Alleviating Effects of Mulberry Fruit Extract on Postprandial Hyperglycemia in Streptozotocin-induced Diabetic Mice

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Postprandial hyperglycemia is an early defect of type 2 diabetes and one of primary anti-diabetic targets. The alpha-glucosidase inhibitors regulate postprandial hyperglycemia by impeding the rate of carbohydrate (such as starch) digestion in the small intestine. This study was designed to investigate the inhibitory actions of mulberry fruit extract (MFE) on α-glucosidase and α-amylase activities, and its alleviating effect on postprandial hyperglycemia activities in vitro and in vivo. Male four-week old ICR mice and streptozotocin (STZ)-induced diabetic mice were treated with mulberry fruit extract. MFE showed strong inhibitory effects against α-glucosidase and α-amylase activities, with half-maximal inhibitory concentration (IC₅₀) values of 0.16 and 0.14 mg/ml, respectively, and was more effective than acarbose, which was used as a positive control. The increase in postprandial blood glucose levels was more significantly attenuated in the MFE-administered group mice than in the control group mice of both STZ-induced diabetic and normal mice. Moreover, the area under the glucose response curve significantly decreased following MFE administration in diabetic mice. These results indicate that MFE may be a potent inhibitor of α-glucosidase and α-amylase, and helpful in suppressing postprandial hyperglycemia in diabetic mice. The mulberry fruit extracts may be considered as a potential candidate for the management of diabetes.

Key words: α-Amylase, α-Glucosidase, diabetic mice, mulberry fruit extract, postprandial hyperglycemia

Introduction

The prevalence of diabetes mellitus, a metabolic disease characterized by hyperglycemia resulting from defects in both regulations of insulin secretion and/or insulin action, is increasing worldwide [9]. The possibility that postprandial hyperglycemia is associated with the development of diabetic complications has recently received much attention [30]. An acute hyperglycemia after a meal may result in endothelial dysfunction and oxidative stress [11]. Moreover, studies have shown an association between postprandial glucose levels and the pathogenesis of atherosclerosis [32]. Therefore, management of postprandial hyperglycemia is considered a major therapeutic strategy for type 2 diabetes treatment, and can be achieved by delaying the release of glucose through the inhibition of carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase in the digestive tract [26].

α-glucosidase inhibitors such as acarbose and miglitol are hypoglycemic agents that slow the digestion and absorption of carbohydrates in the small intestine and hence, reduce the increase in blood glucose levels after a meal. However, it is well acknowledged that administration of such drugs is frequently associated with adverse effects including flatulence, abdominal pain, and diarrhea [5, 29]. Thus, many natural products having minimal side effects have been investigated with respect to their inhibition of glucose production from carbohydrates in the gut or glucose absorption from the intestine [3].

Mulberry (Morus alba L.) is a genus of flowering plants in the family Moraceae, and its fruits and leaves have been used as traditional oriental medicines to treat diabetes [4]. The mulberry fruit, called Oddi in Korea, is a 2- to 3 cm long multiple fruit. Immature fruits are white, green, or pale yellow. The fruits turn pink and then red while ripening, and finally dark purple or black with a sweet flavor when fully ripe. Mulberry fruits contain large amounts of anthocyanins. Cyanidin-3-glucoside (C3G) and cyanidin-3-rutinoside (C3R) are the most important active components of mul-
Inhibitory effect of mulberry fruits on intestinal α-glucosidase, either
ing a readily available yeast enzyme [33]. Briefly, yeast α-
glucosidase (0.7 units, Sigma, St. Louis, MO, USA) was dis-
solved in 100 mM phosphate buffer (pH 7.0) containing 2
μl bovine serum albumin and 0.2 g/l NaN3 to form the
enzyme solution. The substrate solution was prepared by
dissolving p-nitrophenyl-α-D-glucopyranoside (5 mM) in the
same buffer. Next, 50 μl of enzyme solution and 10 μl of
sample dissolved in dimethyl sulfoxide (5 mg/ml) were
mixed in a well of a microtiter plate and the absorbance
at 405 nm was measured with a microplate reader at time
zero. After incubation for 5 min, the substrate solution (50
μl) was added and the mixture was incubated for another
5 min at room temperature. The increase in absorbance from
time zero was measured. The inhibitory activity of MFE at
varying concentrations was expressed as 100 minus the ab-
sorbance change of test compounds relative to the absorb-
ance change of the control (%), where the test solution was
replaced by the carrier solvent. The measurements were per-
formed in triplicate and the IC50 value (the concentration of
MFE that results in 50% inhibition of maximal activity) was
determined.

