Gastroprotective effect of cardamom, *Elettaria cardamomum* Maton. fruits in rats

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Abstract

Cardamom, the fruits of *Elettaria cardamomum* Maton. (Zingiberaceae) commonly known as “Heel khurd” is used in Unani system of medicine to treat gastrointestinal disorders. A crude methanolic extract (TM), essential oil (EO), petroleum ether soluble (PS) and insoluble (PI) fractions of methanolic extract, were studied in rats at doses of 100–500, 12.5–50, 12.5–150 and 450 mg/kg, respectively for their ability to inhibit the gastric lesions induced by aspirin, ethanol and pylorus ligation. In addition their effects on wall mucus and gastric acid output were recorded. All fractions (TM, EO, PS, PI) significantly inhibited gastric lesions induced by ethanol and aspirin but not those induced by pylorus ligation.

TM proved to be active reducing lesions by about 70% in the EtOH-induced ulcer model at 500 mg/kg. The PS fraction reduced the lesions by 50% at 50 and 100 mg/kg (no dose response was observed) with similar effect than the PI fraction at 450 mg/kg. In the aspirin-induced gastric ulcer, the best gastroprotective effect was found in the PS fraction, which inhibited lesions by nearly 100% at 12.5 mg/kg. In our experimental conditions, the PS extract at doses ≥12.5 mg/kg proved to be more active than ranitidine at 50 mg/kg.

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Keywords: Cardamom; Antiulcer; *Elettaria cardamomum*; Zingiberaceae

1. Introduction

Peptic ulcer is one of the most common gastrointestinal diseases. In recent years, a widespread search has been launched to identify new antiulcer-drugs from natural sources. A number of spices, namely ginger (Alahyda et al., 1999), turmeric (Rafatullah et al., 1990), large cardamom (Jafri et al., 2001) among others have been shown to possess significant gastroprotective activity. Fruit of *Elettaria cardamomum* is used as spice throughout the world. In Unani system of medicine, it is known as Heel khurd or Choti ilaichi. Its seed powder (3.5–7 g) is frequently prescribed in the treatment of gastrointestinal disorders and is used as stomachic, resolvent, retentive, digestive, antiemetic and carminative. It has also been mentioned in the treatment of acid peptic disorders and gastritis (Azam Khan, 1313 A.H.).

Literature survey revealed that the fruit of *E. cardamomum* has been reported to contain essential oil (Baruah et al., 1973; Korikanthimathm et al., 2001), sterol (Kataoka et al., 1987), phenolic acids (Vairiyar and Bandyopadhyay, 1995) and lipids (Gopalakrishnan et al., 1990). The essential oil, isolated from the fruits of *E. cardamomum* showed antimicrobial (Islam et al., 1990; Pruthi et al., 1980; Venkataraman et al., 1978) antiinflammatory, analgesic and antispasmodic (Al-Zuhair et al., 1996) activities. The effect of essential oil on the cardiovascular system in rats, nictitating membrane of cats, isolated rabbit jejunum, isolated guineapig ileum and frog sciatic nerve preparation have been reported (Tahir Keh et al., 1997). Huang et al. (1999) investigated the effect of cardamom on the transdermal delivery of indomethacin. Aqueous extract of *E. cardamomum* increased the gastric acid secretion in pentobarbitone anaesthetised rats (Vasudevan et

There was no scientific report available on the traditional claims of the effects of cardamom in gastrointestinal disorders. Therefore, we investigated the antiulcerogenic effects of various fractions of *E. cardamomum* in different models of gastric lesions, induced by alcohol, aspirin and pylorus ligation in rats.

2. Materials and methods

2.1. Plant material

The dried fruits of *E. cardamomum* were purchased from the local market in New Delhi and authenticated by matching with the specimen available in the pharmacognosy section of Department of Ilmul-Advia, Faculty of Medicine (Unani). A voucher specimen (AJ-EA-2002) was also deposited in the same section of the department.

