Antidiabetic effect of long-term supplementation with *Siraitia grosvenori* on the spontaneously diabetic Goto–Kakizaki rat

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*Siraitia grosvenori* Swingle (SG) is a traditional Chinese fruit used as a folk medicine. Its extract (SG-ex) contains potent sweet elements with a sweetness several hundred times higher than table sugar. We investigated the antidiabetic effect of SG-ex in the type 2 diabetic Goto–Kakizaki (GK) rat. Diabetic 7-week-old GK rats were fed a diet supplemented with 0.4% of the SG-ex for 13 weeks, and its antidiabetic effects were evaluated. SG-ex had no effect on food intake or body weight. In oral glucose tolerance tests (OGTT), SG-ex supplementation improved the insulin response at 15 min (control, 63 (SEM 6) pM; SG-ex, 107 (SEM 20) pM; P<0.05) and reduced the plasma glucose level at 120 min after the glucose administration (control, 18.5 (SEM 0.8) mM; SG-ex, 14.8 (SEM 0.7) mM; P<0.05). The total amount of insulin in whole pancreas taken from fasting rats was higher in the SG-ex-supplemented group, which may explain the greater capacity to secrete insulin during the OGTT. Thiobarbituric acid-reactive substances in both the liver and the plasma were lower in the SG-ex-supplemented group, suggesting that an absorbable component in SG-ex has an antioxidative effect on lipid peroxidation, thereby counteracting the oxidative stress caused by a diabetic state. Excreted urine volume and urinary albumin level for 24 h were both reduced in the SG-ex-supplemented group, suggesting the attenuation of kidney damage that is caused by diabetes. These data indicate that SG-ex supplementation may prevent complications and attenuate pathological conditions for type 2 diabetes, along with its sweet characteristics.

*Siraitia grosvenori* Swingle: Antidiabetic effects: Diabetes: Insulin response: Sugar substitutes

The inhibitory effects of SG-ex on the initiation and promotion of cancer have also been reported (Takasaki et al. 2003). In addition, SG-ex has been found to have anti-allergic effects (Hossen et al. 2005). We also found that SG-ex reduced hyperglycaemia after a single oral administration of maltose in the rat (Suzuki et al. 2005). *In vitro*, SG-ex, as well as its constituents mogroside V, mogroside IV, mogroside III and siamenoside I, have been found to inhibit α-glucosidase (Suzuki et al. 2005). The α-glucosidase inhibitors are known to delay carbohydrate digestion in the small-intestinal tract and thereby to reduce rises of postprandial plasma glucose and of plasma insulin levels (Clissold & Edwards, 1988; Toeller, 1994). Voglibose and acarbose are well-known α-glucosidase inhibitors and in fact have been shown to be beneficial for treating type 2 diabetes as drugs (Saito et al. 1998; Rury et al. 1999). We thus hypothesised that long-term administration of SG-ex is beneficial for type 2 diabetes.

The Goto–Kakizaki (GK) rat is an animal model of spontaneous non-insulin-dependent diabetes mellitus (Goto et al. 1975). The diabetic state was generated by selective breeding repeated over many generations with glucose intolerance as a selection index, starting from a colony of non-diabetic Wistar...
rats (Goto & Kakizaki, 1981). The pathogenesis of diabetes in
the GK rat includes an impaired insulin secretion (Portha et al.
1991), insulin resistance (Bisbis et al. 1993) and abnormal
blood glucose metabolism (Ostenson et al. 1993). In contrast to
many other rodent models of type 2 diabetes (Janssen et al.
1999), GK rats do not become obese (O’Rourke et al. 1997)
and do not develop hyperlipidaemia (Zhou et al. 1995).
These characteristics are similar to the typical Asian-type dia-
abetes, and thus we found it an appropriate model to study the
effect of SG-ex on type 2 diabetes.

Accordingly, the present study was performed to examine the
effect of SG-ex supplementation in the diet on attenuating the
pathological status of type 2 diabetes.

Materials and methods

Preparation of the extract from Siraitia grosvenori

S. grosvenori (SG-ex) was prepared in Guilin S&T New Tech
Company (Guilin, China) as described previously (Suzuki
et al. 2005). Briefly, fresh fruits of S. grosvenori were crushed
and boiled in water and the water-soluble fraction was concen-
trated until soluble solids of a 64-0 Brix paste, measured by
refractometry at 20°C. Mogrosides V, 11OM-V, mogroside
IV, mogroside III and siamenoside I contents in SG-ex deter-
mimed by HPLC method (Suzuki et al. 2005) were approxi-
mately 2.1, 0.2, 0.8, 0.7 and 0.3 %, respectively.

