Hypoglycemic Effect of the Rhizomes of *Smilax glabra* in Normal and Diabetic Mice

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Received July 4, 1996; accepted September 10, 1996

The hypoglycemic effect of the rhizomes of *Smilax glabra* Roxburgh (Liliaceae) was investigated in normal and KK-Ay mice, one of the animal models of non-insulin dependent diabetes mellitus (NIDDM) with hyperinsulinemia. The methanol extract of rhizomes of *Smilax glabra* Roxburgh (SM, 100 mg/kg body weight) reduced the blood glucose of normal mice 4 h after intraperitoneal administration (p < 0.05), and also significantly lowered the blood glucose of KK-Ay mice under similar conditions (p < 0.001). However, SM did not affect the blood glucose in streptozotocin-induced diabetic mice, one of the animal models of insulin-dependent diabetes mellitus (IDDM) with hyperinsulinemia. SM also suppressed epinephrine-induced hyperglycemia in mice. SM-treated KK-Ay mice significantly decreased the blood glucose in an insulin tolerance test. We concluded that the hypoglycemic effect of SM raised insulin sensitivity.

**Key words** hypoglycemic effect; KK-Ay mouse; *Smilax glabra*; Liliaceae; insulin sensitivity

The rhizomes of *Smilax glabra* Roxburgh (Liliaceae) are used in the Orient as a traditional medicine for chronic skin disease and syphilis.1) The constituents of this plant have been chemically investigated, and include astilbin, isoengeletin, succinic acid, palmitic acid, and β-sitosterol.2) However, there is no experimental evidence of the hypoglycemic effect of these materials. In the present study, we examined the effect of Smilax Rhizome on blood glucose in normal and diabetic mice, and also the effect of insulin resistance in order to clarify the hypoglycemic mechanism.

**MATERIALS AND METHODS**

Rhizomes of *Smilax glabra* Roxburgh which was obtained in a Chinese market by Tochimoto Tenkaido Co., Ltd. (Osaka, Japan) was used in the present experiment. Five hundred grams of rhizomes were extracted with 2 l of methanol (65 °C, 2 h, 4 times). The methanol extracts were lyophilized (SM) and stored at 4 °C until use.

**Animals** Adult male ddY mice (SLC, Shizuoka, Japan) weighing 22–25 g and KK-Ay mice (Clea, Tokyo, Japan), 12 weeks old, were used. Streptozotocin-induced diabetic mice (STZ mice) were produced by intravenous injection with 150 mg/kg body weight of streptozotocin (Sigma, Tokyo, Japan). KK-Ay and STZ mice with blood glucose level above 300 mg/100 ml were considered to be diabetic and used in this study. The mice were housed in an air-conditioned room at 22 ± 2 °C with a 12 h light–12 h dark cycle. The animals were kept in the experimental animal room for 7 d with free access to food and water. Blood samples were withdrawn from the cavernous sinus with a capillary to determine blood glucose levels. SM was dissolved in saline.

**Epinephrine-Induced Hyperglycemic Mice** The adult ddY mice were given SM intraperitoneally (i.p.) and, 4 h later, the epinephrine (0.6 mg/kg body weight) solution was also administered i.p. Blood samples were collected 1 h thereafter.

**Oral Glucose Tolerance Test** After overnight (18 h) fasting, KK-Ay mice were given SM i.p. and, 4 h later, the glucose (2 g/kg body weight) solution was administered orally. Blood samples were collected before the administration of the glucose and 30, 60, 120 and 180 min later.

**Insulin Tolerance Test** After overnight (18 h) fasting, KK-Ay mice were given SM i.p. and, 4 h later, the insulin (0.5 U/kg body weight) solution was administered subcutaneously (s.c.). Blood samples were collected before the administration of the insulin and at 30 and 120 min later.

**Determination of Blood Glucose and Insulin** Blood glucose levels in mice were determined by glucose oxidase method,3) and plasma insulin was measured by double antibody method.4) All the data were expressed as mean ± S.E., and Student’s t-test was used for the statistical analysis. The values were considered to be different when the p value was less than 0.05.

**RESULTS**

**Effect of SM on Blood Glucose in Normal Mice** The mean blood glucose levels of mice after i.p. administration are shown in Fig. 1. These levels were compared to those found when 0.9% saline was administered alone (control group) and also to those found when tolbutamide (50 mg/kg body weight), a known hypoglycemic agent, was administered. The hypoglycemic effect of SM was dose-dependent. The plasma insulin levels in SM (100 mg/kg)-treated mice did not change at 4 h (Fig. 2). Tolbutamide-treated mice showed lower blood glucose level during the period 4 to 7 h after administration.

