Digestive stimulant action of three Indian spice mixes in experimental rats

Kalpana Platel¹, Alkananda Rao², G. Saraswathi² and K. Srinivasan¹

The present study examined the favourable influence of three spice mixes derived from a few commonly consumed spices of known digestive stimulant action on digestive enzymes of pancreas and small intestine, and on bile secretion and composition in experimental rats. The common ingredients of these mixes were coriander, turmeric, red chilli, black pepper and cumin, while the spice mix II additionally had ginger, and spice mix III containd onion. All the three spice mixes favourably enhanced the activities of pancreatic lipase, chymotrypsin and amylase when consumed during the diet. In addition, these spice mixes brought about a pronounced stimulation of bile flow and of bile acid secretion. Among the three spice mixes examined, spice mix III which is customised so as to include spices that are desirable from the point of view of stimulation of digestion, had the highest stimulatory influence particularly on bile secretion, bile acid output and the activities of pancreatic enzymes. While activities of pancreatic lipase, amylase and chymotrypsin were elevated by 40, 16 and 77%, respectively, the bile volume as well as the bile acid secretion were almost doubled in spice mix III treatment. The higher secretion of bile especially with an elevated level of bile acids and a beneficial stimulation of pancreatic digestive enzymes, particularly of lipase could probably be the two mechanisms by which these combinations of spices aid in digestion.

1 Introduction

The digestive stimulant action of spices has been recognised since a long time. Several pharmacological preparations available to correct digestive disorders employ certain spices besides other plant substances. Spices such as mint (Mentha spicata), garlic (Allium sativum), ajowan (Trachyspermum ammi), fennel (Foeniculum vulgare), and coriander (Coriandrum sativum) are the usual ingredients of such digestive stimulant preparations both commercial and as home remedies. Spices have been generally believed to intensify salivary flow and gastric juice secretion, thereby aiding digestion [1]. Earlier studies from our laboratory have revealed that certain spices or their active principles stimulate bile flow and increase biliary bile acids which has an important role in digestion and absorption of food lipids [2–5]. The digestive stimulatory action of spices is also probably through a stimulation of activities of enzymes that participate in digestion. Hence, in the present investigation, we examined the favourable influence of three spice mixes derived from a few commonly consumed spices of known digestive stimulant action on digestive enzymes of pancreas and intestinal mucosa in experimental rats [6–9].

While these spices are experimentally evidenced to exert a beneficial influence on digestion individually, it would be interesting to understand the influence of combinations of such spices, since they are usually consumed as mixes in our dietary. Hence, in the present investigation, we examined the digestive stimulant influence of dietary intake of three combinations of spices (spice mixes), either selected from the prevalent recipes or customized to include spices desirable from the point of view of digestion. These spice components are: turmeric (Curcuma longa), red pepper (Capsicum annum), black pepper (Piper nigrum), coriander (Coriandrum sativum), cumin (Cuminum cyminum), ginger (Zingiber officinale) and onion (Allium cepa).

2 Materials and methods

2.1 Spice mixes

Spices were procured from the local market. They were roasted lightly before blending into spice mixes. In the case of ginger, fresh rhizomes were cleaned, ground to a paste, dried at 60 °C and powdered. Similarly, in the case of onion, fresh bulbs were freeze-dried and powdered. Spice mixes were prepared by blending the component spices in different proportions as per recipes given below and powdering.

<table>
<thead>
<tr>
<th>Spice</th>
<th>Spice mix I</th>
<th>Spice mix II</th>
<th>Spice mix III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coriander</td>
<td>40</td>
<td>5</td>
<td>39</td>
</tr>
<tr>
<td>Red pepper</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Black pepper</td>
<td>5</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>Cumin</td>
<td>20</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Ginger</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Onion</td>
<td>–</td>
<td>–</td>
<td>20</td>
</tr>
<tr>
<td>Mustard</td>
<td>5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Fenugreek</td>
<td>2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>1</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>Clove</td>
<td>1</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>Bay leaves</td>
<td>–</td>
<td>5</td>
<td>–</td>
</tr>
</tbody>
</table>

2.2 Chemicals

Benzoyl-l-arginine-p-nitroanilide, succinyl phenylalanine-p-nitroanilide, enterokinase, diethyldithiocarbamate, 2,4-dinitroso cyclic acid, and 3α-hydroxysteroid dehydrogenase were procured from Sigma Chemical (St. Louis, MO, USA). All other chemicals used were of analytical grade. Solvents were distilled before use.
2.3 Animal treatment