Study design

Male GK rats (aged 5 weeks) were obtained from Clea Japan
( Osaka, Japan). The animals were kept on a standard pellet
diet (CE-2; Clea Japan, Osaka, Japan) and water ad libitum
to acclimatise to their environment for 1 week. The rats were
fed the control artificial diet (Table 1) for another week, then
randomly allocated into two groups; control and SG-ex
groups. They were housed individually at controlled tempera-
ture (23 ± 2° C), humidity (60 ± 10 %) and lighting (09.00 to
21.00 hours), and allowed free access to the designated artificial
diet and water for the subsequent 13 weeks. The content of the
designated artificial diet is shown in Table 1. Food intake and
body weight were measured every other day. Average food
intake was about 15 g/d in both groups. Excess amounts of
diet (25 g/d) were given at 09.00 hours every day. Blood was
collected in non-fasting conditions at 10.00 hours every other
week from the tail vein into heparinised tubes, and centrifuged
at 3000 g for 10 min at 4°C. The supernatant fraction (plasma)
was collected and stored at −20°C until analysed. Plasma glu-
cose levels were measured by a glucose oxidase method using a
commercial kit (Glucose B-test Wako; Wako Pure Chemical
Industries, Osaka, Japan). At week 13 of treatment (age 20
weeks) after 16 h starvation, rats were anaesthetised with
diethyl ether and killed. Blood was collected from the vena
cava, and the heart, liver, kidney, spleen, pancreas and small
intestine tissues were collected, weighed, frozen in liquid N2
and stored at −80°C until analysed. The animals used were
maintained in accordance with the guidelines of the National
Research Council (1985).

Oral glucose tolerance test

An oral glucose tolerance test (OGTT) was performed at week
7 of treatment (age 14 weeks). The tail vein blood was col-
llected in heparinised microtubes after 16 h starvation as a con-
trol blood sample at 0 min. Glucose (1 g/kg body weight) was
intubated orally, and the tail vein blood was collected in a
heparinised microtube at 30, 60, 90 and 120 min after the intu-
bation. Plasma glucose levels and plasma insulin levels
(ELISA kit, rebis insulin-rat U type; Shibayagi, Gunma,
Japan) were determined for each time point.

Urine analysis

During weeks 11 and 12 of supplementation (age 18–19
weeks), urine was collected. A rat was individually placed in
a metabolism cage for 3 d before the urine collection in
order to acclimatise the rat to the environment. The rat
could freely access the water and food. The urine was col-
llected at 24 h intervals for 2 d. Urinary albumin levels were
determined by a sandwich ELISA kit (Shibayagi, Gunma,
Japan).

Pancreatic insulin level

Fasting pancreatic insulin levels were determined by a sand-
wich ELISA kit (Shibayagi, Gunma, Japan). Briefly, the pan-
creas (150 mg) was homogenised in 600 μL PBS containing
0.05 % Triton X-100 by a polytron homogeniser. The hom-
genate was serially diluted with the buffer provided in the
ELISA kit, and the insulin level was measured according to
the instructions.

Thiobarbituric acid-reactive substance level

Thiobarbituric acid-reactive substance (TBARS) levels of the
liver, kidney, pancreas and plasma were determined (Ohkawa
et al. 1979). Briefly, each tissue (liver, kidney, pancreas;
150 mg) was homogenised in 600 μL KCl (1-15 %) solution
by a polytron homogeniser. SDS (40 μL; 8-1 %), 300 μL
sodium acetate buffer (20 %; pH 3-5) and 300 μL thiobarbituric
acid (0.8 %) were added to 80 μL of the homogenate or 80 μL
of the plasma, and incubated at 95°C for 20 min. After
cooling down the reaction mixture to room temperature,
200 μL of the deionised water and 1 mL of the n-butanol-pyridine
mixture (15:1; v/v) were added, mixed and centrifuged at
4000 rpm for 10 min. The absorbance of the supernatant fraction

<table>
<thead>
<tr>
<th>Table 1. Ingredients of the experimental diets</th>
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<tbody>
<tr>
<td><strong>Ingredient</strong></td>
</tr>
<tr>
<td>Maize starch</td>
</tr>
<tr>
<td>Milk casein</td>
</tr>
<tr>
<td>Sucrose</td>
</tr>
<tr>
<td>Soyabean oil</td>
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<tr>
<td>AIN-93 VX mineral mix</td>
</tr>
<tr>
<td>AIN-93G vitamin mix</td>
</tr>
<tr>
<td>Methionine</td>
</tr>
<tr>
<td>Choline chloride</td>
</tr>
<tr>
<td>Cellulose</td>
</tr>
<tr>
<td>Siraitia grosvenori Swingle extract*</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

* For the control, Siraitia grosvenori Swingle extract was
  replaced by cellulose.
was measured at 535 nm in a spectrophotometer. 1,1,3,3-Tetraethoxypropane (0.5, 1.25, 2.5 nmol/80 μl) was used as a standard. Protein concentrations for these tissues were determined by a BCA assay (Pierce, Rockford, IL, USA).

