Inhibition of Increases in Blood Glucose and Serum Neutral Fat by *Momordica charantia* Saponin Fraction

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[Focusing on a functional component of *Momordica charantia*, saponin, we investigated its effects on serum glucose and neutral fat levels. Saponin was extracted as a butanol-soluble fraction (saponin fraction) from hot blast-dried *Momordica charantia* powder. The disaccharidase-inhibitory activity and the pancreatic lipase-inhibitory activity of the saponin fraction were measured, and *in vivo* sugar- and lipid-loading tests were performed. The saponin fraction inhibited disaccharidase activity and elevation of the blood glucose level after sucrose loading. The fraction also markedly inhibited pancreatic lipase activity and elevation of the serum neutral fat level after corn oil loading. Based on these findings, the main active component related to the anti-diabetic effect of *Momordica charantia* is present in the butanol fraction, and it may be saponin. The blood glucose and serum neutral fat-lowering effects of *Momordica charantia* were closely associated with its inhibitory activity against disaccharidase and pancreatic lipase.

Key words: *Momordica charantia*; saponin fraction; blood glucose; serum neutral fat; rats

Diabetes is a serious metabolic disease. Diabetic patients have been rapidly increasing in number worldwide, and prevention of the advancement of diabetes is a major issue in the 21st century. A chronic hyperglycemic condition leads to insulin resistance and induces the complications characteristic of diabetes. The prevention of these complications and inhibition of their advancement are possible by the control of blood glucose and improvement of hyperlipidemia.

In addition to the restriction of energy intake and the promotion of exercise, the usefulness of functional foods in daily life for the prevention of diabetes is recognized. For this purpose, studies of functional food components with blood glucose-controlling and serum lipid-lowering effects are in progress, and many useful components have been discovered in plants.

*Momordica charantia* (MC) belongs to the gourd family and is widely distributed in Asia, East Africa, and South America. It has a unique bitter taste, and has been used in folk remedies for diabetes for a long time. Functional studies have confirmed the anti-diabetic effect of MC, and blood glucose- and serum lipid-lowering effects of MC in STZ-induced diabetic rats have also been reported. As the action mechanism of the hypoglycemic effect, inhibition of glucose absorption, promotion of glucose uptake in skeletal muscles, increased insulin sensitivity, and improvements in insulin secretion and action have been reported. Hypoglycemic effects of methanol and water extracts of MC have also been reported, but the blood glucose-lowering component of MC has not yet been identified. Moreover, there have been very few reports concerning the serum lipid-lowering component and its action mechanism.

We focused on saponin, which is contained in MC and is soluble in n-butanol. Many saponin-rich plants are used for medicinal purposes. Saponin has been found to reduce elevation of the blood glucose level and lipid metabolism, particularly lipid absorption.

In this study, we investigated the inhibitory effects of the MC saponin fraction on disaccharidase and blood glucose increase. To clarify the mechanism of the serum lipid-lowering effect of MC, the inhibitory effects of the MC saponin fraction on pancreatic lipase and increases in the serum neutral fat level were also investigated.

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Abbreviation: MC, *Momordica charantia*
Materials and Methods

Preparation of the saponin fraction (butanol-soluble fraction) and water-soluble fraction. Hot blast-dried MC (2,000 g) was powdered using a food processor. This dried powder was combined with 70% methanol (6 liters) and kept at room temperature for 2 d. The extract was filtered and the residue was combined with 70% methanol (6 liters) for re-extraction. The two extracts were combined, filtered, concentrated using a rotary evaporator, and freeze-dried, and methanol extract (409.2 g, 20.5%) was obtained. This extract was dissolved in pure water (2 liters) and partitioned with diethyl ether (1 liter) twice, and the partitioned ether layer was removed. The water layer was partitioned with n-butanol (1 liter) twice, and the partitioned butanol and water layers were separated. Each extract was concentrated and dried under reduced pressure. A saponin fraction (25.1 g, 1.3%) was prepared as a butanol-soluble component from the butanol layer. The concentration of saponin in this fraction was 15.4% as saikosaponin C (Wako Pure Chemical Industries, Osaka, Japan). From the water layer, a water-soluble fraction (255.2 g, 12.8%) was prepared (Fig. 1).

