ORIGINAL ARTICLES

Influence of intragastric perfusion of aqueous spice extracts on acid secretion in anesthetized albino rats

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Background: The effect of spices on gastric acid secretion is variable. Their mechanism of action is also not well established. Aim: To study the effect of spices on gastric acid secretion in anesthetized rats. Methods: Aqueous extracts (10% w/v) of red pepper (Capsicum annum), fennel (Foeniculum vulgare), omum/ajwan (Carum copticum), cardamom (Elettaria cardamomum), black pepper (Piper nigrum), cumin (Cuminum cyminum) and coriander (Coriandrum sativum) were prepared. The stomach of pentobarbitone-anesthetized rats was perfused at 0.15 mL/min with aqueous extracts of spice or acetylcholine (1 µg/mL or 10 µg/mL solutions, in 40 min blocks, twice in each experiment bracketed by saline perfusions. The acid content in the samples was estimated by titration with 0.1N NaOH with phenolphthalein as indicator. Atropine 1 µg/mL was added to the perfusion fluid in 28 experiments. In 32, acute gastric mucosal injury was induced by leaving aspirin 125 mg/Kg in the stomach for 2 h before perfusion. Results: All the spices tested increased acid secretion in the following declining order: red pepper, fennel, omum, cardamom, black pepper, cumin, coriander. Red pepper increased acid secretion (mean [SEM] 0.93 [0.16] mL 0.1N HCl to about 7 times the basal secretion (0.14 [0.05]; p<0.005). The increase in acid secretion by the other spices was as follows: fennel 0.42 (0.11) mL 0.1 N HCl from basal secretion (0.12 [0.03]) (p<0.02); omum 0.33 (0.05) from 0.09 (0.02) (p<0.01); cardamom 0.28 (0.04) from 0.10 (0.03) (p<0.005); black pepper 0.19 (0.03) from 0.04 (0.01) (p<0.005); cumin 0.12 (0.02) from 0.08 (0.01) (p<0.05); coriander 0.18 (0.03) from 0.09 (0.02) (p<0.005). Atropine abolished the acid secretion induced by acetylcholine and significantly reduced acid induction by red pepper, omum and coriander, but not that by fennel. In experiments with aspirin-induced mucosal injury the basal acid secretion was low; acid secretion by red pepper and fennel was reduced significantly, but not that by acetylcholine. Cumin and coriander increased acid secretion in injured stomachs. Conclusion: The spices tested increased gastric acid secretion, in some by a cholinergic mechanism but by other mechanism(s) as well. Red pepper produced maximum increase in acid secretion, but this was significantly reduced in injured stomachs. Cumin and coriander increased gastric secretion in injured stomachs. [Indian J Gastroenterol 2000;19:53-56]

Key words: Gastric acidity

Spices are commonly used in food, home remedies and indigenous systems of medicine. Patients with peptic ulcer are generally advised against consuming spices lest they increase gastric acidity. We had reported that aqueous extracts from several roasted spices contained large amounts of acetylcholine (ACH) and choline.1,2 The present investigation was undertaken to evaluate the effect of spices on gastric acid secretion in anesthetized rats using the method of perfused rat stomach.3 Atropine (atropine sulfate; Boehringer Ingelheim) was added to the perfusion fluid in some experiments to determine the cholinergic component of the effect. In some rats, spices were perfused after injuring the mucosa with aspirin.

Methods

This study was approved by the animal welfare committee of our institution. Seeds of red pepper (Capsicum annum), fennel (Foeniculum vulgare), omum or ajwan (Carum copticum), cardamom (Elettaria cardamomum), black pepper (Piper nigrum), cumin (Cuminum cyminum) and coriander (Coriandrum sativum) were procured from seed stocks of the Tamil Nadu State Government Agriculture Department. Aqueous extracts were prepared from roasted spice seeds.1,2 One gram of spice seeds was dry heated in a conical flask to a light brown color, 10 mL distilled water was added while fuming and the flask taken off the flame. After cooling, the supernatant fluid was filtered through a Whatman No.1 filter paper.

Experimental procedures

Fasting male albino rats (Wistar strain), weighing 160-180 g, were anesthetized with intraperitoneal pentobarbitone sodium (Loba) 40 mg/Kg and fixed supine to the operation board. A pediatric nasal catheter was introduced through the mouth into the esophagus with its tip reaching the stomach, to serve as inflow cannula. The neck was opened by a midline incision and the trachea cannulated. A ligature was placed around the esophagus and tightened over the esophageal cannula, to exclude back flow from the stomach. The abdomen was opened in the midline. A polyvinyl cannula with multiple holes

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at its tip was introduced into the pyloric portion of the stomach, through a niche in the duodenum near the pyloro-duodenal junction. A ligature was placed over the cannula (exit cannula) at the pyloro-duodenal junction, excluding the blood vessels, to prevent any leakage or back flow of fluid. The stomach was replaced in the abdomen and closed with clips leaving out the exit end of the outflow cannula. A group of 5 to 7 rats was used for each test solution. A total of 109 rats were used for perfusion, including 28 with atropine and 32 for studies on injured stomach. At the end of each experiment the animal was sacrificed by a large dose of pentobarbitone.

