Inhibitory Activities of Some Mucilages and Gums Against Certain Intestinal Disaccharidases

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Abstract: The inhibitory activities of some plant mucilages (taro, okra, Jew’s mellow and aloe vera mucilages) and some gums (arabic, tragacanth, olibanium and mastic gums) were tested on certain intestinal disaccharidases in vitro. Different concentrations (50, 100, 150 and 200 ppm) of each mucilage and gum were employed to evaluate their potentials on intestinal disaccharidases after pre-incubation with enzyme. The obtained data showed that the mucilages and gums under investigation possessed inhibitory activities for certain intestinal disaccharidas es (i.e. intestinal invertase, maltase or lactase). The inhibitory activities of mucilages and gums were very varied each to other. The inhibitory activities of mucilages and gums were proportioned with the concentration of polysaccharide. In conclusion, the mucilages and gums under investigation can be used in medical and pharmaceutical fields as an adjunct to the dietary management of obesity and diabetes.

Key words: Inhibitory activities, mucilages, gums, intestinal disaccharidases

INTRODUCTION

The plant mucilages and gums are polysaccharides which form colloidal solution in water from which they can often precipitated. They found in a widespread number of plants and also in some microorganisms. They can exist either as a secondary membrane thickening material or as intracellular substance. They are used in medical and pharmaceutical purposes (Smith and Montgomery, 1959). In the recent years, plant polysaccharides especially mucilages and gums has been reported to be having biological activity in human and animal. Antitumor, immunological, anticomplementary, anti-inflammatory, anticoagulant, antiviral, hypcholesterolemic and hypoglycemic activities have been observed in a wide range of polysaccharides. Polysaccharides constitute the subject matter of several excellent researches; either in vivo or in vitro. It has been observed that plant polysaccharide, interact with digestability of foods, either in vitro or in vivo. It is also well known to us that increasing the dietary fiber content of diets (including gums and mucilages) affects the metabolism of nutrients (i.e. carbohydrates, proteins and lipids) either in humans or in animals. The presence of “indigestible polysaccharides” in casein diets fed to rats was reported to significantly increase fecal nitrogen excretion and significantly decrease the digestibility of casein. In other words, polysaccharides of soluble dietary fiber did not reduce protein digestibility only but also reduced starch digestibility (Kelsay et al., 1978; Harmuth-Hoene and Schwerdtfeger, 1979; Ikeda and Kusano, 1983 and Kashef et al., 2008).

Dietary fibers, including gums and mucilages, are able to alter the digestion rate by various mechanisms which depend on the chemical composition of the polysaccharides and their physical properties such as viscosity. Among the various fiber sources, some are reported to reduce the enzyme activity, while others have no effect (Gagne and Acton, 1983; Peter, 1989 and Harland, 1989). The inhibitory activities of mucilages and gums under investigation were studied on some digestive enzymes in vitro. Data showed that pre-incubation of digestive enzyme (i.e. pancreatic amylase, trypsin or lipase) or enzyme substrate with different concentrations of each mucilage or gum led to significant inhibition of enzyme activity (Hassan, 2004 and Afify et al., 2004). Few studies were concerning the inhibitory activities of most polysaccharides on intestinal disaccharidases, especially mucilages and gums of our study. Infante et al. (2008) studied the effect of legume dietary fiber on rat disaccharidase in vitro. The results suggested that dietary fiber may impair carbohydrate availability and may contribute to the low glycemic index proper of these foodstuffs. Modulatory effect of fenugreek seed mucilage and spent turmeric on intestinal and renal disaccharidases in streptozotocin induced diabetes.
diabetic rats was studied by Nandini et al. (2000) and Kumar et al. (2005). They found that fenugreek seed mucilage and spent turmeric supplemenations were beneficial in alleviating the reduction in maltase activity during diabetes, however not much change in the activities of sucrase and lactase was observed upon feeding. The present study was planed to investigate the inhibitory activities of some mucilages and gums against certain intestinal disaccharidases (invertase, maltase and lactase). Although the effects were assayed in vitro, the results of this work should be relevant to the human body.

MATERIALS AND METHODS

Materials:

Plant Material:
Three plant samples, taro tubers (Colocassia esculenta, family Araceae); okra fruits (Hibiscus esculenta L., family Malvaceae); and Jew’s mellow leaves (Corchorous olitorius, family Tiliaceae), and four gums, gum arabic (from Acacia Senegal, family Leguminosae); gum tragacanth (from Astragalus gummifer, family Leguminosae); gum olibanium (from Boswellia carterii, family Frankincense); and gum mastic (from Pistacia lentiscus, family Anacardiaceae), were purchased from a local market at Giza, Egypt. Aloe leaves (Aloe vera, family Liliaceae) were collected from Orman garden at Giza, Egypt.

