Extracts of *Momordica charantia* Suppress Postprandial Hyperglycemia in Rats

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Summary  *Momordica charantia* (bitter melon) is commonly known as vegetable insulin, but the mechanisms underlying its hypoglycemic effect remain unclear. To address this issue, the effects of bitter melon extracts on postprandial glycemic responses have been investigated in rats. An aqueous extract (AE), methanol fraction (MF) and methanol insoluble fraction (MIF) were prepared from bitter melon. An oral sucrose tolerance test revealed that administration of AE, MF or MIF each significantly suppressed plasma glucose levels at 30 min as compared with the control. In addition, the plasma insulin level at 30 min was also significantly lower after MF administration than in the control in the oral sucrose tolerance test. By contrast, these effects of bitter melon extracts were not observed in the oral glucose tolerance test. In terms of mechanism, bitter melon extracts dose-dependently inhibited the sucrase activity of intestinal mucosa with IC50 values of 8.3, 3.7 and 12.0 mg/mL for AE, MF and MIF, respectively. The fraction with a molecular weight of less than 1,300 (LT 1,300) obtained from MF inhibited the sucrase activity most strongly in an uncompetitive manner with an IC50 value of 2.6 mg/mL. Taken together, these results demonstrated that bitter melon suppressed postprandial hyperglycemia by inhibition of α-glucosidase activity and that the most beneficial component is present in the LT 1,300 fraction obtained from MF.

Key Words  α-glucosidase inhibitor, *Momordica charantia*, postprandial hyperglycemia

Diabetes mellitus is a major global health problem currently affecting more than 194 million people worldwide. Diabetes mellitus is characterized by chronic hyperglycemia and, in particular, postprandial hyperglycemia is closely related to risk of micro- and macro-vascular complications and death (1, 2). In addition, delayed digestion of carbohydrates could be beneficial to avoid postprandial hyperglycemia in diabetes mellitus (3). Specific inhibitors of α-glucosidase, such as acarbose and voglibose, have been utilized to delay carbohydrate digestion and to suppress postprandial hyperglycemia (4, 5). The STOP-NIDDM trial has also shown that acarbose treatment significantly decreases the risk of progression to type 2 diabetes mellitus (6). These findings indicate that α-glucosidase inhibitors are beneficial for the prevention and treatment of diabetes mellitus.

*Momordica charantia* (bitter melon), ni-ga-u-ri in Japanese, is a commonly consumed vegetable in Japan and other Asian countries. Recently, bitter melon has been shown to possess hypoglycemic properties in diabetic animal models (7–9) and in patients with type 2 diabetes mellitus (10). A component of bitter melon extract appears to be related structurally to animal insulin (11). Aqueous, chloroform and methanol extracts of bitter melon treatment decreased blood glucose levels in type 1 diabetic and normal rats (12). In addition, protein extract from bitter melon has been shown to have insulinomimetic and insulin secretagogue effects (13). However, bitter melon reduces serum insulin and blood glucose levels in diet-induced obese rats and in type 2 diabetic mice, but does not affect normal mice (14–16). This effect of bitter melon conflicts with its insulinomimetic and insulin secretagogue effects. Thus, these data suggest that bitter melon contains several components that suppress hyperglycemia and the incidence of diabetes mellitus.

Among dietary carbohydrates, sucrose can induce postprandial hyperglycemia and hyperinsulinemia more strongly than starch (17). Therefore, in the present study, the effects of bitter melon extracts on postprandial glycemic responses and on the sucrase activity of intestinal mucosa have been investigated in rats.
**MATERIALS AND METHODS**

