anaesthesia. Care was taken not to bleed or occlude the blood vessels. Aqueous suspension of Black pepper was administered intraduodenally, immediately after pylorus ligation. The animals were sacrificed, 6 hour after the pylorus ligation. The stomach was removed, contents collected, volume of contents measured, centrifuged at a speed of 2500 RPM for 10 minutes. The pH of supernatant was adjusted to 7 by addition of 0.01 N sodium hydroxide. The titrable acidity is calculated by the following formula:

Titrable acidity = Sodium hydroxide consumed x 100 ÷ Volume of gastric juice

Gastric lesions induced by indomethacin
Indomethacin was suspended in 1% carboxy-methylcellulose in water (6 mg/ml) and administered orally to the fasted rats at a dose of 30 mg/kg., (0.5 ml/100 g). Control rats were treated similarly with an equivalent volume of the vehicle (17). The animals were sacrificed 5 hr after the treatment (11). The stomach of the animals were removed, rinsed with normal saline, and studied according to the procedure (describe this procedure) of Szabo et al., (18).

Statistical Analysis
The results are expressed as mean ± SEM. The mean determination of treatment groups were statistically analysed by One-Way ANOVA and Post hoc Tukey-Kramer multiple comparisons.

Results

Effect of Black pepper on Gastric mucosa in untreated rats
Aqueous suspension of Black pepper failed to cause gastric ulcers, erosions or hyperaemia in the gastric mucosa of normal rats at doses of 250 and 500 mg/kg (Table 1).

Effect on the Gastric lesions induced by different necrotizing agents
The treatment with Black pepper was found to significantly reduce the gastric ulcers induced by ethanol and NaCl ($P<0.001$) at both (250 and 500 mg/kg) doses of Black pepper. The ulcers caused by NaOH were found to be protected significantly ($P<0.01$) and ($P<0.001$) at lower and higher doses of Black pepper respectively (Table 2).

Effect on gastric wall mucus
The treatment with ethanol caused a significant ($P<0.001$) decrease in the mucus content of gastric wall in rats. The depletion was found to be protected after pretreatment with Black pepper at both the doses (250 and 500 mg/kg.) studied. These changes were statistically significant ($P<0.01$) as compared to the values obtained in the ethanol treated control group (Table 3).

Estimation of NP-SH groups in the gastric tissue
Ethanol caused a significant ($P<0.05$) decrease in concentrations of NP-SH in the gastric tissue. An increase of these levels was observed after the pre-treatment with Black pepper at both the doses. However, the protection was statistically insignificant ($P>0.05$) at any of these doses as compared to the values obtained in the ethanol treated control (Table 4).

Effect on histopathological gastric lesions
Pre-treatment with Black pepper (500 mg/kg) was found to completely protect the different histopathological changes (congestion, haemorrhage, oedema, necrosis, inflammatory and dysplastic changes, erosions and ulceration) caused in the gastric mucosa of ethanol treated rats. However, the lower dose of Black pepper was found to protect edema, dysplastic changes and ulcerations completely. The intensity of other lesions (congestion, haemorrhage, necrosis and erosions, caused by ethanol, was reduced after the pretreatment with Black pepper at the lower dose (250 mg/kg.). The inflammatory changes induced by ethanol were not affected by the treatment of Black pepper (Table 5, Figures 1-4).

Effect on the Anti-secretary activity
Treatment with Black pepper, after pylorus ligation resulted in a significant decrease in the volume of the gastric contents ($P<0.01$) and ulcer index ($P<0.001$) at both the doses (250 and 500 mg/kg). There was a total reduction of the titrable acid at the higher dose, whereas the reduction at the lower dose was statistically insignificant ($P>0.05$) (Table 6).

Effect on gastric lesions induced by Indomethacin
Pretreatment with Black pepper was found to reduce the gastric ulcers caused by indomethacin. The inhibition of ulceration was statistically significant at 250 ($P<0.05$) and at 500 mg/kg. ($P<0.001$) (Table 7).

