Hypoglycemic and Aldose Reductase Activity of *Polichia campestris*

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The hypoglycemic activity of 80% methanol extract of *Polichia campestris* (PM) was investigated in both normal and streptozotocin-induced diabetic mice, one of the insulin-dependent diabetic mellitus. PM (300 mg/kg) reduced the blood glucose of normal mice from 2 to 7 h after oral administration. It also decreased hyperglycemia from 594 ± 46 to 472 ± 31 mg/dl 4 h after oral administration in streptozotocin-induced diabetic mice (p < 0.05). PM strongly increased glucose uptake in adipose tissues. In addition, the petroleum ether fraction of PM inhibited aldose reductase activity. These experimental results suggest that the antidiabetic activity of *Polichia campestris* supports its use in traditional medicine.

**Key words** antidiabetic activity; *Polichia campestris*; Caryophyllaceae; oriental traditional medicine; streptozotocin; aldose reductase activity

*Polichia campestris* (Caryophyllaceae) is used as a traditional medicine for diabetes (polyuria and polydipsia) in East Africa; however, there is no experimental evidence of the antidiabetic activity of this plant. The purpose of this study was to examine the antidiabetic activity using a diabetic model animal. We also studied the aldose reductase activity implicated in many diabetic complications such as neuropathy and retinopathy.

**MATERIALS AND METHODS**

**Materials** Plant materials used were of leaves of *Polichia campestris* collected in Kenya in Africa. Fresh leaves were extracted with 80% methanol in a heating bath. The methanol extracts were lyophilized (abbreviated as PM) and stored at 4°C until just before use. The petroleum ether, benzene, AcOEt, CHCl₃, n-BuOH and H₂O layer were separated by conventional methods. PM was successively partitioned into petroleum ether, benzene, AcOEt, CHCl₃, n-BuOH and H₂O, and each layer was evaporated to dryness.

**Animals** Adult male ddY mice weighing 22–25 g were housed in an air-conditioned room at 22 ± 2°C with a 12 h light and 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 Blood samples were withdrawn from the cavernous sinus with a capillary to determine blood glucose levels.

The animals were divided into two groups. One was injected intravenously with 150 mg/kg body weight of streptozotocin (STZ), freshly dissolved in citrate buffer pH 4.5, and the other group was given buffer alone and used as a control. Eight days after injection of STZ, the blood glucose levels of all the mice were determined. Mice with a blood glucose level above 300 mg/dl were considered to be diabetic and were used in the study. Five to six animals were used for each group. The *in vivo* studies were started at 10:00—11:00 am.

**Oral Glucose Tolerance Test** After overnight (18 h) fasting, mice were given PM orally and, 4 h later, glucose (2 g/kg body weight) solution was administered orally. Blood samples were collected before the administration of the glucose and at 0.5, 1 and 2 h afterward. Blood samples for serum insulin determination were also taken at 0.5 h after the administration of glucose.

**Preparation of Isolated Adipocytes** Isolated adipocytes were prepared from whole epididymal fat pads of *ad libitum* fed animals. Briefly, the whole epididymal fat pads from 3 mice were removed, minced, and digested at 37°C with 1 mg/ml of crude collagenase type 1 ( Worthington, NJ) in 5% bovine serum albumin (BSA)–HEPES (10 mM HEPES, 2.5 mM NaH₂PO₄, 50 mg/ml BSA) buffer, pH 7.4. Following 40—60 min of digestion, the liberated cells were washed 4 times with 5% BSA–HEPES buffer, pH 7.4, and then centrifuged (1000 rpm, 2—3 min).

**Glucose Uptake Activity** Isolated mice adipocytes (2 × 10⁶) were incubated for 1 h at 37°C in Krebs-Ringer bicarbonate HEPES containing 0.1 mM glucose, 0.1 μCi/ml 1-[14C]glucose, and either PM or insulin in polyethylene bottles in a metabolic shaker bath; 0.5 ml of 6 N H₂SO₄ was then added, and incubation continued. After 2 h, the amount of glucose uptake was measured in an automated scintillation counter.

**Aldose Reductase Activity** Mice lens were homogenated in 135 mM Na, K-phosphate buffer (pH 7.0), followed by centrifugation at 15000 rpm for 15 min. The supernatant was used as crude aldose reductase enzyme fraction. Aldose reductase activity was measured according to the method of Dufrane et al. Incubation was carried out in 1 ml of medium (135 mM Na, K-phosphate buffer (pH 7.0)), 0.1 mM LiSO₄, 0.1 mM glyceraldehyde) at 30°C for 30 min, and terminated by the addition of 0.3 ml of 0.5 N HCl. One milliliter of 6 N NaOH containing 10 mM imidazole was added, and fluorescent nicotinamide adenine dinucleotide phosphate (NADP) was measured by fluorescence spectrophotometry at an emission wavelength of 360 nm.