**Plasma analysis**

Plasma TAG (triglyceride E-test), plasma cholesterol (cholesterol E-test), transaminase (transaminase C II-test), γ-glutamyl transpeptidase (γ-glutamyl transpeptidase C-test), lactase dehydrogenase (lactate dehydrogenase C II-test) and alkaline phosphatase (alkaline phospha K kit) were determined according to the manufacturer’s instructions (Wako Pure Chemical Industries, Osaka, Japan).

**Statistical analysis**

Data on OGTT were analysed by two-way ANOVA for repeated measures, and post hoc analyses were done by Fisher’s least significant difference test. These statistical analyses were performed with GB-Stat 5.4 (Dynamic Microsystems, Silver Spring, MD, USA). Student’s t test was performed for urine, pancreatic insulin, TBARS and plasma analysis. Results are expressed as mean values with their standard errors. Statistical significance was defined as $P<0.05$.

**Results**

**Food intake, body weight, tissue weight and non-fasting plasma glucose**

There were no significant differences between the control and the SG-ex-supplemented rats for both food intake and body weight (data not shown), suggesting that SG-ex at this dose for a 13-week supplementation period does not have any adverse effects on feeding performance. Average food intake was about 15 g/d for both groups. The non-fasting plasma glucose levels ($PG_{NF}$) were measured every other week (Fig. 1). The $PG_{NF}$ were almost identical between the two groups at the beginning of the study until 4 weeks of treatment. However, at week 8 of treatment, the $PG_{NF}$ in the SG-ex group became lower than that in the control group, and this trend was maintained until the end of the study. Although the difference did not reach statistical significance ($P>0.05$), this result suggests that the longer supplementation with SG-ex may attenuate the elevated $PG_{NF}$ caused by diabetes.

**Oral glucose tolerance test**

OGTT were performed after 7 weeks of treatment. Fasting plasma glucose was identical between the control and the SG-ex-supplemented group (Fig. 2 (A)). The fluctuation patterns of plasma glucose levels after the glucose administration were similar between the two groups until 60 min. The control group exhibited the typical pattern for diabetes: glucose administration caused a quick enhancement of plasma glucose and it stayed high once increased. It was, however, found to be significantly lower ($P<0.05$) at 120 min in the SG-ex group than in the control group, which suggests that orally supplemented SG-ex may improve the ability to control postprandial plasma glucose levels. The fasting plasma insulin level
was similar in both groups (Fig. 2 (B)). Strikingly, the SG-ex group showed a marked, short-lived increase at 15 min in the plasma insulin levels that returned to the control levels after 30 min. This marked rise of plasma insulin could influence liver and peripheral tissues to facilitate glucose uptake and result in the decrease of plasma glucose levels at 120 min in the SG-ex group. Furthermore, these results also suggest that GK rats supplemented with SG-ex ameliorate the pancreatic insulin storage capacity during fasting conditions or the insulin-releasing capacity from the pancreas. We thus examined the fasting pancreatic insulin contents.

Pancreatic insulin content

The total amount of insulin in whole pancreas taken from fasting rats is shown in Fig. 3. As we had expected, pancreatic insulin contents were significantly higher in the SG-ex group than in the control group ($P = 0.013$), suggesting that the pancreas is capable of storing more insulin in SG-ex-supplemented rats, which can then be released promptly responding to the rise in plasma glucose levels and properly regulate plasma glucose levels.

Urine analysis

The volume of urine and the urinary albumin levels are shown in Fig. 4. The excreted urine volume was significantly lower in the SG-ex group than in the control one ($P = 0.036$). Accordingly, the urinary albumin level was significantly lower in the SG-ex group than in the control one ($P = 0.044$). These results suggest that SG-ex is likely to attenuate the kidney functions, which are often damaged under the case of the diabetic complication.