*Preparation of bitter melon extracts.* The preparation protocol of bitter melon extracts is delineated in Fig. 1. Bitter melon (Mob Bao) was imported from China and lyophilized for 24 h using a freeze dryer (FDV-540, Tokyo Rikakikai, Co., Ltd., Tokyo, Japan). Lyophilized bitter melon extracts (400 g) were extracted twice with distilled water at 60℃ for 1 h and filtered. The filtrate, concentrated and freeze dried, was used as a bitter melon aqueous extract (AE: 128.6 g). Next, a sample of AE (50.7 g) was extracted twice with methanol and dissolved with an ultrasonic disintegrator. The filtrate, concentrated and freeze dried, was used as a bitter melon methanol insoluble fraction (MF: 10.8 g), and the residue, freeze dried, was used as a bitter melon methanol insoluble fraction (MIF: 35.5 g). Furthermore, MF was subjected to size-exclusion chromatography (Bio-Gel P-2 Gel Fine, Bio-Rad Japan, Tokyo, Japan) with distilled water as the eluent, and fractionated by molecular weight. We used vitamin B12 (MW 1,355) as the molecular weight marker to obtain fractions corresponding to approximate molecular weights of 1,300 (AP 1,300) and less than 1,300 (LT 1,300).

**Animals.** Male Sprague-Dawley rats at 8 wk of age were purchased from the Japan SLC (Hamamatsu, Japan). All rats were housed in cages under a 12-h light (8:00–20:00), 12 h dark cycle and constant temperature (23±2℃). The rats had free access to water and diet (MF Oriental Yeast Co., Ltd., Tokyo, Japan). These studies were approved by the Tokushima University Animal Use Committee, and the rats were maintained according to the guidelines of Tokushima University for the care of laboratory animals.

**Effects of bitter melon extracts on postprandial glucose and insulin levels in rats.** After 2 wk acclimation, catheterization was performed as described previously (18, 19). The rats were anesthetized with diethylether. A silicon rubber catheter (PT-025, Bio-Medica, Osaka, Japan) was inserted into the left femoral vein and led out through subcutaneous tissue to an intravenous hyperalimentation (IVH) kit (Bio-Cannula, Bio-Medica). Catheterized rats were kept in a special IVH cage (BG-781, Bio-Medica) and continuously infused with physiological saline until the tests were carried out. All tests were performed 4 d after catheterization. After overnight fasting (16–18 h), AE (0.6 g/kg BW) was orally administered either alone (test 1) or with glucose (1 g/kg BW, test 2). We then performed a sucrose (1 g/kg BW) tolerance test with AE, MF or MIF (0.6 g/kg BW, test 3). The control group was given distilled water as an alternative to the bitter melon extracts. Blood samples were drawn from the femoral vein at 0, 10, 20, 30, 60 and 120 min in test 1 and at 0, 15, 30, 60, and 120 min in tests 2 and 3. Plasma glucose levels were determined by the glucose dehydrogenase method (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Plasma insulin levels were measured by an enzyme linked immunosorbent assay (ELISA) kit (Morinaga, Yokohama, Japan).

**Intestinal mucosa homogenate.** Ten male Sprague-Dawley rats at 20 wk of age were anesthetized with diethylether and the small intestine was removed. The intestinal lumens were flushed with ice-cold saline. The mucosa of the small intestine were collected by scraping with a glass slide and homogenized ten times in 100 mmol/L sodium maleate buffer solution (pH 6.0) with a teflon homogenizer. After centrifugation at 3,000×g for 10 min, the supernatant was used as the crude enzyme solution.

**Effect of bitter melon extracts on α-glucosidase and alkaline phosphatase activity in rat intestinal mucosa.** Alpha-glucosidase activity was measured by the Dahlqvist method (20, 21) with slight modifications. The activity of α-glucosidase was assayed with 28 mmol/L sucrose or maltose with or without bitter melon extracts at different concentrations, with miglitol used as a positive control. After incubation at 37℃ for 60 min, reactions were stopped by heating at 100℃ for 3 min, and the quantity of glucose produced during incubation was measured by a commercial kit (Wako Pure Chemical Industries). For each extract, the concentration required to inhibit 50% of sucrase activity was defined as the IC50 value. The alkaline phosphatase activity of the enzyme solution after incubation with 5 mg/mL of each bitter melon extract was also measured by a commercial kit (Wako Pure Chemical Industries). The amount of protein in the sample was determined by Bradford assay (Bio-Rad Japan).

**Kinetics analysis of sucrase inhibition.** In order to examine the mechanism of inhibition by the bitter melon extracts, sucrase activities were measured with increasing concentrations of sucrose (3.125–200 mmol/L) with or without bitter melon extracts at two different concentrations (5 and 25 mg/mL). Manners of inhibition by the bitter melon extracts were assessed by Lineweaver-Burk plot analysis.