**Determination of Blood Glucose and Insulin** Blood glucose levels in diabetic animals were determined by glucose oxidase method and serum insulin was measured by a double-antibody radioimmunoassay. All the data were expressed as means ± S.E.M. and Student's *t* test was used for the statistical analysis. The values were viewed as significantly different when the *p* value was less than 0.05.

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0.05.

RESULTS

Effect of PM on Blood Glucose in Normal Mice  
The mean blood glucose levels of normal mice at various time intervals after oral administration of PM are shown in Fig. 1. These levels were compared with the values in control mice administered saline alone and also in animals receiving 50 mg/kg body weight of tolbutamide, a known sulfonylurea hypoglycemic agent (Fig. 1). PM at 100 and 300 mg/kg lowered blood glucose from 0 to 7 h after the administration.

Effect of PM on Blood Glucose in STZ-Induced Diabetic Mice  
The hypoglycemic effects of PM on the blood glucose of STZ-induced diabetic mice are shown in Fig. 2. PM at 300 mg/kg-treated mice showed a decrease in blood glucose after 4 h compared with the basal values (PM 300; p < 0.05). Insulin-treated mice (5 U/kg body weight) exhibited a significant decrease in blood glucose at 2 h compared with the basal values (p < 0.001).

Oral Glucose Tolerance Test  
The glucose tolerance of PM after oral glucose loading is shown in Table 1. PM-treated animals (300 mg/kg body weight) showed a significant decrease in blood glucose levels after 120 min compared with controls (PM 142 ± 6 mg/dl, control 174 ± 9 mg/dl, p < 0.05). No differences in serum insulin were observed between the levels at the points before, 30 and 120 min after glucose administration compared with control (control; before 12 ± 1, 30 min 58 ± 3, 120 min 26 ± 3, PM 300 mg/kg; before 13 ± 1, 30 min 63 ± 17, 120 min 22 ± 2 µU/ml).

Glucose Uptake Activity  
PM increased adipocyte glucose uptake in a dose-dependent manner (control 2.5 ± 0.1, 50 µg/ml PM 4.5 ± 0.4 p < 0.05, 100 µg/ml PM 5.0 ± 0.5 p < 0.05, 200 µg/ml PM 5.5 ± 0.4 µmol p < 0.001). Insulin (500 µU/ml) stimulated glucose uptake (4.2 ± 0.2 µmol p < 0.001) (Fig. 3).

Aldose Reductase Activity  
The greatest inhibitory effect of aldose reductase activity is petroleum ether fraction (10 µg/ml, 36.8 ± 0.8%). Benzene (23.8 ± 1.6%), AcOEt (25.8 ± 0.9%), CHCl₃ (25.8 ± 2.1%), n-BuOH (28.1 ± 0.9%) layer also inhibited the activity (Fig. 4).

Table 1. Glucose Tolerance Test of PM

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>Blood glucose level (mg/dl)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>p.o.</td>
<td>76 ± 9</td>
</tr>
<tr>
<td>PM</td>
<td>100</td>
<td>p.o.</td>
<td>88 ± 6</td>
</tr>
<tr>
<td>PM</td>
<td>300</td>
<td>p.o.</td>
<td>75 ± 4</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E. Significantly different from the control, * p < 0.05.  

a) N: number of mice.

Fig. 1.  Effect of PM on Blood Glucose in Normal Mice

Fig. 2.  Effect of PM on Blood Glucose in STZ-Induced Diabetic Mice

Fig. 3.  Glucose Uptake Activity of PM

Fig. 4.  Inhibition of Aldose Reductase Activity

Each value represents the mean ± S.E. of 3 separate experiments.
DISCUSSION

The present study clearly shows that PM produces consistent hypoglycemic effects in both normal and STZ-induced diabetic mice, one of the insulin dependent diabetes mellitus (IDDM). The hypoglycemic activity was observed without any changes in serum insulin. The therapeutic effect of PM on hyperglycemia in STZ-induced diabetes in mice, one of the animal models of IDDM, was also examined. After treatment of mice with PM, the resulting hypoglycemia was observed without any change in serum insulin at 4 h (control 28±4 μU/ml, PM at 300 mg/kg 30±4 μU/ml). PM (300 mg/kg) significantly reduced the blood glucose levels in STZ-induced diabetic mice (Fig. 2), and strongly increased adipocyte glucose uptake. From these findings, it seems likely that PM may exhibit its hypoglycemic effects by stimulating glucose uptake. Pioglitazone, a new synthetic drug, stimulated glucose uptake.\(^5\) The petroleum ether fraction of PM inhibited the aldose reductase activity implicated in many diabetic complications such as neuropathy and retinopathy. It thus has the advantage of combining anti-diabetic activity with aldose reductase inhibition.

These results appear to indicate the validity of clinical use of *Polichia campestris* in the treatment of diabetes mellitus.

REFERENCES