Antihyperglycemic, antioxidant and anti-inflammatory effects of umbelliferone in high-fat diet/streptozotocin-induced type 2 diabetic rats

Jarinyaporn Naowaboot*, Nuntiya Somparn*, Suphaket Saentaweesuk**, Patchareewan Pannangpetch***

Abstract

Introduction: Oxidative stress and chronic inflammation are important pathological processes that can cause insulin resistance and type 2 diabetes mellitus, and eventually complications of diabetes mellitus. For this study, we aimed to investigate the effect of umbelliferone (UMB) in the regulation of blood glucose, hyperlipidemia, oxidative stress, and chronic inflammation in high-fat diet/streptozotocin-induced type 2 diabetic rats.

Method: Male Wistar rats were fed on 45 kcal % fat diet for 3 weeks followed by intraperitoneally injected with a single dose of streptozotocin (35 mg/kg). Diabetic rats were fed with UMB for 6 weeks. The fasting blood glucose (FBG), lipid profiles, malondialdehyde (MDA), and inflammatory cytokine; tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) and monocyte chemoattractant protein-1 (MCP-1) were determined.

Result: Type 2 diabetic rat model showed elevated blood glucose, impaired glucose tolerance, increased total cholesterol, triglyceride and non-esterified fatty acid, increased MDA, and increased TNF-\(\alpha\) and MCP-1.

The results of the present study have been shown that the UMB treatment reduced blood glucose level, improved glucose tolerance, and decreased the levels of all of lipid profiles. In addition, serum and tissues MDA as well as levels of TNF-\(\alpha\) and MCP-1 in serum were decreased.

Discussion: These results suggest a beneficial effect of UMB in reducing the blood glucose, hyperlipidemia, oxidative stress and inflammatory mediators in type 2 diabetic rats.

Key words: Umbelliferone, Type 2 diabetic rat, Oxidative stress, Inflammation

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Introduction

Type 2 diabetes mellitus (T2DM) is one of the world’s most common chronic diseases as changing lifestyles lead to reduced physical activity and increased obesity. Early phenomenon of T2DM is insulin insensitivity, which not only has negative metabolic consequences but also contributes subsequent pancreas β-cell exhaustion, resulting in the onset of clinical hyperglycemia.

Oxidative stress resulting from increased production of reactive oxygen species (ROS) have a key function in the pathogenesis of late diabetic complications. ROS resulting from hyperglycemia are thought to contribute to the initiation of lipid peroxidation. Malondialdehyde (MDA) is an end product of lipid peroxidation. There has been a report that hyperglycemia associated with hyperlipidemia could be the causative factor for the increased production of free radicals and lipid peroxides, that is, MDA. Moreover, hyperglycemia has been shown to induce the expression of proinflammatory cytokines and chemokine genes in monocyte cells. Certain inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), impair insulin action in peripheral tissue and have a direct function in obesity-associated insulin resistance.

Monocyte chemotactant protein (MCP)-1 is a C-C chemokine family with a potent chemotactic factor for monocytes. MCP-1 biosynthesis is induced by inflammatory cytokines, or oxidatively modified low density lipoprotein (LDL) in monocytes, endothelial cells, and vascular smooth muscle cells. It has been demonstrated that high glucose concentration stimulates the expression of MCP-1 and the formation of ROS. As well as, the antidiabetic drug, pioglitazone, causes decreased gene expression of MCP-1 in adipose tissue, along with an improvement in insulin resistance.

Many kinds of plants and their bioactive compounds have been shown to improve metabolic abnormalities, such as insulin resistance, obesity, and diabetes mellitus. Coumarins constitute a very large class of compounds present in several species belonging to different botanical families. Coumarin and its derivatives have been found to have a wide range of bioactivities, such as antifungal, antitumor, antithrombotic, vasodilatory, anti-inflammatory, and antioxidant activities. Umbelliferone (UMB) or 7-hydroxycoumarin is a widespread natural product of the coumarin family with anti-inflammatory activity. Recent studies have shown that UMB can act as a potent antioxidative and anti-inflammatory agent in streptozotocin-induced type 1 diabetic rats. However, the anti-inflammation activity of UMB in hyperglycemic with oxidative stress condition in type 2 diabetic animal model is still not completely investigated. Also as mentioned above the incidence of type 2 diabetes is increasing, therefore, this study was aimed to determine these effects in T2DM induced by high-fat diet with low dose of streptozotocin (STZ).

Method

Chemicals and reagents

Umbelliferone and all fine chemicals were obtained from Sigma-Aldrich (St. Louise, MO, USA). Pioglitazone was purchased from Berlin Pharmaceutical Industry Co., Ltd. (Latkrabang, Bangkok, Thailand).