**Statistical analysis.** Values are expressed as the mean±SE. Differences between two groups were assessed by an unpaired two-tailed t-test and those among more than two groups by ANOVA or the Kruskal-Wallis test. When a significant difference was found with ANOVA or the Kruskal-Wallis test, post-hoc analyses were performed with Students-Newman-
Keules protected least-significant difference test. Differences were considered significant at $p<0.05$. Analyses were performed using StatView software (version 5.0-J for Windows, SAS Institute, Inc., Cary, NC).

RESULTS

Effects of bitter melon extracts on postprandial glucose and insulin levels in rats

Administration of AE alone did not affect plasma glucose or insulin levels (data not shown). In the oral glucose tolerance test, there were no significant differences in plasma glucose or insulin levels between the AE group and control (distilled water) group (Fig. 2). In the oral sucrose tolerance test, however, the plasma glucose levels of the AE and the MF group were significantly lower than those of the control group at 15 and 30 min (Fig. 3A; $p<0.05$). The plasma glucose level of the MIF group was lower than that of control group only at 30 min (Fig. 3A; $p<0.05$). The plasma glucose levels of the AE, MF, MIF and control groups were 6.5±0.2, 6.4±0.1, 7.3±0.4 and 8.1±0.3 mmol/L at 15 min and 6.3±0.2, 6.7±0.2, 7.2±0.3, 8.4±0.3 mmol/L at 30 min, respectively. Moreover, plasma insulin levels of the MF group were significantly lower than those of the control group (Fig. 3B; $p<0.05$). The plasma insulin levels of the MF and control groups were, respectively, 371.1±32.2 and 718.6±109.8 pmol/L at 15 min and 379.0±25.8 and 515.8±41.8 pmol/L at 30 min after sucrose administration. The total incremental area (area under the curve: AUC) of plasma glucose levels over 120 min for the AE and MF groups was, respectively, 27% and 26% lower than that for the control group (Fig. 4A). The AUC of plasma insulin levels over 120 min for the AE and MF groups were, respectively, 18% and 47% lower than that for the control group (Fig. 4B).

Effect of bitter melon extracts on α-glucosidase and alkaline phosphatase activity in rat intestinal mucosa

All bitter melon extracts, namely AE, MF and MIF, as well as miglitol effectively inhibited sucrase activity dose-dependently and MF was more effective than the other two bitter melon fractions (the IC$_{50}$ values of AE, MF and MIF against sucrase were 8.3, 3.7 and 12.0 mg/mL, respectively, Table 1). Miglitol, as a positive control, inhibited sucrase activity and the IC$_{50}$ value was $1.4\times10^{-3}$ mmol/L. To elucidate the active components, MF was fractionated according to molecular weight. LT 1,300 showed greater inhibition than AP 1,300 with an IC$_{50}$ value of 2.6 mg/mL (Table 1). In addition, the bitter melon extracts AE, MF and MIF at 25 mg/mL inhibited maltase activity by 28.2, 46.2 and 26.9%, respectively. To verify the specificity of inhibitory effects of bitter melon extracts against α-glucosidase, we assessed their effect on the alkaline phosphatase activity. None of the bitter melon extracts had an effect on

![Fig. 2](image-url)  
**Fig. 2.** Effect of bitter melon extract on plasma glucose (A) and plasma insulin (B) levels after oral glucose administration. Values are means±SE ($n=5$). Data points show control (white square) and AE (black square).

![Fig. 3](image-url)  
**Fig. 3.** Effect of bitter melon extracts on plasma glucose (A) and plasma insulin (B) levels after oral sucrose administration. Values are means±SE ($n=8–10$). Data points show control (white square), AE (black square), MF (white circle) and MIF (gray triangle). *$p<0.05$ (control vs. AE, MF), †$p<0.05$ (control vs. MIF), ‡$p<0.05$ (control vs. MF).
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alkaline phosphatase activity (data not shown).