Thiobarbituric acid-reactive substance level

In order to analyse lipid peroxidation, TBARS levels of the liver, kidney, pancreas and plasma were measured (Table 2). TBARS level was standardised by malondialdehyde, a metabolite of oxidised lipid, and thus reflects the extent of lipid peroxidation. Average TBARS values were higher in control than in SG-ex-supplemented rats in all four tissues that we measured in the present study. In particular, TBARS values in both liver and plasma were significantly lower in SG-ex-supplemented rats, suggesting that lipid peroxidation was inhibited by long-term administration of SG-ex.

Plasma analysis

Glutamic oxaloacetic transaminase and γ-glutamyl transpeptidase were significantly lower in the SG-ex group. In addition,

| Table 2. Thiobarbituric acid-reactive substance (TBARS) levels (nmol/mg) in various tissues* |
|-----------------|-----------------|-----------------|-----------------|
|                  | Control (n 10)  | SG-ex (n 10)    |                  |
|                  | Mean   | SEM   | Mean   | SEM   | P    |
| Liver            | 1·55   | 0·23  | 0·77   | 0·02  | 0·007|
| Plasma           | 0·146  | 0·009 | 0·113  | 0·014 | 0·017|
| Pancreas         | 58·5   | 2·9   | 50·8   | 3·6   | 0·067|
| Kidney           | 0·72   | 0·08  | 0·66   | 0·05  | 0·309|

* *Siraitia grosvenori* Swingle extract.

Rats were fed the control (without SG-ex) or the experimental (with SG-ex) diet for 13 weeks (see Table 1). TBARS levels of the liver, kidney, pancreas and plasma were determined. Results are expressed as a malondialdehyde equivalent (nmol malondialdehyde/mg protein).
average values of TAG, glutamic pyruvic transaminase, lactic dehydrogenase and alkaline phosphatase were lower in the SG-ex group (Table 3), although these differences were not statistically significant. These results suggest that the decline of liver function caused by diabetes is attenuated by long-term supplementation of SG-ex in rats.

Discussion

We demonstrated that a diet which is supplemented with SG-ex exerted antidiabetic effects that appear to moderately ameliorate various tissues from the typical diabetic pathological status. In addition, a 13-week supplementation of SG-ex did not show any adverse effects in GK rats, including feeding behaviour, body weight and various biochemical parameters in various organs.

We observed a trend of a lower PGF in the SG-ex-supplemented GK rats compared with the control rats, but it did not reach the statistically significant level. The effect of long-term administration with voglibose, an α-glucosidase inhibitor, on the GK rat has been reported (Wada et al. 1999). Treatment was started at 12 weeks of age and the PGF became significantly lower in the voglibose-treated GK rat than that in the control GK rat at 24 weeks of age. Although the α-glucosidase-inhibitory effect of SG-ex has been previously shown to be effective to inhibit the elevation of postprandial plasma glucose levels in Wistar rats (Suzuki et al. 1999), the α-glucosidase inhibitory effect of SG-ex is likely due to its function as well as α-glucosidase inhibitor. Thus, oxidative components in SG-ex were targeted to the pancreas and helped to repair oxidative properties in the liver and the plasma.

In the present experiment, pancreatic insulin of adult GK rats at week 13 of the treatment (age 20 weeks) was 8.1 nmol/pancreas, while the typical pancreatic insulin of adult Wistar rats (age 18 weeks) is 42.4 nmol/pancreas (Movassat et al. 1997). We observed a significant increase of pancreatic insulin in the SG-ex-supplemented GK rat, although it was about 11.4 nmol/pancreas and still much lower than the normal level in Wistar rats. The GK rat has been shown to develop an alteration of β-cells as early as embryonic day 21.5, and the deficit of total pancreatic β-cell mass in the GK rat has been shown to be maintained in the adult animal (Movassat et al. 1997). It is therefore possible that the β-cell functions are hardly recovered to the normal level after pancreatic β-cells were severely impaired during embryonic development.

Kidney function in streptozotocin-induced type 1 diabetic rats was shown to be disrupted and urinary albumin was reported to be increased in the diabetic rat (Adachi et al. 2000). Urine volume in the Wistar rat is reported to be about 6.9 ml/24 h (Adachi et al. 2000) and that in GK rats in the present study was 10.0 ml/24 h. Thus, the urine volume of control GK rats being higher than that of normal Wistar rats causes the control GK rat to excrete more albumin. Therefore, our observations, where SG-ex-supplemented GK rats excreted less urine and urinary albumin than control GK rats, suggest that kidney dysfunction caused by diabetes was attenuated by the supplementation with SG-ex.