Chemicals:
Glucose kit (for determination of glucose) was from Biocon, Germany. All other chemicals were of analytical reagent grade.

Animals:
Male Wistar white rats were purchased from Research Institute of Ophthalmology, Giza, Egypt.

Methods:

Extraction of Crude Mucilages:
The crude mucilage of taro was extracted from corms using the procedure reported by Lin and Huang (1993). The crude mucilages of okra and Jew’s mellow were extracted from the fruits and leaves using the procedure reported by El-Mahdy and El-Sebaiy (1984). The crude mucilage of aloe vera was extracted from the leaves using the procedure reported by Gowda et al. (1979).

Preparation of Gums:
The crude gums were prepared from gum arabic, gum tragacanth, gum olibanium and gum mastic using the procedure reported by Tischer et al. (2002).

Assay of Intestinal Disaccharidases Inhibitory Activity:
The intestinal disaccharidases inhibitory activity was assayed for polysaccharides under investigation based on the method of Dahlqvist (1968).

Animals:
Male Wistar white rats, weighing 200-250 g, were used for the experiment. The animals were housed in a controlled environment and fed, ad libitum, on a regular laboratory diet.

Preparation of Intestinal Homogenate:
Rats were decapitated, and the intestine was removed by cutting of both the upper end of the duodenum and the lower end of the ileum. The entire intestine content was washed with cold saline solution (0.9% NaCl, w/v). The intestine was turned inside out and the mucosa is scraped off with a piece of glass. Mucosa was homogenised with four parts of cold distilled water by ultrasonic probe (frequency at 10 kHz) for 1 min. The tube was chilled with crushed ice before and during homogenization. The homogenate was centrifuged at 4000 rpm for 10 min and supernatant was used for disaccharidases inhibitory activity assay.

Procedure:
A known volumes (10, 20, 30 and 40 μl) of each polysaccharide solution (0.1%) were added to 50 μl of diluted supernatant (dilution factor = d). The solutions were mixed well and incubated at 37°C for 30 min. 100 μl of substrate-buffer solution (0.056 M disaccharide in 0.1 M sodium maleate buffer, pH 6.0) was added
and mixed well. After exactly 60 min, the reaction is interrupted by incubating at 100°C for 5 min, then 200 μl of distilled water were added and mixed well. The liberated glucose was determined by glucose kit. Activities of sucrase, maltase and lactase were calculated from the following equation. The inhibitory activities were calculated by the difference.

\[
\text{Disaccharidase activity} = \frac{a \cdot d}{n \cdot 1080}
\]

Where: \(a\) is amount of glucose (μg) liberated in 60 min (sample-blind), \(d\) is dilution factor for the enzyme solution (supernatant, 50 μl) and \(n\) is number of glucose molecule per molecule of disaccharide.

**Determination of Glucose:**

Glucose content was determined using the method described by Trinder (1969) with Biosub Glu kit (Biocon, Germany).

**Statistical Analysis:**

The results were analysed by an analysis of variance (\(P<0.01\)) and the means separated by Duncan’s multiple range test. The results were processed by CoStat computer program (1986).

## RESULTS AND DISCUSSION

Mucilages and gums under investigation were tested for their inhibitory activities (in vitro) against certain intestinal disaccharidases (i.e. intestinal invertase, maltase or lactase). In this study, the enzyme activities were assayed after pre-incubation of individual mucilage or gum at various concentrations with enzyme for suitable period.

### Intestinal Invertase:

Four different concentrations (50, 100, 150 and 200 ppm) of each mucilage and gum were employed to evaluate their potentials on intestinal invertase after pre-incubation with enzyme. The obtained results are presented in Table (1). Data showed that there were inhibitory effects of mucilages and gums at various concentrations. Inhibitory activities of mucilages and gums were elevated with higher concentrations. The maximum inhibitory effect (41.5%) was observed with taro mucilage at higher concentration (200 ppm, IC\(_{50}\) = 217 ppm). The lower inhibitory activity (24.8%) was observed with gum arabic at the same concentration (IC\(_{50}\) = 412 ppm). At higher concentration (200 ppm), the inhibitory activities of mucilages and gums were in the following decreasing order: taro mucilage > okra mucilage > gum mastic > Jew’s mellow mucilage > aloe vera mucilage > gum elbanium > gum tragacanth > gum arabic.