**Effect of SM on Blood Glucose in Diabetic Mice** The hypoglycemic effect of SM on the blood glucose of KK-Ay mice is shown in Table 1. SM of 20, 100 and 500 mg/kg-treated mice showed a significant decrease in blood glucose after 4 h when compared with 0 h (SM 20 mg/kg: p < 0.05, 500 mg/kg: p < 0.01, 100 mg/kg: p < 0.001). The mean plasma insulin of mice at 4 h after i.p. administration

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Table 1. Effect of SM on Blood Glucose in KK-Ay Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>0 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>5</td>
<td>666 ± 19</td>
<td>614 ± 53</td>
</tr>
<tr>
<td>SM</td>
<td>100</td>
<td>5</td>
<td>514 ± 65</td>
<td>291 ± 48*</td>
</tr>
<tr>
<td>Normal</td>
<td>500</td>
<td>5</td>
<td>690 ± 10</td>
<td>276 ± 9***</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. Significantly different from 0 h, *p < 0.05, **p < 0.01, ***p < 0.001. N, number of mice.

Fig. 1. Effects of SM and Tolbutamide on Blood Glucose in Normal Mice

- Control; - SM 0.8 mg/kg; -- SM 4 mg/kg; --- SM 20 mg/kg; Δ Δ Δ SM 100 mg/kg; — Δ Δ Δ tolbutamide 50 mg/kg. Each value represents the mean ± S.E. of 5–6 mice. Significantly different from prev value, *p < 0.05, **p < 0.01, ***p < 0.001.

Fig. 2. Effect of SM on Insulin in Normal and KK-Ay Mice

Each value represents the mean ± S.E. of 5–6 mice. Significantly different from prev value, *p < 0.05, ***p < 0.001. N.S. not significant.

Fig. 3. Effect of SM on Glucose Tolerance Test in KK-Ay Mice

- Control; • • • SM 100 mg/kg. Each value represents the mean ± S.E. of 5 mice. Significantly different from control, **p < 0.01, ***p < 0.001.

Fig. 4. Effect of SM on Insulin Tolerance Test in KK-Ay Mice

- Control; • • • SM 100 mg/kg. Each value represents the mean ± S.E. of 5–6 mice. Significantly different from control, *p < 0.05.

(100 mg/kg) is shown in Fig. 2. SM reduced the plasma insulin level from 253 ± 33 to 124 ± 6 μU/dl (*p < 0.05, Fig. 2), while at 100 or 500 mg/kg SM did not affect the blood glucose in STZ mice (data not shown).

Effect of SM on Blood Glucose in Epinephrine-Induced Hyperglycemic Mice The effect of SM injected i.p. to
epinephrine-induced hyperglycemic mice is shown in Table 2. SM-treated animals (100, 500 mg/kg body weight) showed lower blood glucose levels than the untreated group (100 mg/kg, p < 0.05; 500 mg/kg, p < 0.05).

**Oral Glucose Tolerance Test** SM-treated mice (100 mg/kg body weight, i.p.) showed a significant decrease in blood glucose after 30, 60 and 120 min compared with controls (Fig. 3).

**Insulin Tolerance Test** SM-treated mice (100 mg/kg body weight, i.p.) showed a significant decrease in blood glucose after 120 min compared with controls (p < 0.05, Fig. 4).

**DISCUSSION**

This study clearly showed that the methanol extract of the rhizomes of *Smilax glabra Roxburgh* (SM) produces a consistent hypoglycemic effect in normal mice. This effect was observed without any changes in plasma insulin. We also examined the therapeutic effects of SM on hyperglycemia in KK-Ay mice, one of the animal models of non-insulin-dependent diabetes mellitus (NIDDM). After treatment of mice with SM, hypoglycemia resulted with reduced plasma insulin and improved glucose tolerance. In terms of the hypoglycemic effect, the blood glucose in KK-Ay mice was lower than that in normal mice. From these findings it would appear that SM can be useful in therapy for NIDDM.

STZ mice, however, showed no decrease in blood glucose when treated with SM (data not shown). SM thus requires the presence of insulin to display its activity.

SM-treated KK-Ay mice also had lower blood glucose in the insulin tolerance test and hyperinsulinemia was improved. Insulin (0.5 U/kg)-treated KK-Ay mice did not have lower blood glucose, because of insulin resistance in the peripheral tissues, suggesting that SM lessens insulin resistance. SM also decreased blood glucose in epinephrine-induced hyperglycemic mice. From this finding, it seems likely that the hypoglycemic effect of SM may result from its decreasing hepatic glucose output in both normal and epinephrine-induced hyperglycemic mice. Further study would show how SM could become a useful drug in the treatment of diabetes through this unique therapeutic mechanism.

Many active components associated with the hypoglycemic activity were discovered: ginsenoside-Rb2 isolated from *Panax ginseng* C. A. Mayer (triterpenoid),

panaxans A, B, C, D and E isolated from *Panax ginseng* C. A. Mayer (glycan),

anemarans A, B, C and D isolated from *Anemarrhena asphodeloides* Bunge (glycan),

Atractans A, B and C isolated from *Atractylodes japonica* Koizumi (glycan),

and PO-2 isolated from *Polygonatum falcatum* (stereoid glycoside). We studied active components using bioassay-guided fractionation. It is not clear, however, that *Smilax glabra Roxburgh* contains components with similar structures. Further work will identify the active components associated with the hypoglycemic effect.

**Acknowledgment** The authors thank Dr. D. Mayer for assistance in preparing the manuscript.

**REFERENCES**