In one experiment to study the influence of spice mixes on digestive enzymes, groups of eight female Wistar rats weighing 120 ± 5 g were housed in individual cages and maintained on three experimental diets along with a parallel basal control diet for 8 weeks with free access to food and water. The basal diet consisted of: 21% casein; 10% cane sugar; 54% corn starch; 10% refined peanut oil; 1% NRC vitamin mixture and 4% Bernhardt-Tommarelli salt mixture. The experimental diets consisted of respective spice mixes incorporated into this basal diet at 2% level substituting an equivalent amount of corn starch. In a separate experiment, to study the influence of spice mixes on bile secretion, groups of ten female Wistar rats weighing 180 ± 5 g were similarly maintained for 8 weeks on the three spice mix diets.

2.4 Enzyme activity determinations

At the end of the feeding trial, the animals were sacrificed under light ether anaesthesia. Pancreas and small intestine (20–25 cm long segments between jejunum and caecum leaving about 5 cm on either side) were immediately excised. Intestines were flushed with ice-cold 0.9% saline. Intestinal segments were cut open longitudinally; mucosa was scraped with a microscopic slide [10]. The pancreatic tissue samples and intestinal mucosal scrapings were homogenized in ice-cold 0.9% saline and used for various enzyme assays. Standard methods were employed for determining the activities of lipase [11], amylase [12], trypsin and chymotrypsin [13, 14]. Lipase was assayed by aerobically incubating the pancreatic homogenate with olive oil suspension in saline in a buffered medium of pH 8.5, and reacting the released free fatty acids with copper nitrate; the copper-bound free fatty acids extracted into chloroform were then determined by a colour reaction with diethyl dithiocarbamate [11]. Activity of amylase was estimated by incubating the pancreatic homogenate with starch solution at pH 6.9 and reacting the released maltose with dinitrosalicyleic acid [12]. Trypsin and chymotrypsin were determined using benzoyl-L-arginine-p-nitroanilide and succinyl phenylalalanine-p-nitroanilide, respectively, as substrates [14] after preactivation with enterokinase according to the procedure of Lewis et al. [13]. Disaccharidases (succrase, lactase and maltase) were assayed by incubating the mucosal preparation with the respective disaccharide in maleate buffer (pH 6.0) and measuring the released glucose by the glucose oxidase method [15]. Alkaline and acid phosphatases in the mucosal preparations were measured using sodium β-glycerophosphate as the substrate at appropriate pH and measuring the released inorganic phosphorus by the method of Hubscher and West [16]. Protein content was determined by the modified phenol procedure using bovine serum albumin as standard [17].

2.5 Bile analysis

At the end of the feeding trial, the animals which were not fasted overnight were anaesthetized by an intraperitoneal injection of ethyl urethane (1.2 g/kg body wt.). Laparotomy was performed and the common bile duct was cannulated with PE-10 tubing (Thomas Scientific Co., USA) [18]. Bile duct cannulation was made on the bile duct about 2 cm from duodenum; the inserted cannula was pushed about 2 cm towards the liver from this site which is well past the point of entry of pancreatic juice. Bile was collected for 2.5 h. Bile volumes were measured and kept frozen pending analysis. Bile solids were determined by gravimetry. Lipids were extracted with chloroform-methanol by the method of Bligh and Dyer [19]. The chloroform layer was used for the determination of cholesterol and phospholipids according to Searcy and Bergquist [20] and Charles and Stewart [21], respectively. Total bile acids in the methanolic phase were estimated by employing 3α-hydroxysteroid dehydrogenase according to the procedure of Turley and Dietschy [22]. Statistical evaluation of the analytical data was done using Student’s t-test and a P-value of <0.05 was considered to be significant [23].

3 Results

In the present study, the three spice mixes examined have been fed to the animals at levels corresponding to about 3 times the average dietary intake of the spice constituents among Indian population. The 2% dietary levels used here, is based on calculated dietary intake of the constituent spices in the form of curry powder and on dietary survey conducted in India [24]. The food intake was essentially similar in various spice mix groups and the corresponding control group. Similarly, the gain in body weights during 8 weeks of spice treatment was comparable to controls (data not given).

