Suppression by Water Extracts of Sophora Plants of Sucrose-induced Hyperglycemia in Rats and Inhibition of Intestinal Disaccharidases In Vitro

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Note

Partially purified hot-water extracts of the roots of plants of the Sophora family suppressed the increase in blood glucose concentration of rats in the oral sugar tolerance test. The extracts also inhibited rat intestinal sucrase and maltase. The most potent sample was about 15 times more active than catechin, a positive control, in these experiments.

Key words: Sophora extract; blood glucose level; disaccharidase inhibition; sucrase; oral sugar tolerance test

Hot water extracts of Sophora radix, the root of plants of the Sophora family, which grow in hot and wet climates such as that of Fujian in China, are used as a folk medicine against various diseases. The chemical structures of some constituents of these extracts have been described.1,2) When the extract is used primarily against a diabetogenic skin disease, it also shows an anti-hyperglycemic effect. One of the causes of hyperglycemia is that an excess glucose is produced by a high disaccharidase activity in the intestine.3,4) In this connection, we surveyed Sophora radix from various sources in tests to suppress the increase in blood glucose concentration in rats and to inhibit sucrase and maltase prepared from rat small intestines. We report here that partially purified materials of radix from four regions among 41 samples suppressed the increase in the blood glucose concentration induced by an oral dose of sucrose in rats and also inhibited the intestinal disaccharidase activities.

The shade-dried Sophora radix (100 g), most of which were supplied by the Fujian Provincial Bureau of Public Health in China, and others of which were purchased from oriental medical shops in China or Japan, were boiled in water for about 10 hr and the resulting water-soluble materials were concentrated in vacuo to dryness. The residue was called SOPHORA. To the residue, ethyl acetate and water were added and then separated into layers. The organic layer was concentrated to dryness to give another residue, to which water and ethyl acetate were again added. The residue obtained from the water layer after concentration to dryness in vacuo was called the SEW fraction in this study.

A water solution (2 ml) containing sucrose (2 g/kg rat) and various amounts of SOPHORA or SEW was given by a stomach tube to 5-week-old KWL Wistar male rats (about 115 g) after 14 hr of starvation. Blood was taken at intervals from the tail vein and the blood glucose level was measured enzymatically (a New Blood Sugar Test Kit, Boehringer Mannheim-Yamanouchi, Co.).5) Figure 1 shows the effects of SOPHORA 2 on the blood glucose concentration in rats given sucrose. The statistical analysis was done by the ANOVA method and the Tukey multiple comparison test using the software GB stat (R). Without SOPHORA, the glucose concentration increased with time to about 5.2 mm after 30 min of the administration of the sugar, and then decreased. When SOPHORA was given simultaneously, the increase in the blood glucose concentration was suppressed significantly. Using data of the increments of the glucose concentration 30 min after dosing, the ED50, which is the dose of SOPHORA to suppress the increase in the relative blood glucose level to 50% of the control, was evaluated as 6.8 mg/kg rat. Along with this value, ED50 values of other SOPHORA and SEW obtained from 6 Sophora radix are listed in Table I. SOPHORA samples of four kinds of Sophora radix (No. 1-4) showed a similar level of activity in suppressing the blood glucose level. They were more active than catechin, a positive control,6) so that their ED50 values were almost 60 times smaller than that of catechin, as shown in Table I. SOPHORA samples 5 and 6 had almost no activity at 15 mg/kg rat. SEW 1 was more potent than the corresponding SOPHORA, but SEW 2 and 3 were less potent than the corresponding SOPHORA samples. Since SEW samples 7-9 and 32 others did not inhibit disaccharidases, as will be described below, SEW 7-9 and the corresponding SOPHORA were not tested for the effects on the blood glucose level.

SEW samples were also tested with crude disaccharidases prepared from rat small intestines. The enzyme activity was assayed by the method of Dahlqvist.7) The inhibitory activity was measured by incubating the enzyme (50 μl) with 25 mM sucrose for the sucrase (7 μg protein/μl) or 12.5 mM maltose for the maltase (3.5 μg protein/μl) in 50 mM potassium phosphate buffer (pH 6.5, 50 μl) and a water solution (10 μl) containing various amounts of SEW at 37°C for 15 min. The reaction mixture was
then heated for 2 min in a boiling water bath to stop the reaction, and then the amounts of glucose produced were measured by the Somogyi-Nelson method in the sucrase assay and by the glucose oxidase method in the maltase assay. Protein was measured by the Bradford method.

**Fig. 1.** Effects of Hot-water Extracts (SOPHORA 2) on the Blood Glucose Concentration in Sucrose-loaded Rats.
Sucrose (2 g/kg rat) and SOPHORA 2 were administered to rats as a water solution (2 ml) at time zero. The blood glucose concentration is expressed as the increment of the concentration from the time zero value. The results are expressed as mean ± SEM for n=6. The blood glucose concentrations at time zero were in ranges of 4.3±0.3 and 4.5±0.2 mEq after 14 hr of starvation, respectively. The blood glucose concentrations were significantly (p<0.05) influenced by dietary treatments according to the two-way ANOVA followed by the Tukey multiple comparison test. Means bearing different letters are significantly different (p<0.05) from the control. —-a--, sucrose alone; —-b--, sucrose plus SOPHORA (2.5 mg/kg rat); —-c--, sucrose plus SOPHORA (7.5 mg/kg rat); —-d--, sucrose plus SOPHORA (15 mg/kg rat).