Disaccharidase-inhibitory activity. The method reported by Konishi et al.\textsuperscript{15} was modified and used to prepare crude enzyme solution. Rat small intestinal acetone powder (Sigma Chemical, St. Louis, MO) was suspended in 20 volumes of 0.1 M phosphate buffer pH 6.0 (Wako). The suspension was sonicated and centrifuged (13,000 rpm, 20 min, 4 °C), and a crude enzyme solution was prepared. The protein content of the crude enzyme solution was measured by the Lowry method and stored at −40 °C until the test. Enzyme-inhibitory activity was measured according to the method reported by Matsuda et al.\textsuperscript{16} For the enzyme reaction, 50 μl of the sample solution was added to 100 μl of 74 mM sucrose solution in 0.1 M phosphate buffer (pH 6.0) and pre-heated at 37 °C for 2 min, followed by the addition of 50 μl of the enzyme solution for the reaction. After the reaction had proceeded for 10 min, 800 μl of pure water was added, and the reaction solution was heated in a boiling water bath for 2 min to inactivate the enzyme. To the control, buffer was added instead of the sample. As for the blank, 800 μl of pure water was added immediately after the addition of the enzyme solution, followed by heating in a boiling water bath for 2 min to inactivate the enzyme. Glucose production was measured using Glucose C II Test Wako (Wako). As an index of inhibitory activity, the sample concentration (mg/ml) at which the enzyme reaction was reduced by 50% (IC\textsubscript{50} value) was used.

Inhibition of blood glucose increase. Six-week-old male Wistar rats (Clea Japan, Tokyo) were housed in stainless steel cages and maintained under conditions of 23 ± 1 °C room temperature, 55 ± 5% humidity, and a 12-h lighting cycle (light-on, 8:00–20:00). The rats were given free access to MF pellets (Oriental Yeast, Tokyo) and water, and used in a sugar-loading test after acclimation for 1 week. Six rats per group were used in a sucrose-loading test. The rats were fasted for 18 h, and MC extracts were administered as test samples. The doses of the water-soluble fraction were 100 and 1,000 mg/kg body weight, and the doses of the saponin fraction were 50 and 100 mg/kg body weight. Each fraction was dissolved in pure water and administered orally using a stomach probe. To the control group, pure water was administered. Ten min after test-sample administration, 2 g/kg body weight sucrose was administered orally using a stomach probe, and blood was collected every 30 min for 120 min. Blood from the tail vein was collected using a heparinized hematocrit
capillary tube. The blood sample was immediately centrifuged (3,000 rpm, 15 min), and the serum was collected. The serum glucose level was measured using Glucose C II Test Wako (Wako).

**Lipase-inhibitory activity.** Lipase activity was measured by the method reported by Han et al. In brief, a substrate solution was prepared by sonicating 80 mg of triolein, 10 mg of lecithin (Wako), and 5 mg of cholic acid (Sigma) in 9 ml of 0.1 M TES (Dojindo Laboratories, Kumamoto, Japan) buffer (pH 7.0) for 10 min. For the enzyme reaction, 100 µl of sample solution and 50 µl of porcine pancreatic lipase (10 U) were added to 100 µl of substrate solution, and this was reacted at 37 °C for 30 min. After completion of the reaction, the reaction solution was heated in a boiling water bath for 2 min to inactivate the enzyme. The blank of each sample was heated in a boiling water bath for 2 min immediately after the addition of the enzyme solution to inactivate the enzyme. Released fatty acids were measured with NEFA C Test Wako (Wako). The activity of each sample was calculated taking fatty acid production in the absence of the sample to be 100%.

**Lipid-loading test.** Six-week-old male Wistar rats (Clea Japan) were housed in stainless steel cages and maintained under conditions of 23 ± 1 °C room temperature, 55 ± 5% humidity, and a 12-h lighting cycle (light-on, 8:00–20:00). The rats were given free access to MF pellets (Oriental Yeast) and water, and used in a lipid absorption inhibition test after acclimation for 1 week. Six rats per group were fasted for 18 h. Three groups, a control and 50 and 100 mg/kg saponin fraction treatment groups, were established. The control group received 10 ml/kg body weight of pure water, followed by oral administration of 1 ml of corn oil (Hayashi Chemical, Tokyo) after 10 min. The treatment groups received orally 10 ml/kg body weight of the saponin fraction (1 and 0.5 g/dl), followed by oral administration of 1 ml of corn oil after 10 min. Blood was collected from the tail vein before corn oil administration and 60, 120, 180, and 240 min after administration. Blood samples were centrifuged (3,000 rpm, 15 min) to separate sera. The serum neutral-fat level was measured with Triglyceride-E-Test Wako (Wako).