The effect of dietary intake of various spice mixes on pancreatic lipase, amylase, and proteases is presened in Table 2. Pancreatic lipase was significantly stimulated by all three spice mixes. The enzyme activity was 13, 25 and 40% higher than control in animal groups maintained on spice mix I, spice mix II and spice mix III, respectively. Pancreatic amylase activity was higher (by about 16%) in animals fed spice mix I and spice mix III. Among the pancreatic proteases, chymotrypsin activity was increased by feeding spice mix I and spice mix III. The increase in the enzyme activity was as high as 77% in spice mix III treatment, while it was 25% in spice mix I treated animals. Pancreatic trypsin was, however, diminished in activity in all the three spice mix fed groups.

The influence of dietary spice mixes on digestive enzymes of small intestine is presented in Tables 3 and 4. Among the three spice mixes tested, spice mix I and spice mix II increased the activity of intestinal amylase. The enzyme activity was 40 and 19% higher than control in these animal groups. The enzyme activity was, however, lower in animals fed with spice mix III. While intestinal lipase was not affected by spice mix

<table>
<thead>
<tr>
<th>Animal/diet group</th>
<th>Lipase&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Amylase&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Trypsin&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Chymotrypsin&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>35.1 ± 1.69</td>
<td>0.298 ± 0.006</td>
<td>7.36 ± 0.41</td>
<td>0.65 ± 0.05</td>
</tr>
<tr>
<td>Spice mix I</td>
<td>39.5 ± 1.14*</td>
<td>0.345 ± 0.006*</td>
<td>4.40 ± 0.52**</td>
<td>0.81 ± 0.05*</td>
</tr>
<tr>
<td>Spice mix II</td>
<td>44.0 ± 1.56*</td>
<td>0.297 ± 0.011</td>
<td>3.40 ± 0.34**</td>
<td>0.80 ± 0.07</td>
</tr>
<tr>
<td>Spice mix III</td>
<td>49.3 ± 3.69**</td>
<td>0.345 ± 0.020*</td>
<td>4.21 ± 0.13**</td>
<td>1.15 ± 0.14*</td>
</tr>
</tbody>
</table>

Mean values ± SEM of eight animals per group

<table>
<thead>
<tr>
<th>Source of fat/min/mg protein</th>
<th>Lipase&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Amylase&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Trypsin&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Chymotrypsin&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) mmol FFA/min/mg protein</td>
<td>0.298 ± 0.006</td>
<td>7.36 ± 0.41</td>
<td>0.65 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>b) mmol maltose/min/mg protein</td>
<td>0.345 ± 0.006*</td>
<td>4.40 ± 0.52**</td>
<td>0.81 ± 0.05*</td>
<td></td>
</tr>
<tr>
<td>c) mmol p-nitroanilide/min/mg protein</td>
<td>0.297 ± 0.011</td>
<td>3.40 ± 0.34**</td>
<td>0.80 ± 0.07</td>
<td></td>
</tr>
</tbody>
</table>

* Mean values were significantly higher than control value (P < 0.05).
** Mean values were significantly lower than control value (P < 0.05).
Table 3. Influence of dietary spice mixes on digestive enzymes of small intestine

<table>
<thead>
<tr>
<th>Animal/diet group</th>
<th>Lipase&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Amylase&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Alkaline phosphatase&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Acid phosphatase&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>194.9 ± 20.4</td>
<td>37.3 ± 1.01</td>
<td>17.6 ± 1.57</td>
<td>0.97 ± 0.09</td>
</tr>
<tr>
<td>Spice mix I</td>
<td>96.6 ± 16.7**</td>
<td>52.3 ± 9.31*</td>
<td>13.1 ± 0.65**</td>
<td>0.78 ± 0.06</td>
</tr>
<tr>
<td>Spice mix II</td>
<td>231.9 ± 8.09</td>
<td>44.3 ± 3.80*</td>
<td>16.2 ± 1.16</td>
<td>0.84 ± 0.06</td>
</tr>
<tr>
<td>Spice mix III</td>
<td>236.4 ± 29.9</td>
<td>21.7 ± 5.57**</td>
<td>12.3 ± 1.15**</td>
<td>0.61 ± 0.02**</td>
</tr>
</tbody>
</table>

Mean values ± SEM of eight animals per group
a) nmol FFA/min/mg protein
b) nmol maltose/min/mg protein
c) μmol Pi/h/min/mg protein
* Mean values were significantly higher than control value ($P < 0.05$).
** Mean values were significantly lower than control value ($P < 0.05$).