2.2. Animals

Albino rats of the Wistar strain weighing between 160 and 220 g were obtained from the Central Animal House, Jamia Hamdard. The animals described as fasted were deprived from food but allowed free access to water. Carboxymethyl cellulose (CMC) 1% (w/v) in distilled water was used as vehicle. All the treatments (except ethanol) suspended in vehicle were administered orally in a volume of 10 ml/kg in all the experiments. These experiments were conducted after getting approval from the Institutional Animal Ethical Committee (IAEC) duly constituted according to CPCSEA (Control and Prevention of Cruelty and Supervision in Experiments on Animals) guidelines of Government of India.

2.3. Extracts

The dried fruits of *E. cardamomum* were crushed thoroughly and extracted with methanol in a Soxhlet apparatus over steam bath for 24 h. The solvent was removed under reduced pressure and the extract yield was 10% (w/w) in terms of starting material. Recovery of the solvent under reduced pressure gave the petroleum ether soluble fraction (PS). The residue left after extraction with petroleum ether was coded as petroleum ether insoluble fraction (PI). The yields of PS and PI in terms of dry starting material were 1 and 9%, respectively.

The essential oil from the dried fruits of *E. cardamomum* was obtained by steam distillation using a Klevenger apparatus. The essential oil (EO) yield was 4.8% (v/w).

2.4. Gastroprotective effect of extracts and fractions on ethanol induced gastric ulcers

In one set of experiment, 24 h fasted animals were randomly allotted into four groups of six animals each. The total methanolic extract (TM) at 500, 300 and 100 mg/kg was administered to three of the groups. Control group animals received only vehicle. After 30 min ulceration was induced by oral administration of ethanol (96%, v/v; 5 ml/kg). The animals were sacrificed after 1 h of ethanol administration (Paiva et al., 1998). The stomachs were removed and opened along the greater curvature to determine the ulcer index as given below

<table>
<thead>
<tr>
<th>Erosions Score</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mm or less</td>
<td>1</td>
</tr>
<tr>
<td>1 mm to 2 mm</td>
<td>2</td>
</tr>
<tr>
<td>More than 2 mm</td>
<td>3</td>
</tr>
</tbody>
</table>

The overall score was divided by a factor 10, which was designated as the ulcer index (Main and Whittle, 1975).

To find out the fraction containing the gastroprotective principles, experiments were undertaken with the fractions and EO. Animals received by gavage either PS (50, 100, 150 mg/kg), PI (450 mg/kg) or EO (30 mg/kg), respectively under identical experimental conditions than the TM extract.

2.5. Gastroprotective effect of extracts and fractions on aspirin induced gastric ulcers

The gastroprotective effect of the extracts and fractions were investigated using 36 h fasted animals allotted in groups of 6 animals each. In the first experiment, the animals of one group received TM (500 mg/kg) while the control animals received vehicle only. After 30 min, aspirin (500 mg/kg) was administered to all the animals (p.o.). After 4 h, the animals were sacrificed (Parmar and Desai, 1993) to determine the ulcer index as described earlier. The same protocol was adopted for the experiment with the PS and PI fractions as well as EO. The PS (37.5, 25 and 12.5 mg/kg), EO (12.5 mg/kg) and ranitidine (50 mg/kg) were administered to different animal groups.

2.6. Gastroprotective effect of TM and PS on gastric ulcers in pylorus ligated rats

The animals, fasted over 36 h, were randomly allotted into four groups of six rats each. The animals of four different groups were given vehicle (10 ml/kg), ranitidine (50 mg/kg), TM (500 mg/kg) and PS (50 mg/kg), respectively. After 30 min of their respective treatment, pylorus was ligated as described by Shay et al. (1945). After 4 h of ligation the rats were sacrificed by an overdose of ether vapours. The stomachs were removed and opened along the greater curvature, washed with distilled water and examined for lesions.

2.7. Effect of EO on gastric contents, gastric wall mucus and gastric ulcers in alcohol induced gastric ulceration in Shay rats.