Animals and induction of diabetes

Animal experiments were performed according to the animal care guidelines and were approved by the Animal Ethics Committee of Thammasat University, Pathum Thani, Thailand (Rec.No. AE 008/2013). Male Wistar rats weighting 120-150g were obtained from the National Laboratory Animal Center of Mahidol University, Nakhon Pathom, Thailand. Two animals were housed per cage in a room with a 12/12-hour light/dark cycle and an ambient temperature of 22 to 25°C. They were fed with standard normal diet (SND) or HFD containing 45 kcal % fat (soybean oil and lard that included 0.95 mg cholesterol per g of lard), 35 kcal % carbohydrates and 20 kcal % protein (Research diets, Brunswick, NJ, USA) ad libitum during 3 weeks. After 3 weeks of the HFD, rats were fasted for 12 hours (free access to water) and were injected with a single low dose of STZ (35 mg/kg intraperitoneally). Rats were allowed to develop diabetes for a week. After that, fasting blood glucose (FBG) levels were determined...
from tail vein blood using glucometer (Accu-Check, Roche Diagnostics, Manheim, Germany). The rats with FBG above 126 mg/dL were considered diabetic and included in the experiments.

Experimental design

The rats were divided into six groups with six rats in each group and treated as follows: group 1: normal control rats (SND feeding) with 5% Gum Arabic; group 2: diabetic control rats with 5% Gum Arabic; group 3: diabetic positive control rats with pioglitazone 10 mg/kg per day; group 4 to 6: diabetic rats with UMB 10, 30, and 60 mg/kg per day, respectively. Pioglitazone and UMB were suspended in 5% Gum Arabic. All treatments were daily administrated orally using feeding tube for 6 weeks. The FBG level of all rats was measured after 6 weeks of treatment. After 6 weeks of UMB treatment, rats were fasted overnight and anesthetized with ether. The heart, liver, kidney and thoracic aorta were rapidly excised to perform biochemical examinations as described below in detail. During fasting, rats were deprived of food for 12 hours but had free access of water.

Oral glucose tolerance test (OGTT)

After 5 weeks of treatment, an OGTT was performed to evaluate the effect of each treatment on glucose tolerance. After 12 hours of fasting, rats were orally loaded with glucose (1.0 g/kg of body weight) and blood glucose levels were determined from the tail vein blood before and after glucose loading at 30, 60, and 120 min. The area under the curve (AUC) of blood glucose was calculated from the blood concentration-time relationships.

Determination of serum lipid profiles

After 6 weeks of UMB treatment, serum triglyceride, total cholesterol and non-esterified fatty acid (NEFA) levels were determined using enzymatic colorimetric method (Wako, Osaka, Japan).

Determination of MDA in liver, kidney, heart and thoracic aorta

The tissues (heart, liver, kidney and thoracic aorta) were finely sliced and homogenized in cold phosphate buffered saline with magnesium chloride (pH 7.4). After 10 minutes centrifugation (14,000 × g, 4 °C), the clear supernatant was collected for MDA assay. MDA was examined using TBARS parameter assay kit (R&D Systems, Minneapolis, MN, USA). The MDA concentration in the tissues was normalized against the protein concentration. Protein was determined by the Bradford’s method.

Determination of serum TNF-α and MCP-1 secretion

After 6 weeks of UMB treatment, serum TNF-α and MCP-1 concentrations were assayed using rat TNF-α ELISA kit (R&D Systems, Minneapolis, MN, USA) and rat MCP-1 ELISA kit (Thermo Scientific, Rockford, IL, USA).

Statistical analyses

Results were expressed as the mean values with their standard errors of each group. Multiple comparisons were analyzed by analysis of variance (ANOVA) and Tukey’s post-hoc test. The statistical analyses were performed using computer-based software SigmaStat (Systat Software, CA, USA). The level of significance was uniformly set at p < 0.05.

Result

Effect of UMB on fasting blood glucose

After a period of 6 weeks, the FBG level of the diabetic control group was still high. However, all doses of UMB (10, 30, and 60 mg/kg) significantly reduced blood glucose levels (35%, 44%, and 29% respectively; p < 0.05) (Figure 1A). An oral administration of positive control 10 mg/Kg pioglitazone reduced blood glucose levels of diabetic rats by 46% (Figure 1A).
Figure 1 Effect of UMB on fasting blood glucose (A), OGTT (B) and AUC in OGTT (C) in high-fat diet/streptozotocin-induced type 2 diabetic rats. Values were mean ± S.E.M. (n = 6). *p < 0.05 when compared to normal control group; "p < 0.05 when compared to diabetic control group. NR: normal control rat treated with 5% Gum Arabic; DM: diabetic control rat treated with 5% Gum Arabic; DM + Pio: diabetic rat treated with pioglitazone 10 mg/kg; DM + UMB: diabetic rat treated with umbelliferone 10, 30, and 60 mg/kg.
Effect of UMB on OGTT