**Inhibition assay for α-amylase activity in vitro**

The α-amylase inhibition assay was performed in the
same way as described for the α-glucosidase inhibition assay
except that porcine pancreatic α-amylase (100 units, Sigma)
and p-nitrophenyl-α-D-maltopentaglucoside were used as
the enzyme and substrate, respectively.

**Experimental animals**

Four-week-old, male ICR mice (Orient Bio Inc., Seong-
nam, Korea) were used as test animals and were housed
individually in a light (12 hr on/12 hr off) and temper-
ature-controlled room with ad libitum access to pelleted food
and water. After a 2-week adjustment period, diabetes was
induced as described below. All procedures were approved
by the animal ethics committee of our university. All animal
experiments were treated in accordance with guidelines of
the Pusan National University for the care and use of labo-
ratory animals (ED-PNU2014-0663).

**Induction of diabetes**

To induce diabetes, mice were fasted for 18 hr before ad-
ministration of a single intraperitoneal (i.p.) injection of 60
mg/kg STZ prepared in 0.1 M sodium citrate buffer (pH
4.5). One week after injection of STZ, fasting blood glucose
levels were periodically measured using a glucometer
(Roche Diagnostics GmbH, Mannheim, Germany). Mice with
blood glucose values of 250 mg/dl or higher were consid-
ered diabetic.

**Measurement of blood glucose level**

Normal mice and STZ-induced diabetic mice fasted over-
night (deprived of food for at least 12 hr but allowed free
access to water) were each randomly divided into 3 groups

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**Materials and Methods**

**Materials**

Mulberry (M. alba L.) fruits were collected from the coast
of Sangju-si Gyeongsangbuk-do, South Korea. The fruits
were individually washed with tap water, rinsed carefully
with fresh water, and freeze-dried. The dried sample was
ground and sifted through a 50 mesh standard testing sieve.
The sample was extracted three times with ten volumes of
80% methanol for 12 hr at room temperature. The filtrate
was then vacuum-evaporated to obtain the extract. After the
mulberry fruit extract (MFE) was thoroughly dried, it was
stored in a deep freezer (-80°C). The composition of antho-
cyanins in the MFE was analyzed by high-performance liq-
uid chromatography (HPLC), and C3G and C3R were found
to be the major anthocyanins. The levels of C3G and C3R
in the MFE were 34.1 and 14.3 mg/1.5 g, respectively [4].

**Inhibition assay for α-glucosidase activity in vitro**

The α-glucosidase inhibition assay was conducted by the
chromogenic method described by Watanabe et al. (1997) using
a readily available yeast enzyme [33].
of 7 mice (a total 6 groups) and treated as follows: 1) control: mice received oral administration of soluble starch (2 g/kg body weight [BW]) alone; 2) MFE: mice received oral administration of starch with MFE (300 mg/kg BW); 3) acarbose: mice received oral administration of starch with acarbose (100 mg/kg BW). Blood samples were taken from the tail vein at 0, 30, 60, and 120 min after oral administration. Blood glucose level was measured using a glucometer (Roche Diagnostics GmbH) and the area under the glucose response curve (AUC) was calculated using the trapezoidal rule [13].