Kinetics analysis of sucrase inhibition

To determine the inhibitory mechanism of bitter melon extracts against sucrase activity, we performed a kinetics analysis. Lineweaver-Burk plots of the results revealed that AE and MF inhibited sucrase activity in a mixed manner, MIF inhibited it in an uncompetitive manner (data not shown). Furthermore, AP 1,300 inhibited sucrase activity in a noncompetitive manner, whereas LT 1,300 inhibited it in an uncompetitive manner (Fig. 5).

Table 1. Glucose production quantity, IC\textsubscript{50} value and kinetics analysis of bitter melon extracts on sucrase activity in intestinal mucosa.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Glucose production* (mmol/min·mg protein)</th>
<th>IC\textsubscript{50} (mg/mL)</th>
<th>Inhibition type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.412±0.015</td>
<td>0.015</td>
<td>—</td>
</tr>
<tr>
<td>Bitter melon extracts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AE</td>
<td>0.256±0.005</td>
<td>8.3</td>
<td>Mixed-type</td>
</tr>
<tr>
<td>MF</td>
<td>0.181±0.003</td>
<td>3.7</td>
<td>Mixed-type</td>
</tr>
<tr>
<td>AP 1,300</td>
<td>0.334±0.006</td>
<td>NT</td>
<td>Noncompetitive</td>
</tr>
<tr>
<td>LT 1,300</td>
<td>0.112±0.004</td>
<td>2.6</td>
<td>Uncompetitive</td>
</tr>
<tr>
<td>MIF</td>
<td>0.289±0.006</td>
<td>12.0</td>
<td>Uncompetitive</td>
</tr>
</tbody>
</table>

\*Sucrase inhibition tests were run at 5 mg/mL bitter melon. Values are expressed as the mean±SE (n=3–4). NT: not tested.

showed that all three extracts, namely AE, MF and MIF, inhibited the sucrase activity in rat intestinal mucosa. Moreover, MF showed two types of inhibitory activity, uncompetitive and noncompetitive, against sucrase. In addition, we confirmed that these bitter melon extracts also inhibited maltase activity as well as sucrase activity. But, the inhibitory effects against maltase were weaker than that against sucrase.

The results of our present study are linked to two different studies conducted by Matsuura et al. (22) and Kumar Shetty et al. (23). First, Matsuura et al. isolated an \(\alpha\)-glucosidase inhibitor from bitter melon (22). However, they did not examine the effect of bitter melon on the postprandial glycemic response after carbohydrate administration in vivo. Second, Kumar Shetty et al. have reported that treatment with air-dried bitter gourd for 45 d significantly reduced the activity of intestinal maltase but not sucrase, and improved hyperglycemia in streptozotocin-induced diabetic rats (23). The mechanism underlying improvement, however, has remained unclear. In contrast, in our present study, bitter melon extracts inhibited sucrase activity and suppressed postprandial hyperglycemia after sucrose administration.

Fig. 4. Effect of bitter melon extracts on AUC after 120 min for plasma glucose (A) and plasma insulin (B) levels after oral sucrose administration. Values are means±SE (n=8–10). Bars show control (white), AE (black), MF (hatched) and MIF (gray). *Significantly different vs. control (p<0.05).

Fig. 5. Kinetics analysis of sucrase inhibition by bitter melon extracts. Results are plotted according to Lineweaver-Burk plot analysis. Data points show vehicle (white), 5 mg/mL AP 1,300 (black) and 5 mg/mL LT 1,300 (gray).
The difference might come from the type of carbohydrate used in each study. Kumar Shetty et al. used starch but not sucrose, whereas we used sucrose in the present study. In addition, we demonstrated that at least three components of bitter melon have sucrase inhibitory activity and that these components suppressed postprandial hyperglycemia in cooperation with each other.