The test solution, either aqueous extract of a spice or acetylcholine 1 µg/mL or 10 µg/mL solution (acetylcholine chloride: Sigma), was perfused (0.15 mL/min) through the inflow cannula. The solution in the reservoir was changed every 40 min to give a perfusion sequence of saline - test solution - saline - test solution - saline over a 200-min perfusion period. The outflow from the exit cannula was collected in centrifuge tubes over every 20 min period; its volume was measured and acid content estimated. The acid content was measured by titration with 0.1N NaOH using phenolphthalein as indicator, and expressed as mL 0.1N HCl. Mean acid content of the first two 20 min saline samples was designated S1 and represented the basal acid secretion. The acid stimulating effect was slow in onset and reached peak only in the second sample with spice/ACh. Hence S3, S7 samples with test solutions were evaluated against saline samples S1, S5 respectively for statistical analysis. The results from 5 to 7 such individual experiments with the same test solution were pooled and mean (SEM) was obtained for samples S1 to S9. Atropine 1 µg/mL was added in 28 experiments both to saline and test solutions.

### Intrinsic acidity of solutions

The pH and acid content in 3 mL (volume infused in 20 min) of each of the solutions used for perfusion was determined on three occasions and average obtained. Their pH (in parenthesis) and intrinsic acid equivalent (expressed as mL 0.1N HCl) were as follows: normal saline (6.53) 0.01 mL, ACh (6.53) 0.01 mL, red pepper seeds (5.05) 0.19 mL, fennel (6.03) 0.05 mL, umon (6.5) 0.015 mL, black pepper (6.96), cumin (6.3) 0.063 mL, cardamom (6.21) 0.04 mL and coriander (6.46) 0.026 mL. These intrinsic acid values were deducted from the observed acid values in respective samples to arrive at the true gastric acid secretion during perfusion.

### Aspirin-induced mucosal injury

In anesthetized rats prepared as described above, aspirin 125 mg/Kg as 10% solution in 0.5% carboxy methylcellulose was left in the stomach for 2 hours. The stomach was then emptied, repeatedly washed with 20 mL saline, and then perfused with saline - test solution - saline for 40 min each. The stomachs were examined postmortem after each experiment by opening along the lesser curvature and the presence of injury confirmed under illuminated magnifier. Hemorrhagic patches of 2-3 cm² were observed in the pyloric portion. The damage was seen as patchy loss of glistening surface in the thin antral region. The stomachs in the experiments without aspirin were not studied.

### Statistical analysis

Student’s t test for paired and unpaired data was used for comparing means, as appropriate. All values given are mean (SEM).

### Results

**Basal acid secretion**: Gastric acid secretion showed a progressive decline during the 200 min perfusion. In 5 preliminary experiments with only saline perfused throughout, the acid secretion fell from 0.17 (0.03) in S1 to 0.004 (0.001) in S9. This observation was further confirmed by changing the solution to saline by the addition of ATR.

### Table 1: Acid content (mean [SEM]) in 20 min samples of gastric perfusate with normal saline (NS) alternating with test substance (T)

<table>
<thead>
<tr>
<th>Test substance</th>
<th>S1 (NS)</th>
<th>S3 (T)</th>
<th>S5 (NS)</th>
<th>S7 (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine 1 µg/mL</td>
<td>0.06 (0.02)</td>
<td>0.14 (0.03)</td>
<td>0.09 (0.02)</td>
<td>0.12 (0.01)</td>
</tr>
<tr>
<td>Acetylcholine + ATR</td>
<td>0.12 (0.01)</td>
<td>0.09 (0.02)</td>
<td>0.11 (0.02)</td>
<td>0.23 (0.02)</td>
</tr>
<tr>
<td>Fennel + ATR</td>
<td>0.11 (0.02)</td>
<td>0.11 (0.02)</td>
<td>0.12 (0.03)</td>
<td>0.12 (0.02)</td>
</tr>
<tr>
<td>Cumin + ATR</td>
<td>0.09 (0.02)</td>
<td>0.09 (0.02)</td>
<td>0.12 (0.02)</td>
<td>0.12 (0.02)</td>
</tr>
<tr>
<td>Cardamom + ATR</td>
<td>0.12 (0.02)</td>
<td>0.12 (0.02)</td>
<td>0.12 (0.02)</td>
<td>0.12 (0.02)</td>
</tr>
<tr>
<td>Black pepper + ATR</td>
<td>0.11 (0.02)</td>
<td>0.11 (0.02)</td>
<td>0.12 (0.03)</td>
<td>0.12 (0.03)</td>
</tr>
<tr>
<td>Coriander + ATR</td>
<td>0.10 (0.02)</td>
<td>0.10 (0.02)</td>
<td>0.10 (0.02)</td>
<td>0.10 (0.02)</td>
</tr>
</tbody>
</table>