II and spice mix III, spice mix I brought about a significant reduction of this enzyme activity (nearly half). Alkaline phosphatase activity was also lower in spice mix I treatment. Similarly, diminished phosphatase activity (both alkaline and acid) was evidenced in spice mix III fed animals. Activities of intestinal disaccharidases (lactase, maltase and sucrase) were generally lowered by all the spice mixes tested. The decrease in lactase activity brought about by spice mixes was in the range of 32–50%. Maltase activity was lowered by about 27% by spice mix I and spice mix III. A 41–50% decrease in sucrase activity was seen as a result of spices mixes treatment.

The influence of spice mixes on bile flow rate and biliary solids is shown in Fig. 1. All the three spice mixes studied brought about a significant increase in bile flow rate, the extent of increase being 30, 41 and 74% in spice mix I, spice mix II and spice mix III fed animals, respectively. Bile solids secreted per hour were also higher in all the three experimental groups. Spice mix III brought about an enormous stimulation of bile solids secretion.

Table 4. Influence of dietary spice mixes on intestinal disaccharidases

<table>
<thead>
<tr>
<th>Animal/diet group</th>
<th>Lactase</th>
<th>Maltase</th>
<th>Sucrase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.94 ± 0.19</td>
<td>268.4 ± 9.00</td>
<td>8.26 ± 0.51</td>
</tr>
<tr>
<td>Spice mix I</td>
<td>1.32 ± 0.15**</td>
<td>196.2 ± 17.1**</td>
<td>4.89 ± 0.98**</td>
</tr>
<tr>
<td>Spice mix II</td>
<td>0.97 ± 0.12**</td>
<td>256.0 ± 1.06</td>
<td>4.40 ± 0.42**</td>
</tr>
<tr>
<td>Spice mix III</td>
<td>1.07 ± 0.06**</td>
<td>193.2 ± 7.37**</td>
<td>4.17 ± 0.08**</td>
</tr>
</tbody>
</table>

Mean values ± SEM of eight animals per group
Activity unit: nmol glucose/min/mg protein
** Mean values were significantly lower than control value ($P < 0.05$).
The effect of dietary spice mixes on bile acid secretion is shown in Fig. 2. The bile acids secreted by the animals fed on spice mix III were more than double those secreted by the animals in the control group (106% higher). Spice mix II also brought about a very high stimulation, viz. 88% of bile acids secretion while spice mix I stimulated the same to an extent of 38%. Biliary lipid values of spice mix fed animals are presented in Table 5. None of the spice mixes examined exerted any influence on secretion of biliary cholesterol. Spice mix III brought about a significant increase (54%) in biliary phospholipid secretion, while the other two spice mixes did not exert any influence.

**Discussion**

Any food additive which has a digestive stimulant action is expected to stimulate the digestive secretions (gastric, bile and pancreatic) and/or stimulate the digestive enzymes of gastric, pancreatic and intestinal juices. In addition, they may also enhance biliary secretion of bile acids which play a major role in fat digestion and absorption. Recent studies on experimental animals have established a positive influence of several common spices or their active principles on pancreatic and intestinal digestive enzymes [6–9] and on bile flow and bile acid secretion [2–5].

Whereas these studies have employed individual spices, the present study examines selective combinations of spices for their influence on digestion. Spice mix I and spice mix II used in this study are based on recipes commonly used in Indian households. Incidentally, these two mixes contain spices that have been evidenced to have a positive influence on digestion. The constituent spices of these spice mixes are turmeric, red pepper, black pepper, cumin and coriander, and also ginger in the case of spice mix I. Spice mix III has been formulated using those spices which have been found to exert the maximum beneficial influence on digestive enzymes and bile secretion individually, viz. turmeric, red pepper, black pepper, cumin, coriander, ginger, and onion. This study makes a comparison of the digestive stimulant potential of these three spice mixes by virtue of their component spices.

Among the digestive enzymes, those of pancreatic origin contribute more to the overall process of digestion. Pancreatic lipase plays a vital role in digestion of dietary fat. The prominent enhancing influence of the three spice mixes on this enzyme suggests their beneficial role in digestion. The stimulatory influence of spice mixes was also seen on amylase and chymotrypsin, this effect being particularly enormous on the latter by spice mix III. The influence of all the three spice mixes on trypsin activity, however, was contrary. The negative influence of spice mixes on pancreatic trypsin is probably compensated by their stimulatory influence on the other protease, namely chymotrypsin. Stimulation of pancreatic amylase by spice mix could be significant in the Indian context, where starch being the major ingredient of cereals contributes over 75% of dietary energy intake.