Discussion
Results of our present study clearly demonstrates that Black pepper failed to cause any changes in the normal gastric mucosa of rat at the doses used for the study. Our data, on lack of any effect of Black pepper on the gastric mucosa of normal rats, contradicts literature reports on the clinical studies. These investigations found Black pepper to significantly increase the parietal secretions, pepsin secretion and potassium loss (4). The discordance between these results may be due to the mode of induction of the secretions (pylorus ligation) in the present study. Furthermore, in view of the effect of Black pepper on cytochromes and the related metabolism (19) it is difficult to analyze the variations between these studies, unless controlled experimental conditions are used in the clinical studies.
The results of our study on antigastric ulcer activity revealed that Black pepper at lower dose confers a dose-dependent protection against the gross damaging action of necrotizing agents (sodium chloride and sodium hydroxide) on gastric mucosa of rats. The protection against the gastric damage caused by these agents might be related to the inhibition of gastric motor activity (20) and the stimulation of prostaglandin synthesis (9) caused by mild irritants. Thus, it is assumed that these gastric irritants might have activated the defensive mechanism and preserved the integrity of gastric mucosa (21).

Ethanol was the major gastric necrotizing agent used in the present study. The induced gastric damage was related with ulceration and the induction of histopathological changes. Our results revealed Black pepper to lend a dose-dependent protection against the gross damaging action of ethanol as revealed by reduction of the intensity of gastric ulcers and the prevention of the histological aberrations (congestion, haemorrhage, oedema, necrosis, dysplastic changes and erosions) in the gastric tissue. Another gastric damage, the ethanol caused was the inhibition of gastric mucus. The gastric mucus coating is thought to be important in both preventing damage and in facilitating the repair of gastric epithelium (22, 23). The pre-treatment with Black pepper, in the present study was found to prevent ethanol induced gastric wall mucus depletion. Nevertheless, the pre-treatment with black paper failed to replenish the ethanol-induced depletion of NP-SH content in stomach of rats. These data contradicts the antioxidant activity of the constituents of black pepper (6, 7, 24, 25). The masking of the activity of these constituents might be due to the influence of piperine on cytochromes. Previous studies have shown piperine to enhance the serum levels of drugs and nutrients in animals and humans by interfering with the metabolism (19, 26, 27, 28).

The other gastric lesion-inducing agent used in the present study was indomethacin, a nonsteroidal anti-inflammatory drug (NSAID). This drug is known to cause gastric lesions by increasing the gastric motor activity and inhibition of the synthesis of endogenous prostaglandins (29, 30). Our study on the indomethacin-induced gastric ulcers revealed Black pepper to affect the motor activity and prostaglandin synthesis in the gastric tissue. The data on gastro protective activity is further supported by our observation on the effect of Black pepper to inhibit the pylorus ligation-accumulated secretions and the related ulcers. The gastric anti-secretory activity observed may be due to peripheral parasympathetic blockade, as the Black pepper does not antagonize the acetylcholine induced contraction of the smooth muscle of guinea pig ileum (31, 32). However, in Black pepper-treated Shay rats, the severity of ulcers was significantly reduced and this protection could be due to its effect in reducing the volume and acidity of gastric secretions (33). Our study confirms the previous observation that suppressants of gastric acid secretion increase the healing of gastric ulcers in both humans and experimental animals (34, 35).

The results of our present study establish the antigastric ulcer and antisecretary activity of Black pepper and confirm its traditional use against gastric disorders (1). The exact constituent/(s) responsible for the gastro protective activity of black pepper is not known. Nevertheless, it is assumed that the influence of piperine on the endogenous levels of coenzyme Q10 (19) might be crucial in the protection of gastric ulcers. Earlier studies found coenzyme Q10 to increase the supply of blood and micelle formation in the gastrointestinal system (36). Johri et al., (37) also reported coenzyme Q10 to modify the epithelial cell wall of the digestive system. Thus Black pepper appears to protect against the gastric injury probably by stimulation of the bioenergetic processes in the gastrointestinal epithelium (38) by the combined effect of piperine and coenzyme Q10. Further work on the effect of Black pepper and its different constituents on the endogenous levels of coenzyme Q10 and the healing of gastric ulcers would be interesting.

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