### Intestinal Maltase:

The inhibitory activities of mucilages and gums at different concentrations (50, 100, 150 and 200 ppm) on intestinal maltase are shown in Table (2). Significant reductions in intestinal maltase activity were obtained by addition of polysaccharides to enzyme. The highest inhibition of these polysaccharides was noticed with gum mastic (34.60%, IC\(_{50}\) = 289 ppm) at concentration of 200 ppm. Okra mucilage also possessed high inhibitory activity but less than that of gum mastic at the same concentration, where inhibition reached 31.80% (IC\(_{50}\) = 317 ppm). The inhibition percentages of intestinal maltase were more than 20% with gum arabic, gum elbanium, taro mucilage and Jew’s mellow mucilage (28.50, 26, 25.60 and 22.30%, respectively). Inhibitory activities of gum tragacanth and aloe vera mucilage less than 20% were 18.40 and 15.60%, respectively.

### Table 1: Inhibitory activity of mucilages and gums for intestinal invertase after pre-incubation of enzyme with polysaccharide

<table>
<thead>
<tr>
<th>Polysaccharide</th>
<th>Concentration of polysaccharide (ppm)</th>
<th>Inhibitory unit</th>
<th>Inhibition %</th>
<th>Inhibitory unit</th>
<th>Inhibition %</th>
<th>Inhibitory unit</th>
<th>Inhibition %</th>
<th>Inhibitory unit</th>
<th>Inhibition %</th>
<th>L.S.D</th>
<th>IC(_{50})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taro mucilage</td>
<td>50</td>
<td>4.35 +0.09a</td>
<td>21.55</td>
<td>6.72 +0.14b</td>
<td>33.27</td>
<td>7.61 +0.13c</td>
<td>37.68</td>
<td>8.39 +0.28c</td>
<td>41.5</td>
<td>0.84</td>
<td>217</td>
</tr>
<tr>
<td>Okra mucilage</td>
<td>100</td>
<td>0.83 +0.02a</td>
<td>15</td>
<td>1.31 +0.02b</td>
<td>23.7</td>
<td>1.64 +0.01c</td>
<td>29.5</td>
<td>2.12 +0.06d</td>
<td>38.2</td>
<td>0.22</td>
<td>258</td>
</tr>
<tr>
<td>Jew’s mellow mucilage</td>
<td>150</td>
<td>1.67 +0.06a</td>
<td>9</td>
<td>2.91 +0.14b</td>
<td>15.6</td>
<td>4.54 +0.16c</td>
<td>24.4</td>
<td>5.42 +0.20d</td>
<td>29.1</td>
<td>0.73</td>
<td>334</td>
</tr>
<tr>
<td>Aloe vera mucilage</td>
<td>200</td>
<td>1.45 +0.06a</td>
<td>7.7</td>
<td>2.47 +0.12b</td>
<td>13.2</td>
<td>3.84 +0.16c</td>
<td>20.6</td>
<td>5.30 +0.13d</td>
<td>28.5</td>
<td>0.58</td>
<td>358</td>
</tr>
<tr>
<td>Gum arabic</td>
<td>50</td>
<td>0.87 +0.04a</td>
<td>6.2</td>
<td>1.62 +0.09c</td>
<td>11.5</td>
<td>2.45 +0.08c</td>
<td>17.5</td>
<td>3.48 +0.14d</td>
<td>24.8</td>
<td>0.44</td>
<td>412</td>
</tr>
<tr>
<td>Gum tragacanth</td>
<td>100</td>
<td>2.81 +0.13a</td>
<td>15.2</td>
<td>3.49 +0.12b</td>
<td>18.8</td>
<td>3.86 +0.14c</td>
<td>20.8</td>
<td>4.60 +0.16c</td>
<td>25</td>
<td>0.70</td>
<td>406</td>
</tr>
<tr>
<td>Gum elbanium</td>
<td>150</td>
<td>0.76 +0.03a</td>
<td>3.6</td>
<td>3.11 +0.13b</td>
<td>14.7</td>
<td>3.74 +0.14b</td>
<td>17.7</td>
<td>5.75 +0.16c</td>
<td>27.2</td>
<td>0.63</td>
<td>373</td>
</tr>
<tr>
<td>Gum mastic</td>
<td>200</td>
<td>0.38 +0.02a</td>
<td>7.1</td>
<td>0.95 +0.04b</td>
<td>17.4</td>
<td>1.44 +0.04c</td>
<td>26.4</td>
<td>1.79 +0.10d</td>
<td>32.7</td>
<td>0.28</td>
<td>296</td>
</tr>
</tbody>
</table>

* Values are means of three replicates ± SE, numbers in the same row followed by the same letter are not significant different at P< 0.01.