All the three spice mixes tested either had no influence or a negative influence on the terminal digestive enzymes of the small intestine. The only exception was amylase which was stimulated to a significant extent by spice mixes I and II. The role of intestinal lipase and amylase in the overall process of digestion is normally minimal, and probably gains importance only when the titres of pancreatic enzymes are limiting [25]. Although many of the component spices have been documented in the earlier studies to have a stimulatory influence on one or more intestinal disaccharidases, the spice mixes examined in the current study, did not produce a similar effect on these terminal enzymes of carbohydrate digestion.

The role of bile and biliary bile acids in fat digestion and absorption is well known. Hence, stimulation of bile secretion, especially that of bile with higher levels of bile salts is probably a major mechanism by which spices aid in digestion. In the present study, all the three spice mixes have prominently enhanced the rate of bile juice production as well as that of bile acid output. Thus, it could be inferred that these spice mixes make a significant contribution to the process of fat digestion and absorption through their stimulatory influence on bile enabling the micelle formation. Many of the individual spices that constitute these spice mixes have also been documented to have a similar stimulatory influence on bile flow and/or bile acid secretion [2–5]. The higher secretion of bile solids by the rats fed the spice mixes could be attributed to the higher rate of bile acid output. Biliary lipids essentially remained unaltered except in the case of spice mix III which brought about 54% increase in phospholipid secretion.
Among the three spice mixes examined here, spice mix III had a relatively greater stimulatory influence particularly on bile secretion, bile acid output and the activities of pancreatic lipase, chymotrypsin and amylase. This particular spice mix has been customised so as to include all those individual spices that are desirable from the point of view of stimulation of digestion, based on earlier evidence. The component spices of this spice mix, viz. turmeric, red pepper, black pepper, cumin, coriander, ginger and onion have been included at levels optimal for their beneficial influence. Onion which is exclusively present in this spice mix has been documented to have a pronounced beneficial influence on bile and pancreatic enzymes \[4, 9\]. It may also be mentioned here that spice mixes I and II include small amounts of a few other spices whose digestive stimulant potential has not been experimentally documented.

The results of the present study indicate that the stimulatory influence of the component spices of the spice mixes on digestive enzymes of pancreas and small intestine is not additive. For example, curcumin, capsaicin, piperine (active principles of turmeric, red pepper, and black pepper, respectively) and ginger have been found to stimulate pancreatic lipase, amylase, trypsin and chymotrypsin to a great extent \[8\]. The beneficial influence of their combination in any of the three spice mixes employed in this study, however, is not as high in magnitude as that reported for the individual spices. The activity of trypsin in particular which was enormously enhanced in the case of all the individual spices, was surprisingly reduced by all the three spice mixes studied here. Similarly, contrary to the expected stimulation of the activities of intestinal disaccharidases by the spice mixes, the enzyme activities remained subdued as a result of spice mix treatments.

On the other hand, it is interesting to note that all the three spices mixes examined in this investigation brought about an enormous stimulation of bile flow rate and bile acid output compared to any of the individual spice components reported earlier \[2–5\]. This suggests a tendency towards an additive effect of the individual spices as far as their stimulatory influence on bile is concerned.

In summary, the present study documents the favourable influence on digestion of spice mixes derived from a few commonly consumed spices. This inference emanates from the animal study that has examined secretion of bile and activities of digestive enzymes of both pancreas and small intestine. The higher secretion of bile especially with an elevated level of bile acids and a beneficial stimulation of pancreatic digestive enzymes, particularly of lipase could probably be the two mechanisms by which these combinations of spices aid in digestion. The extent of stimulation of digestive enzymes, however, was lower than that expected of the individual spice components. On the other hand, the stimulation of bile flow and bile acid secretion brought about by these spice mixes was greater than that reported for individual component spices \[2–5\]. While spice mixes I and II employed in this study are commonly used in Indian culinary in preparations such as ‘Rasam’ and ‘Curry’, respectively, spice mix III, which has been specifically customised to include onion, could be used in preparations such as ‘Sambar’ to derive maximum digestive stimulant benefit.

5 References


Received February 20, 2002
Accepted July 24, 2002