The 24 h fasted animals were randomly distributed into two groups of six animals each. The animals of one group...
received EO (50 mg/kg) and other group received vehicle only. After 30 min of their respective treatments, pylorus was ligated as described earlier. Immediately after ligation ethanol (96%, v/v; 5 ml/kg) was given orally to all the rats. Drinking water was withheld and gastric juices were allowed to collect for a period of 4 h. The rats were then sacrificed by an overdose of ether vapours and the stomach was removed after clamping the oesophagus. The gastric contents were collected through the oesophagus and the volume measured. It was centrifuged at 3000 rpm for 20 min. The supernatant was analysed for titrable acidity. The stomachs were opened along the greater curvature, washed with distilled water and examined for lesions. After examination, the stomach was weighed and immediately immersed in Alcian blue solution for determining the mucus wall thickness.

2.8. Effect of PS on gastric contents, gastric wall mucus and gastric ulcers in aspirin induced gastric ulceration in Shay rats

The protocol adopted in the alcohol-induced gastric ulcer in pylorus ligated rats was followed to evaluate the volume of gastric juice, titrable acidity, ulcer index and mucous wall thickness. However, in this study, aspirin (500 mg/kg) instead of ethanol was used as ulcer inducing agent (Akhbar and Munir, 1989).

2.9. Gastric wall mucus (barrier mucus) determination

Gastric wall mucus was determined according to the procedure of Corne et al. (1974). In this method, Alcian blue, a histological dye, is used, which stains only the barrier mucus and does not penetrate the mucosal tissue. The dye complexed with barrier mucus is recoverable. The quantity of Alcian blue recovered per gram of net glandular tissue was calculated.

2.10. Total acidity

For the determination of total acidity of the gastric juice sample, a known volume of the gastric juice was titrated with 0.01 N sodium hydroxide to pH 8.5 using phenolphthalein as indicator (Parmar et al., 1984). The values of the total acidity were expressed as milliequivalents per litre per 4 h (mequiv./l/4 h). The values of the total acidity of the gastric juice samples contaminated with blood were not included in the data.

2.11. Statistical analysis

All values have been expressed as mean ± S.E.M. Statistical significance was determined by using ANOVA followed by Dunnet test.

3. Results and discussion

It is known that ethanol produces necrotic lesions in the gastric mucosa by its direct toxic effect reducing the secretion of bicarbonates and production of mucus (Marhuenda and Martin, 1993). The products of the 5-lipoxygenase pathway may also play a key role in the development of ulcer, induced by irritant agents such as ethanol (Lange et al., 1985). The pretreatment with TM (500, 300 and 100 mg/kg) showed a dose dependent reduction in the severity of the lesions in experimental model of ethanol-induced acute ulcer in rats (Table 1).

The TM was fractionated into petroleum ether soluble (PS) and petroleum ether insoluble (PI) fractions. These two fractions PS (50 mg/kg) and PI (450 mg/kg) corresponding to 500 mg of TM (5 g of dried fruits) were tested in ethanol-induced gastric lesion to determine the fraction containing most active principle. The fractions PS and PI showed significant inhibition of ulceration by 50 and 54.8%, respectively. As the dose of PS (50 mg/kg) is quite lower than PI (450 mg/kg), PS may be considered as more active fraction. This fraction in a dose of 100 mg/kg showed significant inhibition of ulceration by 53.3%. It is interesting to note that PS in a dose of 150 mg/kg did not significantly reduce the ulcer index (Table 1). It seems that the dose of PS (100 mg/kg) is producing sub-maximal response.

Essential oils are highly soluble in petroleum ether. To ascertain the role of the essential oil present in PS in gastrotropism, the EO (50 mg/kg) was also tested in ethanol induced gastric ulceration. The EO was found to be more active than PS (73.3 and 50.0% inhibition, respectively). Thus it may be considered that the constituents responsible for gastrotropic action can be obtained by steam distillation of the crude drug.

The EO (50 mg/kg) was found to be effective in decreasing by 65% the ulcer index in alcohol induced gastric ulceration in Shay rats but total volume of gastric secretion, total acidity and mucus wall thickness remained unaffected. The ulcer index (mm) for the control and essential oil-treated animals were 2.08 ± 0.08 and 0.73 ± 0.08, respectively.