The OGTT revealed that glucose tolerance in SG-ex-supplemented GK rats appeared to be ameliorated compared with that in control GK rats. The plasma glucose levels were decreased significantly (P<0.05) at 120 min in SG-ex-supplemented GK rats. The plasma insulin levels in the SG-ex-supplemented GK rats at 15 min were significantly higher than that in the control rats. An antioxidant, α-tocopherol, has been reported to ameliorate glycaemic control in GK rats (Ihara et al. 2000). It was reported that in the OGTT, plasma glucose levels were decreased significantly at 30 and 120 min in the α-tocopherol-supplemented GK rats and plasma insulin levels in α-tocopherol-supplemented GK rats were significantly higher at 30 min, which is quite similar to what we have observed in the SG-ex-supplemented GK rats. 11OM-V, one of the sweet components in SG-ex, has been reported to have an antioxidative effect (Takeo et al. 2002), and thus it may be possible that antioxidative components in SG-ex such as 11OM-V could have a similar effect as α-tocopherol.

It has been shown that the chronic hyperglycaemic state in the GK rat induces oxidative stress on the pancreatic β-cells, which appeared to cause cytotoxicity making pathological conditions worse (Ihara et al. 1999). α-Tocopherol has been found to be accumulated in the pancreas when supplemented in the diet (Ihara et al. 2000). Although it has not yet been directly proven that antioxidative components in SG-ex are absorbed into the circulation, the reductions of lipid peroxidation (measured by TBARS) in the liver, plasma and pancreas of SG-ex-supplemented GK rats suggest that antioxidative components are absorbed and delivered to various tissues. It is therefore possible that antioxidative components in SG-ex were targeted to the pancreas and helped to repair its function as well as α-tocopherol did. This feasible hypothesis still needs to be investigated.

In a recent placebo-controlled large-scale clinical trial, acarbose, an α-glucosidase inhibitor, has been shown to improve sensitivity to insulin and decrease postprandial hyperglycaemia, thereby releasing the stress on the β-cells (Chiasson et al. 1996). The fundamental mechanisms of acarbose and that of SG-ex are similar, i.e. they act as α-glucosidase inhibitors. Therefore it is conceivable that long-term supplementation with SG-ex could have the similar antidiabetic effect in human subjects.

In summary, SG-ex exhibited an antidiabetic effect on the spontaneously diabetic GK rat by improving insulin response in the OGTT, accumulating insulin in the pancreas in the fasting state, ameliorating kidney function, and enhancing antioxidative properties in the liver and the plasma.

Table 3. Biochemical analysis of the plasma* (Mean values with their standard errors)

<table>
<thead>
<tr>
<th></th>
<th>Control (n=10)</th>
<th>SG-ex (n=10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>808 ± 24</td>
<td>779 ± 17</td>
<td>0.426</td>
</tr>
<tr>
<td>(mg/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAG (mg/l)</td>
<td>411 ± 37</td>
<td>391 ± 26</td>
<td>0.332</td>
</tr>
<tr>
<td>GOT (IU/l)</td>
<td>27.5 ± 0.8</td>
<td>24.8 ± 1.1</td>
<td>0.042</td>
</tr>
<tr>
<td>GPT (IU/l)</td>
<td>6.9 ± 0.3</td>
<td>6.4 ± 0.2</td>
<td>0.003</td>
</tr>
<tr>
<td>γ-GTP (IU/l)</td>
<td>3.3 ± 0.1</td>
<td>2.8 ± 0.1</td>
<td>0.011</td>
</tr>
<tr>
<td>LDH (IU/l)</td>
<td>266.2 ± 22.5</td>
<td>235.0 ± 8.4</td>
<td>0.051</td>
</tr>
<tr>
<td>ALP (IU/l)</td>
<td>148.8 ± 8.2</td>
<td>137.6 ± 4.0</td>
<td>0.368</td>
</tr>
</tbody>
</table>

SG-ex, Siraitia grosvenori Swingle extract; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; γ-GTP, γ-glutamyl transpeptidase; LDH, lactic dehydrogenase; ALP, alkaline phosphatase.*Rats were fed the control (without SG-ex) or the experimental (with SG-ex) diet for 13 weeks (see Table 1).
Antidiabetic effect of Siraitia grosvenorii

References


Movassat J, Saulnier C, Serradas P & Portha B (1997) Impaired development of pancreatic β-cell mass is a primary event during the progression to diabetes in the GK rat. Diabetologia 40, 916–925.