Recently, Fujita and Yamagami have reported that α-glucosidase inhibitors obtained from vegetable extracts can suppress postprandial hyperglycemia in rats (24). In addition, Itoh et al. have reported that the ethanol fraction of adzuki beans is an α-glucosidase inhibitor (25). The IC₅₀ value of the adzuki beans extract against sucrase was 7.8 mg/mL. The bitter melon extracts used in the present study showed inhibition equal to that of the adzuki bean extract, whereas miglitol showed more effective inhibition against sucrase. However, Fujisawa et al. have reported that some medicines, such as many α-glucosidase inhibitors, have side-effects (26). Many vegetable and herbal medicines have been used in the prevention and treatment of diabetes mellitus. It is safer to take a natural food extract than to take medication or medicinal drugs for a long time for diabetic therapies. Taking these results together, bitter melon extract might improve postprandial hyperglycemia in type 2 diabetes mellitus and obesity.

Sugar transport in the intestine also plays an important role in postprandial hyperglycemia. Dyer et al. have reported that protein levels of the sodium glucose cotransporter SGLT1 and fructose transporter GLUT5 are higher in diabetic patients (27). This finding indicates that intestinal carbohydrate absorption is increased in diabetes mellitus and that these changes are related to postprandial hyperglycemia. Ahmed et al. have reported whole bitter melon juice reduces Na⁺- and K⁺-dependent glucose absorption in streptozotocin-induced diabetic rats (28). We could not confirm this suppressive effect in normal rats given AE with glucose. Casirola and Ferraris have reported that an α-glucosidase inhibitor prevents the increase in glucose transport induced by a high carbohydrate diet in diabetic mice (29). Thus, the inhibitory effects of bitter melon on sugar transport might be more potent under conditions of higher sugar transport.

Ali et al. (30) and Harinantenaina et al. (31) have reported that bitter melon reduces blood glucose levels in normal rats, and bitter melon protein extracts have been shown to increase insulin secretion from perfused pancreas of rats (32). Moreover, Basch et al. have reported that a component isolated from bitter melon has an insulin-like structure (11). These studies indicate that the insulin-like property of bitter melon is derived from the protein fraction. In the present study, administration of AE alone had no effect on insulin levels in rats. Because we used extract that had been heated at 60°C, the higher order architecture of some proteins may have been disrupted. For the same reason, fresh bitter melon might have more potent activity to ameliorate postprandial hyperglycemia than the bitter melon extracts used in our study. However, at least in part, our data may explain why bitter melon ameliorated serum insulin and blood glucose levels in diet-induced obese rats and in type 2 diabetic mice, but did not in normal mice as previously reported (14–16).

Postprandial hyperglycemia is related to postprandial hyperinsulinemia (32). Acarbose treatment reduces insulin demand and maintains beta cell numbers in pancreatic islets, and also prevents enlargement or atrophy of islets in type 2 diabetic rats (33). Recently, Matsui et al. have reported an α-glucosidase inhibitor, caffeoylsophorose, obtained from fermented sweet potatoes (34). Caffeoylsophorose was found to suppress insulin levels by 48% at 30 min after maltose administration in rats. The postprandial insulin demand in the MF group was 53% lower than that in the control group in our present study. In addition, whole bitter melon juice has been shown to prevent the destruction of the pancreas induced by streptozotocin in rats (35). Collectively, these results suggest that bitter melon abrogates exhaustion of pancreatic beta cells.

Quan et al. (36) and Zhang et al. (37) have reported that saponins have α-glucosidase inhibitory activity. Bitter melon also contains saponin analogs and these might be candidates for the α-glucosidase inhibitors. In general, a saponin analog called charantin has hypoglycemic properties (11). On the other hand, Matsuura et al. have isolated an α-glucosidase inhibitor, d(-)-trehalose, from bitter melon (22). The molecular weight of trehalose is approximately 350. In addition, Kim et al. (38) and Anila and Vijayalakshmi (39), have reported flavonoids with α-glucosidase inhibitory action, and bitter melon contains flavonoids. Furthermore, bitter melon contains 48% dietary fiber (of which 16.6% is soluble) (28, 40). Dietary fiber is well known to delay carbohydrate digestion and absorption (41). These components might be present in the bitter melon extracts used in our present study.

In conclusion, bitter melon contains several components that have α-glucosidase inhibitory activity and these components contribute to the suppression of postprandial hyperglycemia and hyperinsulinemia. The results of our study indicate that bitter melon is useful in the prevention and treatment of diabetes mellitus.

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