The aspirin-induced gastric lesions model was used in this study, considering the different mechanism by which it induces gastric ulceration. The reason being principally attributed to biosynthesis inhibition of cytoprotective prostaglandins (e.g. PGE’s and PGI2), inhibition of the cyclooxygenase pathway of arachidonic acid metabolism resulting in overproduction of leukotriene and other products of 5-lipoxygenase pathway (Rainsford, 1987). We observed that EO (12.5 mg/kg), TM (500 mg/kg) and its PI (450 mg/kg) and PS (50, 37.5, 25, 12.5 mg/kg) fractions showed significant inhibition of ulceration (Table 1). The results also showed that PS is more active than EO and ranitidine. Thus, it may be concluded that constituents other than essential oil present in PS are also involved in the gastrotrophic action.

The pretreatment with PS (50 mg/kg) did not have any significant effect on gastric volume, titrable acidity and gastric...
Table 1  
Gastroprotective effect of extracts and fractions from *Elettaria cardamomum* fruit on ethanol and aspirin induced gastric ulcers in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ethanol induced gastric lesions</th>
<th>Aspirin induced gastric lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ulcer index (mm)</td>
<td>Inhibition (%)</td>
</tr>
<tr>
<td>Control</td>
<td>Vehicle (10 ml/kg)</td>
<td>1.55 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>TM (100 mg/kg)</td>
<td>1.37 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>TM (300 mg/kg)</td>
<td>0.82 ± 0.11*</td>
</tr>
<tr>
<td></td>
<td>TM (500 mg/kg)</td>
<td>0.42 ± 0.08*</td>
</tr>
<tr>
<td>Control</td>
<td>Vehicle (10 ml/kg)</td>
<td>1.5 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>PS (50 mg/kg)</td>
<td>0.75 ± 0.08*</td>
</tr>
<tr>
<td></td>
<td>PS (100 mg/kg)</td>
<td>0.70 ± 0.06*</td>
</tr>
<tr>
<td></td>
<td>PS (150 mg/kg)</td>
<td>1.35 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>PI (450 mg/kg)</td>
<td>0.69 ± 0.08*</td>
</tr>
<tr>
<td>Control</td>
<td>Vehicle (10 ml/kg)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>PS (37.5 mg/kg)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>PS (25 mg/kg)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>PS (12.5 mg/kg)</td>
<td>–</td>
</tr>
<tr>
<td>Control</td>
<td>Vehicle (10 ml/kg)</td>
<td>1.75 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>Ranitidine (50 mg/kg)</td>
<td>0.2 ± 0.05*</td>
</tr>
<tr>
<td></td>
<td>EO (12.5 mg/kg)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>EO (50 mg/kg)</td>
<td>0.47 ± 0.05*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. for six rats in each group. TM: methanolic extract, PS: petroleum ether soluble fraction, PI: petroleum ether insoluble fraction, EO: essential oil.

* Statistically significant *P* < 0.01 (all compared with control).

... (Table continues with similar formatting)

In the course of our experiment with PS and EO, we observed flattening of the mucosal folds, which suggests that the gastroprotective effect of both the fractions may be due to a decrease in gastric motility. It is reported that the changes in gastric motility may play a role in the development and prevention of experimental gastric lesions (Garrick et al., 1986; Mersereau and Hinchey, 1982; Takeuchi and Nobuhara, 1985). Relaxation of circular muscles may protect the gastric mucosa through flattening of the folds. This will increase the mucosal area exposed to necrotizing agents and reduce the volume of the gastric irritants on rugal crest. Such action has been postulated to play a role in cytoprotective effect of prostaglandin (Takeuchi and Nobuhara, 1985). Ethanol produces a marked contraction of the circular muscles of rat fundic strip. Such a contraction can lead to 'mucosal compression' at the site of the greatest mechanical stress i.e. at the crests of mucosal folds leading to necrosis and ulceration (Mersereau and Hinchey, 1982). Thus, it may be postulated that the gastroprotective action of PS and EO is due to decrease in gastric motility. But it needs further exploration.

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References


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