**Table 1.** Inhibitory Effects of SOPHORA and their SEW Fractions of Various Sophora Family Plants on the Blood Glucose Level in the Oral Sucrose Tolerance Test and on Crude Sucrase and Maltase Prepared from Rat Small Intestines

<table>
<thead>
<tr>
<th>No. or compound</th>
<th>Origin</th>
<th>(ED_{90}) (mg/kg rat) (^a)</th>
<th>Sucrase (^c)</th>
<th>Maltase (^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>China, Fujian (Fu Qing)</td>
<td>6.4</td>
<td>19±0.5</td>
<td>115±5</td>
</tr>
<tr>
<td>2</td>
<td>China, Fujian (Yun Xiao)</td>
<td>6.8</td>
<td>115±5</td>
<td>145±5</td>
</tr>
<tr>
<td>3</td>
<td>China, Fujian (Nan An)</td>
<td>6.3</td>
<td>95±5</td>
<td>115±5</td>
</tr>
<tr>
<td>4</td>
<td>China, Fujian (Sheng Xi)</td>
<td>8.0</td>
<td>95±5</td>
<td>115±5</td>
</tr>
<tr>
<td>5</td>
<td>China, Shanghai</td>
<td>&gt;15</td>
<td>&gt;300</td>
<td>&gt;300</td>
</tr>
<tr>
<td>6</td>
<td>China, Guizhao</td>
<td>&gt;15</td>
<td>&gt;300</td>
<td>&gt;300</td>
</tr>
<tr>
<td>7</td>
<td>China, Beijing</td>
<td>&lt;6</td>
<td>&gt;300</td>
<td>&gt;300</td>
</tr>
<tr>
<td>8</td>
<td>Japan</td>
<td>&lt;6</td>
<td>&gt;300</td>
<td>&gt;300</td>
</tr>
<tr>
<td>9</td>
<td>Korea</td>
<td>&lt;6</td>
<td>&gt;300</td>
<td>&gt;300</td>
</tr>
</tbody>
</table>

**Fig. 2.** Effects of SEW 1 and 4, and Catechin on the Rat Small Intestinal Sucrase Activity.
---○--, SEW 1; ---●--, SEW 4; ---×--, catechin. Vertical bar indicates the standard deviation for \(n=5\).

Figure 2 shows the dose-response curves of SEW 1 and 4, and catechin, a positive control, in the inhibition of sucrase activity. The IC\(_{90}\) values of SEW 1 and 4 and catechin were 19, 95 and 291 \(\mu\)g/ml, respectively. The values of these and other samples are also listed in Table 1. SEW 1 was the most active among the samples and was about 15 times more active than catechin in terms of IC\(_{90}\). SEW samples 2-4 were about 5 times less active than SEW 1. Other SEW samples 5-9 did not show the inhibitory effect even at 300 \(\mu\)g/ml. Based on the calculated amounts of sucrase in the rat small intestine, the dose of SEW 2 is considered to be enough for the suppression of hyperglycemia of rats by inhibition of the enzyme. The rat intestinal sucrase occurs as a com-

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\(^a\) Measured with the oral sucrose tolerance test.
\(^b\) SEW for samples 1-9.
\(^c\) Mean±SD for 10 experimental runs.
\(^d\) Mean±SD for 3 experimental runs.
\(^e\) Not measured.
plex of sucrase and isomaltase,\(^{10}\) so the effects of SEW on the isomaltase were also tested. We observed inhibitory effects on the isomaltase similar to those on the sucrase (data not shown). Figure 3 shows the dose-response curves of SEW 1 and 2, and catechin in the inhibition of maltase activity. From these and similar curves, IC\(_{50}\) values were calculated (Table I). The activity of SEW 1 against the maltase was about 6 times less than against the sucrase, but the inhibitory activity of SEW 2-4 and catechin against the enzyme was almost the same level as that against sucrase. SEW 5-9 and the other 32 Chinese samples did not have any inhibitory effect even at 300 \(\mu\)g/ml on the enzyme.

Catechin is known to be an antihyperglycemic chemical to inhibit rat disaccharidases.\(^{6}\) We confirmed its effects. Hot water extracts of \textit{Sophora} radix showed similar trends; when they were inhibitory against rat intestinal disaccharidases, they suppressed the increase in the blood glucose concentration after sucrose feeding in rats. We observed various potencies of the extracts of \textit{Sophora in vivo} and \textit{in vitro} tests. No apparent relationship between producing areas of plants and activities was observed. It may be due to harvest time or post-harvest treatment. Further investigations would be needed to clarify this point. To find antihyperglycemic compounds from SOPHORA, we are now isolating the active components from the extracts of \textit{Sophora} plants and analyzing their chemical structures.

References