**Statistical analysis.** The measured values are presented as the means ± standard error. For significance analysis of differences among the three groups in vivo, Tukey’s t-test was used (p < 0.05). In the statistical analysis of pancreatic lipase activity between the two groups in vitro, Student’s t-test was used (p < 0.01).

**Results**

**Disaccharidase-inhibitory activity**

The inhibitory activities against rat small intestinal disaccharidase are presented in Table 1. MC saponin and the water-soluble fractions inhibited disaccharidase in a concentration-dependent manner. The IC₅₀ values of MC saponin and the water-soluble fractions were 1.0 and 5.5 mg/ml respectively, showing the marked inhibitory activity of the saponin fraction.

**Inhibition of blood glucose increases**

The effects of the saponin and water-soluble fractions on the blood glucose level after sucrose loading are shown in Figs. 2 and 3. In the saponin-fraction treatment groups (50 and 100 mg/kg body weight), the blood glucose level slowly increased, but the elevation of this level fell significantly at 30 min after sucrose loading. In the 100-mg/kg treatment group, this significant reduction persisted at 60 min after sucrose loading. In the 1,000 mg/kg body weight water-soluble fraction treatment group, the blood glucose level was significantly lower than in the control group at 30 min after sucrose loading. In the 100 mg/kg body weight water-soluble fraction treatment group, no significant inhibition of the blood glucose increase after 30 or 90 min was noted, suggesting that the saponin fraction exhibited a stronger inhibitory effect on the elevation of the blood glucose level than the water-soluble fraction.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>IC₅₀ (mg/ml)</th>
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<tr>
<td>Saponin fraction</td>
<td>1.0</td>
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<tr>
<td>Water-soluble fraction</td>
<td>5.5</td>
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**Table 1. Inhibitory Effects of the Saponin Fraction and the Water-Soluble Fraction on Rat Intestinal Disaccharidase**

**Fig. 2. Effect of Saponin Fraction on Blood Glucose Level after Administration of Sucrose Solution.**

Ten min after the saponin fraction (50 or 100 mg/kg body weight) was administrated orally to rats using a stomach probe, 2 g/kg body weight of sucrose was administrated. The glucose level of blood collected from the tail vein was determined by Glucose C II Test Wako. Values are means ± SE, n = 6. Those not sharing a common letter at the same time are significantly different, p < 0.05.
Inhibition of lipase activity by the saponin and water-soluble fractions

The effects of the saponin and water-soluble fractions on pancreatic lipase activity are shown in Fig. 4. The saponin and water-soluble fractions inhibited pancreatic lipase activity in a concentration-dependent manner. On comparison of the inhibitory activity of the two fractions, the inhibitory activity of the saponin fraction was significantly stronger than that of the water-soluble fraction.

Inhibition of lipid absorption

The effects of the saponin and water-soluble fractions on increases in the serum neutral fat level after corn oil loading are shown in Fig. 5. The elevation of the serum neutral fat level was significantly inhibited at 60, 180, and 240 min after corn oil loading in the saponin-fraction treatment groups (100 mg/kg body weight). In the other treatment group by saponin fraction (50 mg/kg body weight), the elevation was significantly inhibited after 60, 120, 180, and 240 min. The elevation in both of these groups was slower than that in the control. These results indicate that the saponin fraction inhibited the elevation of the serum neutral fat level after lipid loading.

Discussion

Momordica charantia is a plant containing a specific saponin. Since many saponin-rich plants are used for medicinal purposes, we prepared an MC saponin fraction and investigated the action mechanism of its blood glucose- and serum lipid-lowering effects.