**Intestinal Malate:**

The inhibitory activities of mucilages and gums at different concentrations (50, 100, 150 and 200 ppm) on intestinal maltase are shown in Table (2). Significant reductions in intestinal maltase activity were obtained by addition of polysaccharides to enzyme. The highest inhibition of these polysaccharides was noticed with gum mastic (34.60%, IC\(_{50}\) = 289 ppm) at concentration of 200 ppm. Okra mucilage also possessed high inhibitory activity but less than that of gum mastic at the same concentration, where inhibition reached 31.80% (IC\(_{50}\) = 317 ppm). The inhibition percentages of intestinal maltase were more than 20% with gum arabic, gum elbanium, taro mucilage and Jew’s mellow mucilage (28.50, 26, 25.60 and 22.30%, respectively). Inhibitory activities of gum tragacanth and aloe vera mucilage less than 20% were 18.40 and 15.60%, respectively.
Intestinal Lactase:

Data in Table (3) show the effect of individual mucilage and gum on intestinal lactase activity after pre-incubation with enzyme. The inhibitory activity was increased with increasing the concentration of each polysaccharide. The inhibitory activities of mucilages and gums were very varied each to other. The obtained data revealed that the mucilages and gums under investigation possessed highest inhibition percentages against intestinal lactase more than 50%. The IC\textsubscript{50} values of polysaccharides were less than 200 ppm. From the obtained results, it could be arranged these polysaccharides according to their inhibition (%) in the following decreasing order: okra mucilage (84.30%) > gum mastic (79.20%) > gum tragacanth (78.30%) > Jew’s mellow mucilage (72.80%) > aloe vera mucilage (72.60%) > taro mucilage (68.20%) > gum arabic (65.40%) > gum olibanium (50.60%).

Table 3: Inhibitory activity of mucilages and gums for intestinal lactase after pre-incubation of enzyme with polysaccharide

<table>
<thead>
<tr>
<th>Polysaccharide</th>
<th>Concentration of polysaccharide (ppm)</th>
<th>Inhibitory activity (% at each concentration)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Taro mucilage</td>
<td>0.79±0.03a</td>
<td>5.6</td>
</tr>
<tr>
<td>Okra mucilage</td>
<td>1.52±0.03a</td>
<td>15.5</td>
</tr>
<tr>
<td>Jew’s mellow mucilage</td>
<td>0.59±0.01a</td>
<td>5.0</td>
</tr>
<tr>
<td>Aloe vera mucilage</td>
<td>3.48±0.07a</td>
<td>29</td>
</tr>
<tr>
<td>Gum arabic</td>
<td>0.63±0.03a</td>
<td>5.7</td>
</tr>
<tr>
<td>Gum tragacanth</td>
<td>1.30±0.09a</td>
<td>12</td>
</tr>
<tr>
<td>Gum olibanium</td>
<td>0.99±0.02a</td>
<td>12</td>
</tr>
<tr>
<td>Gum mastic</td>
<td>1.82±0.05a</td>
<td>13</td>
</tr>
</tbody>
</table>

* Values are means of three replicates ± SE, numbers in the same raw followed by the same letter are not significant different at P< 0.01.

In general, the data obtained concerning the inhibitory activities of mucilages and gums under investigation could be revealed that activities, as indicated by inhibitory units and inhibition percentages, are proportioned with the concentration of each polysaccharide. All mucilages and gums under investigation are possessed inhibitory activities against certain intestinal disaccharidases. Inhibitory activity of each polysaccharide is varied from enzyme to other and also from polysaccharide to other.

The inhibitory activity of mucilages and gums could be attributed to that these polysaccharides are reducing the enzyme-substrate binding or affecting on enzyme directly or altering the enzyme conformation or interacting with enzyme (as protein). These possibilities are in agreement with those of many authors (Felix and Zahola, 1993; Khokhar, 1994; Hassan, 2004 and Kashef et al., 2008). Viscous fibres like guar gum are shown to bring about slow intestinal absorption of sugar by delayed gastric emptying or by interaction with digestive enzymes of the intestine (Schneeman, 1982). It found that, compared with a fiber-free diet in rats, 27 wk of pectin supplementation produced significant decreases in jejunal sucrose and lactase specific activities with the same trend observed for maltase specific activity (Thomsen and Tasman-Jones, 1982 and Koruda et al., 1988). Since proteins will interact to form complexes with polysaccharides of opposite net charge, it is not surprising that enzyme activities can be affected quite markedly by the presence of anionic polysaccharides. The mucilages and gums under investigation are also considered anionic polysaccharides. Kenjiro et al. (2003) found that there was an ionic interaction between acidic polysaccharides (APS) and proteins at the pH range in which APS were negatively charged and proteins were also positively charged, and in enzymes the interaction was detected as a change in the enzyme activity. At pH 4.7, acid phosphatase (pl, 5.4), α-glucosidase (pl, 5.7), and β-glucosidase (pl, 7.3) were inhibited by APS to various extents.

Concluding Remarks:

Finally, it could be concluded that mucilages and gums under investigation can be used in the medical and pharmaceutical fields as an adjunct to the dietary management of obesity and diabetes.
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REFERENCES