Data and statistical analysis
The data are represented as the mean ± standard deviation (SD) of triplicate experiments. The statistical analysis was performed using SAS software ver. 9.1 (SAS Institute Inc., Cary, NC, USA). Differences among groups were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett’s multiple range test. Differences between two groups were compared using Student’s t-test. Values of p less than 0.05 were considered statistically significant.

Results and Discussion
Inhibitory effect of MFE on α-glucosidase and α-amylase in vitro
The inhibitory effect of MFE against α-glucosidase was determined using p-nitrophenyl-α-glucopyranoside as the substrate. α-Glucosidase activity was inhibited by MFE in a dose-dependent manner, with 28.57, 38.10, 48.15, and 59.26% inhibition at MFE concentrations of 0.05, 0.10, 0.15, and 0.20 mg/ml, respectively (Fig. 1). The α-glucosidase inhibitory activity of the MFE was significantly higher than that of the positive control, acarbose. The α-amylase inhibitory activity of MFE on the other hand, was determined using p-nitrophenyl-α-maltopentoglycoside as the substrate. Similarly, the inhibitory activity of MFE against α-amylase was also dose-dependent (34.52, 40.74, 53.70, and 61.57% inhibition at MFE concentrations of 0.05, 0.10, 0.15, and 0.20 mg/ml, respectively) (Fig. 2). Compared with the commercial inhibitor, acarbose, MFE was more effective in inhibiting α-amylase activity. The IC_{50} values of MFE against α-glucosidase and α-amylase were 0.16 and 0.14 mg/ml, respectively, which demonstrated its stronger inhibitory activities over those of acarbose (Table 1).

Postprandial hyperglycemia is a prominent and early defect in the development of diabetes and an important factor that contributes to complications associated with diabetes, such as micro- and macrovascular diseases. Therefore, alleviating hyperglycemia in the postprandial state is a treatment goal for patients with diabetes [27]. Starch is primarily di-

![Fig. 1. Inhibitory effect of mulberry fruit extract (MFE) on α-glucosidase. Each value represents the mean ± SD of triplicate experiments. *Values denoted by different letters are significantly different at p<0.05 as analyzed by Duncan’s multiple range test. Acarbose was used as positive control.](image1)

![Fig. 2. Inhibitory effect of mulberry fruit extract (MFE) on α-amylase. Each value represents the mean ± SD of triplicate experiments. *Values denoted by different letters are significantly different at p<0.05 as analyzed by Duncan’s multiple range test. Acarbose was used as positive control.](image2)

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC_{50} (mg/ml)</th>
<th>α-glucosidase</th>
<th>α-amylase</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFE</td>
<td>0.16±0.01*</td>
<td>0.14±0.01*</td>
<td></td>
</tr>
<tr>
<td>Acarbose</td>
<td>0.34±0.02</td>
<td>0.45±0.04</td>
<td></td>
</tr>
</tbody>
</table>

IC_{50} value is the concentration of sample required for 50% inhibition. Each value represents the mean ± SD of triplicate experiments.

*p<0.05 compared to the control group.
gested in the small intestine through the action of pancreatic α-amylase, producing both linear maltose and branched isomaltose oligosaccharides, which are further hydrolyzed by intestinal α-glucosidase to yield absorbable monosaccharides [17]. α-glucosidase catalyzes the final step in the digestive process of carbohydrates; hence, its inhibitors could impede the absorption of dietary carbohydrates to reduce postprandial hyperglycemia [24]. Thus, controlling postprandial hyperglycemia by inhibiting α-glucosidase and α-amylase is one of the key goals in diabetic therapy [1].

In the present study, the inhibitory activities of MFE on α-glucosidase and α-amylase were investigated to examine the possible use of MFE as an anti-hyperglycemic functional food. MFE has higher inhibitory activities on the aforementioned enzymes than the commercial carbohydrate digestive enzyme inhibitor, acarbose; and this can be attributed to the active compounds present in MFE. Mulberry fruits contain abundant anthocyanins, particularly C3G, C3R, pelargonidin 3-O-glucoside, and pelargonidin 3-O-rutinoside [20, 25].