* p<0.05, ** p<0.02, *** p<0.01, + p<0.005, ++ p<0.001 with paired t test comparing S3 / S1 or S7 / S5 in normal experiments. In + ATR' experiments S3-S1 compared with experiments without ATR (given above) each by unpaired 'T' test.

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firmed by results during saline perfusion in 49 animals perfused with test substances.

**Effect of test solutions**

**Acetylcholine**: Acetylcholine 1 µg/mL and 10 µg/mL produced significant increase in acid secretion (Table 1); with 10 µg/mL, the increase in acid was significant during the second perfusion (S7) also.

**Spice solutions**: Red pepper, fennel, omum, and cardamom in that order produced significant increase in acid secretion both times (S3, S7) in the same experiment (Table 1). During the first perfusion the increase above the basal acid secretion was about 6-fold with red pepper and 3-fold with fennel, omum and cardamom. Even with diminishing basal secretion, the second perfusion raised the acid secretion about 8-fold with red pepper, 4-fold with fennel and omum, and only 2-fold with cardamom. Though there was an increase in acid secretion with black pepper and cumin, it was significant only during the second perfusion with low basal acid secretion. Coriander increased acid secretion during the first perfusion but not the second.

**Influence of atropine**

Atropine significantly reduced the increase in acid secretion induced by red pepper (S3-S1) from 0.79 (0.12) to 0.09 (0.05) (p<0.001; Table 1). A significant reduction of acid secretion with omum and coriander was also observed; however, atropine did not influence the acid stimulatory effect of fennel.

**Perfusion in aspirin-injured stomachs**

The basal acid secretion in S1 saline sample was significantly reduced in experiments with injured stomachs. The acid increase evoked by ACh 10 µg/mL was not different in the normal and ulcerated groups (Table 2). However, the acid increase (S3-S1) induced by red pepper in injured stomachs was significantly lowered (p<0.001) from 0.79 (0.12) in normal to 0.03 (0.01) in injured stomachs. A similar reduction was also observed with fennel (0.30 [0.09] to 0.09 [0.03]). On the other hand, acid secretion was more in injured stomachs with cumin (p<0.01) and coriander.

**Discussion**

In our study, intragastric perfusion of spices in anesthetized rats produced significant, reproducible increases in acid secretion of varying degrees with different spices. The concentration of spices (10%) used was similar to that used in a previous study. High concentrations are generally used initially to elicit a positive biological response. Some recipes have high concentrations of capsicum in foods like pickles, sauces, and chutneys. High concentrations (1% - 10%) are used in amounts of pepper which have been used in human studies.

Aqueous extracts of roasted spices have been reported to contain both ACh and histamine. In freeze-dried extracts, ACh was present in the maximum concentration in cardamom (165.1 nmol/g), followed by red pepper (104.9), omum (93.3), fennel (73.9), cumin (68.4), coriander (43.5), and black pepper (23.3). But their acid-stimulating ability did not follow this order. Cardamom, though high in ACh content, produced less acid stimulation than red pepper, fennel, and omum. The acid induction by red pepper was significantly reduced by atropine. Similar reduction was also observed with omum and coriander, but not with fennel. This suggests that ACh has a role to play in stimulation of acid secretion with some but not all spices. Spices may induce acid secretion through other ingredients and mechanisms.

The reduction of basal acid secretion by half in injured stomachs could be due to denaturation of acid-secreting stomach lining. Marked reduction in acid induction by red pepper to some extent by fennel in injured stomachs compared to normal rats suggested that a normal intact gastric mucosa was necessary for acid stimulation. The increased acid stimulation with cumin and coriander in injured stomachs cannot be explained, indicating that each spice could affect acid secretion differently.

Most reports on spices relate to red pepper and its active ingredient capsaicin. Black pepper was reported to cause significant increase in parietal secretion and potassium loss. Among various spices included in the test meal, only coriander was found to significantly increase acid secretion in duodenal ulcer patients. In our studies also, coriander and cumin increased acid secretion in injured stomachs.