The inhibitory effect on disaccharidase, which has a marked influence on carbohydrate absorption, was significantly stronger by the saponin fraction than by the
water-soluble fraction. In the sugar-loading test, the saponin fraction (50 and 100 mg/kg) significantly suppressed a sucrose load-induced elevation in the blood glucose level at 30 and 60 min after sugar-loading. Inhibition of blood glucose increases by the water-soluble fraction was noted only at 30 min in the high-dose group (1,000 mg/kg).

The saponin fraction markedly reduced disaccharidase activity, and inhibited the elevation of the blood glucose level at a low dose, suggesting that the main component related to the hypoglycemic effect of MC is concentrated in the saponin fraction. Ali et al. found that saponin-free methanol extract of whole MC produced no hypoglycemic effects in normal rats. These controversial results are likely due to variations in the source of MC and the method of preparation. It has been reported that the saponin contained in Aralia elata and Senega is a component of the fraction with an inhibitory action against blood glucose elevation. These findings also suggest that MC saponin was closely associated with the suppression of blood glucose elevation. Furthermore, MC-induced lowering of blood glucose was closely associated with inhibition of carbohydrate absorption by the disaccharidase-inhibitory activity. Samane et al. found that the saponin fraction of Argania spinosa enhances insulin-induced PKB/Akt activation. PKB/Akt mediates the effect of insulin to enhance glucose transport in muscle and adipose tissues and glycogen synthesis in the liver.

To clarify the mechanism of the inhibition of blood glucose increases, further study of the effect of the saponin fraction of MC on PKB/Akt activation in muscle and adipose tissues is necessary.

A component with inhibitory action against carbohydrate load-induced blood glucose increases has been reported simultaneously to inhibit insulin secretion, suggesting that the saponin fraction also inhibited insulin secretion after sucrose loading. Excess postprandial insulin secretion promotes lipid accumulation, causing obesity. Obesity is a basic pathological condition of various disorders including diabetes, and a risk factor for advancement of insulin resistance. The saponin fraction, which inhibits postprandial elevation of the blood glucose level, might be also useful for the prevention of obesity.

In addition, the saponin and water-soluble fractions inhibited pancreatic lipase activity in a concentration-dependent manner, and the inhibitory activity of the saponin fraction was markedly high. Since the lipase reaction occurs at the interface between lipase and oil droplets, reducing the interface volume might inhibit this reaction. The saponin fraction binds to the substrate, fat, which might reduce the contact area between the substrate and lipase, indirectly inhibiting the action of lipase. To investigate the effect of the saponin fraction on elevation of the serum neutral-fat level, an in vivo lipid-loading test was performed. In the saponin-fraction treatment group, lipid load-induced elevation of the serum neutral-fat level was significantly inhibited, suggesting that the saponin fraction inhibited intestinal lipid absorption by reducing pancreatic lipase activity.

The above findings suggest that the component related to the serum lipid-lowering effect of MC is concentrated in the saponin fraction. Inhibition of lipid absorption by saponin components derived from plants, such as tea and platycodin, has been confirmed, suggesting that the active component of MC is saponin. As a mechanism of the serum lipid-lowering effect of MC, inhibition of lipid absorption through the inhibition of pancreatic lipase activity was involved.

Postprandial hyperlipidemia is considered a risk factor for arteriosclerotic diseases. Hence, the saponin fraction, which inhibits lipid absorption, might prevent not only diabetes but also arteriosclerotic diseases. Since lipid absorption-inhibitory saponin has also been reported to have an anti-obesity action, the saponin fraction is expected to exhibit an anti-obesity action through a similar mechanism.

Based on the above findings, the active component with blood glucose- and serum lipid-lowering actions was concentrated in the saponin fraction, suggesting a close involvement with saponin. As a mechanism of the blood glucose- and serum lipid-lowering effects, carbohydrate and lipid absorption was inhibited through digestive enzyme inhibition.

In conclusion, the saponin fraction reduced postprandial increases in blood glucose and neutral fat levels, suggesting that it might be useful for the prevention of diabetes and associated complications. The MC saponin fraction has been confirmed to contain various MC-specific saponins. Their anti-diabetic and obesity-preventive effects have not yet been clarified. Further investigation of the active component causing these effects is necessary.

References

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