These anthocyanins, belonging to the flavonoid class of polyphenolic compounds, are known to bind to a variety of proteins. The hydroxyl groups in polyphenolic compounds may link and form complexes with enzymes, thus inhibiting their activity [14, 31]. McDougall et al. [23] reported that anthocyanins from berry extracts have been implicated in the inhibition of α-glucosidase [23]. Furthermore, Iwai et al. [8] demonstrated that C3G contained in mulberry fruit significantly inhibited the activities of carbohydrases such as sucrose, α-glucosidase, and α-amylase [8]. Thus, we assume that MFE may possess similar inhibitory effects on α-glucosidase and α-amylase owing to the presence of these anthocyanins.

**Effect of MFE on blood glucose level in vivo**

The effect of MFE on postprandial blood glucose levels was determined in STZ-induced diabetic and normal mice. In diabetic mice, postprandial blood glucose levels of the MFE-administered group were lower than those of the control group (Fig. 3). Blood glucose levels of the control group increased to 402.5 mg/dl at 60 min after starch loading, before decreasing to 379.67 mg/dl at 120 min. On the other hand, postprandial blood glucose levels were noticeably lower (p<0.05) when diabetic mice were fed with MFE (351.0, 369.7, and 352.0 mg/dl at 30, 60, and 120 min, respectively). Similarly in normal mice, MFE significantly reduced (p<0.05) postprandial hyperglycemia where the increase in postprandial blood glucose level was suppressed to 137.6, 129.2, and 112.6 mg/dl at 30, 60, and 120 min, respectively (Fig. 4). In diabetic mice, the AUC of glucose response was significantly lower in the MFE-administered group (3330.2±462.79 mmol/min.L⁻¹) than in the control group (5036.5±289.6 mmol/min.L⁻¹) (Table 2), further supporting the hypoglycemic effect of MFE.

![Fig. 3. Blood glucose levels after the administration of mulberry fruit extract (MFE) in STZ-induced diabetic mice. Distilled water (as control), MFE (300 mg/kg), and acarbose (100 mg/kg) were co-administered orally with starch (2 g/kg). Each value represents the mean ± SD of seven mice (n = 21). Values denoted by different letters are significantly different at p<0.05 as analyzed by Duncan’s multiple range test.](image1)

![Fig. 4. Blood glucose levels after the administration of mulberry fruit extract (MFE) in normal mice. Distilled water (as control), MFE (300 mg/kg), and acarbose (100 mg/kg) were co-administered orally with starch (2 g/kg). Each value represents the mean ± SD of seven mice (n = 21). Values denoted by different letters are significantly different at p<0.05 as analyzed by Duncan’s multiple range test.](image2)
Table 2. Area under the curve (AUC) of postprandial glucose responses of normal and streptozotocin-induced diabetic mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal mice</th>
<th>Diabetic mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFE</td>
<td>2586.0±576.6</td>
<td>3300.2±462.8</td>
</tr>
<tr>
<td>Acarbose</td>
<td>1703.7±51.3</td>
<td>2222.1±572.4</td>
</tr>
<tr>
<td>Control</td>
<td>4746.5±275.4</td>
<td>5036.5±289.6</td>
</tr>
</tbody>
</table>

*Values denoted by different letters are significantly different at p<0.05 as analyzed by Duncan’s multiple range test.