Spice-induced gastric secretion is clinically relevant in view of their suspected detrimental effect in peptic ulcer patients. Most human studies with red pepper showed an increase in acid secretion. Red chilli powder instilled

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**Table 2: Influence of acetylcholine and spice extracts in gastric perfusates in normal stomachs or those with mucosal injury. Test (T) solutions in S3 compared with normal saline (NS) in S1.**

<table>
<thead>
<tr>
<th>Test solution</th>
<th>n</th>
<th>Acid content (mean ± SEM) mg of 0.1N HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NS</td>
<td>S1</td>
</tr>
<tr>
<td>ACh</td>
<td>7</td>
<td>0.14 (0.03)</td>
</tr>
<tr>
<td>in injury</td>
<td>9</td>
<td>0.07 (0.01)</td>
</tr>
<tr>
<td>Red pepper</td>
<td>5</td>
<td>0.14 (0.05)</td>
</tr>
<tr>
<td>in injury</td>
<td>5</td>
<td>0.06 (0.03)</td>
</tr>
<tr>
<td>Fennel</td>
<td>6</td>
<td>0.12 (0.03)</td>
</tr>
<tr>
<td>in injury</td>
<td>6</td>
<td>0.07 (0.02)</td>
</tr>
<tr>
<td>Cumin</td>
<td>5</td>
<td>0.12 (0.02)</td>
</tr>
<tr>
<td>in injury</td>
<td>6</td>
<td>0.15 (0.04)</td>
</tr>
<tr>
<td>Coriander</td>
<td>5</td>
<td>0.09 (0.02)</td>
</tr>
<tr>
<td>in injury</td>
<td>6</td>
<td>0.07 (0.02)</td>
</tr>
</tbody>
</table>

ACh: Acetylcholine 10 µg/mL.

p<0.05, **p<0.01, ***p<0.001 for S3 compared to S1 or S3-S1 in NS compared to S3-S1 with intact stomach.
directly into the stomach at 1.6 g/h resulted in significant increase in acid secretion in normal subjects and in patients with duodenal ulcers. Similar administration of test meal with red pepper (0.1-1 g) or black pepper (1.5 g) to healthy volunteers caused a significant increase in parietal secretion. Instillation of 200 mL of 1% red pepper decoction alone or neutralized with alkali in patients with and without duodenal ulcers significantly increased acid secretion, especially in the latter patients. However, another study with red pepper, black pepper and coriander (10 g in 250 mL fractional test meal containing 30 g cream of wheat) instilled into the stomach revealed increase in acidity.

The influence on acid secretion and endocrine changes by red pepper or other spices seems to depend on whether the spices were given in a concentrated form directly into the stomach or combined in meal or as a fractional test meal. In 20 subjects, in whom 3% red pepper extract was instilled, endoscopy 15 min later revealed no change in pH, mild mucosal edema and hyperemia in 3, moderately severe hemorrhagic spots in 3 and actual hematemesis in one. But, in another study when 30 g jalapeno green peppers were given with meal and were compared with bland meal and meal containing 150 mg aspirin, the endoscopic score 12 hours after the last meal was 0 with bland and green pepper meals, but grade ‘C’ with aspirin.

Capsaicin is an important constituent of red pepper. When instilled into the stomach of anesthetized rats at (50-2000 µg/kg) capsaicin produced dose-dependent augmentation of acid output. Hexamethonium and atropine completely abolished this acid secretion. Another antagonist, scopolamine, only partially reduced the response. It was suggested that capsaicin caused an increase in gastric secretion and mucosal blood flow by release of endogenous gastric secretagogues.

At the same time, there are reports of a protective action of red pepper on gastric injury. In rats, the protective action of capsaicin on gastric lesions induced by 100% ethanol, acidified aspirin or water immersion stress was attributed to its hyperemic and antisecretory effects induced by endogenous release of prostaglandins. In human volunteers, 20 g chillies in 200 mL water given orally protected against aspirin-induced (600 mg) gastric damage.

To summarize, in perfused rat stomach, red pepper induced maximum acid stimulation, which was markedly reduced by addition of atropine and in stomachs with mucosal injury. Cardamom had only a moderate effect on gastric secretion. Fennel had greater acid-stimulating effect, but this effect was not reversed by atropine and was reduced to a lesser degree in injured stomachs than that observed with red pepper. Cumin and coriander produced mild acid stimulation, which was blocked by atropine; they increased acid secretion in injured stomachs. We believe that a cholinergic mechanism operates in some of these spices, but other mechanisms(s) also play a role.

References