In diabetes, the postprandial phase is typified by a rapid and large increase in blood glucose levels, and the possibility that these postprandial “hyperglycemic spikes” may have a negative influence on the pathophysiology of late diabetes complications has recently received much attention [30]. Postprandial hyperglycemia reduces not only insulin secretion due to the dysfunction of the pancreas [2] but also insulin sensitivity [15, 16], resulting in a deteriorated diabetic state and late diabetes complications. Therefore, maintaining near-normal levels of glycemic control, both in the fasting and postprandial states, is a treatment goal for patients with type 2 diabetes [27]. In this study, we investigated the anti-hyperglycemic effects of MFE in STZ-induced diabetic mice and normal mice after the consumption of starch together with MFE. Postprandial blood glucose levels were significantly decreased in STZ-induced diabetic mice. Consistent with these findings, Ritta et al. [28] reported that dietary anthocyanins in berries significantly reduced postprandial glucose levels. Therefore, the results of our study suggest that anthocyanin contained in MFE may delay the absorption of dietary starch, thus attenuating the increase in postprandial blood glucose levels. This alleviating effect on postprandial hyperglycemia may be due to the inhibition of carbohydrate digestive enzymes in the small intestine of diabetic mice. Inone et al. (1997) showed that medication that flattens the peak of the postprandial blood glucose levels reduces the AUC of the blood glucose response curve [7]. In this study, MFE significantly decreased blood glucose level at the peak time point and hence, the AUC.

Diabetes is caused by alterations in carbohydrate metabolism leading to resistance to insulin secretion, thus resulting in a state of hyperglycemia [34, 36]. Postprandial hyperglycemia plays an important role not only in the development of type 2 diabetes but also in complications associated with the condition, including microvascular and macrovascular diseases [2]. A large number of intervention trials have demonstrated that improving hyperglycemia achieves considerable reduction of these complications [6]. Although many synthetic compounds such as gliptazide, metformin, and voglibose have been used in the treatment of diabetes, they have in general been associated with marked toxic or undesirable side effects [5]. Therefore, mulberry fruit, containing abundant anthocyanins, may possibly be an excellent source of natural anti-diabetic agents. Our current findings suggest that MFE could potentially be developed as a natural functional food to improve postprandial hyperglycemia and prevent diabetic complications.

In conclusion, MFE inhibited α-glucosidase and α-amylase activities and attenuated the rise in blood glucose levels, leading to a reduction in postprandial hyperglycemia. Moreover, MFE may delay the absorption of dietary carbohydrates in the gut, hence suppressing the increase in blood glucose levels after a meal. Thus, MFE may be a useful food source to control diabetes and helpful in improving postprandial hyperglycemia.

Acknowledgement

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References


초록: STZ으로 유도된 당뇨 마우스에서 오디열매추출물의 식후 고혈당 완화 효과

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식후 고혈당은 제2형 당뇨병 초기 진단과 항당뇨병의 중요한 바이오마커 중 하나이다. α-글루코시다아제 억제제는 소장에서 탄수화물 소화의 속도를 방해함으로써 식후 고혈당을 조절한다. 본 연구에서는 오디열매추출물이 α-글루코시다아제와 α-아밀라아제에 미치는 억제효과 및 스트렙토조토신(STZ)이 유발하는 당뇨병 생쥐의 식후고혈당에 미치는 완화 효과를 조사하였다. α-글루코시다아제와 α-아밀라아제에 대한 오디열매추출물(MFE)의 IC₅₀ 값은 각각 0.16과 0.14 mg/ml의 결과값을 나타내어, 양성대조군인 acarbose보다 더 효과적이었다. STZ으로 유발된 당뇨병 환자의 식후 혈당 수치는 정상 대조군에 비해 오디열매추출물에서 유의적으로 더 낮았다. 더욱이, 오디열매추출물 투여는 당뇨병 환자에서 포도당 반응에 대한 곡선 Integrity 감소와 관련이 있었다. 결론적으로, 오디열매추출물은 α-글루코시다아제와 α-아밀라아제 활성의 강력한 억제제이며 식후 고혈당을 완화할 수 있다는 내용이다. 따라서 오디열매추출물은 당뇨병 치료제 및 기능성식품의 소재로 가치가 높다고 사료된다.