To determine the insulin sensitizing effect of UMB, OGTT treatment was performed at the fifth week of treatment. Oral administration with glucose caused a gradual increase in blood glucose in all the groups at 30 min (Figure 1B). In diabetic control group, the blood glucose was remained at the high level until at 120 min. As compared to the diabetic control rats, the UMB (30 mg/kg) or pioglitazone (10 mg/kg) significantly (p < 0.05) suppressed the blood glucose level at 60 and 120 min. For the lowest concentration of UMB (10 mg/kg), it significantly (p < 0.05) decreased blood glucose only at 120 min. Consistent with the AUC of blood glucose level after glucose administration, which was higher in diabetic control than that of normal control rats, and was decreased by 10 and 30 mg/kg UMB treatments (Figure 1C).
Figure 2 Effect of UMB on MDA production in thoracic aorta (A), heart (B), kidney (C) and liver (D) in high-fat diet/streptozotocin-induced type 2 diabetic rats. Values were mean ± S.E.M. (n = 6). *p < 0.05 when compared to normal control group; *p < 0.05 when compared to diabetic control group. NR: normal control rat treated with 5% Gum Arabic; DM: diabetic control rat treated with 5% Gum Arabic; DM + Pio: diabetic rat treated with pioglitazone 10 mg/kg; DM + UMB: diabetic rat treated with umbelliferone 10, 30, and 60 mg/kg.
Effect of UMB on serum lipid profiles

As shown in Table 1, there were significant (p < 0.05) increases in total cholesterol, triglyceride, and NEFA levels in the serum of diabetic control as compared to normal control rats. However, the diabetic rat treated with UMB (10, 30, and 60 mg/kg) or pioglitazone had the significant decreases in total cholesterol, triglyceride, and NEFA.

Table 1 Effect of UMB on serum total cholesterol, triglyceride, and NEFA in high-fat diet/streptozotocin-induced type 2 diabetic rats.

<table>
<thead>
<tr>
<th>Group of treatment</th>
<th>Total cholesterol (mg/dL)</th>
<th>Triglyceride (mg/dL)</th>
<th>NEFA (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>71.3 ± 4.1</td>
<td>155.5 ± 1.6</td>
<td>26.6 ± 1.1</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>127.4 ± 9.4*</td>
<td>253.1 ± 9.9*</td>
<td>54.4 ± 3.4*</td>
</tr>
<tr>
<td>Diabetic + Pio 10</td>
<td>87.3 ± 5.3*</td>
<td>158.7 ± 8.7*</td>
<td>26.3 ± 3.4*</td>
</tr>
<tr>
<td>Diabetic + UMB 10</td>
<td>89.9 ± 3.8*</td>
<td>171.3 ± 8.1*</td>
<td>28.0 ± 1.2*</td>
</tr>
<tr>
<td>Diabetic + UMB 30</td>
<td>85.4 ± 6.4*</td>
<td>162.4 ± 8.5*</td>
<td>27.5 ± 1.2*</td>
</tr>
<tr>
<td>Diabetic + UMB 60</td>
<td>91.0 ± 4.1*</td>
<td>174.7 ± 1.9*</td>
<td>30.4 ± 4.5*</td>
</tr>
</tbody>
</table>

Values were mean ± S.E.M. (n = 6).
*p < 0.05 when compared to normal control group; *p < 0.05 when compared to diabetic control group. NR: normal control rat treated with 5% Gum Arabic; DM: diabetic control rat treated with 5% Gum Arabic; DM + Pio: diabetic rat treated with pioglitazone 10 mg/kg; DM + UMB: diabetic rat treated with umbelliferone 10, 30, and 60 mg/kg.

Effect of UMB on lipid peroxidation content

The MDA contents in serum (Table 2), thoracic aorta, heart, kidney and liver (Figure 2A, 2B, 2C, and 2D, respectively) of diabetic rats were significantly increased as compared to the normal rats (p < 0.05). It is noteworthy that the diabetic rats receiving all doses of UMB had significantly lower level of MDA in all tissue than that of diabetic control rats.

Effect of UMB on serum TNF-α and MCP-1 levels

As shown in Table 2, the diabetic rats had significantly increased TNF-α and MCP-1 levels. However, after treatment with all doses of UMB, the levels of TNF-α and MCP-1 were significantly reduced. The decreases in TNF-α and MCP-1 by 30 mg/kg UMB was comparable to 10 mg/kg pioglitazone.
Discussion and conclusion

The major findings of the present work were that the daily supplementation with UMB could decrease blood glucose, improve glucose tolerance, and decrease lipid profiles, MDA inflammatory cytokines in HFD/STZ-induced T2DM rats.

The rats that received HFD and low dose of STZ (35 mg/kg) had the hyperglycemic state and impaired glucose tolerance. This model also showed abnormalities of lipid metabolism by increase in serum total cholesterol, triglyceride and NEFA, which are similar to the metabolic changes in human with T2DM. All metabolic changes in our diabetic animal model were consistent with the previous reports from other studies.24-26

To understand the pharmacological effect of UMB, we first started to analyze its effect on blood glucose and lipid profiles. We observed that the high blood glucose in diabetic rats was decreased by UMB treatment. In particular, UMB at 30 mg/kg not only decreased the FBG, but also remarkably improved the glucose tolerance. Several studies suggested that abnormal blood lipids were characteristic of case with insulin resistance, especially high circulating free fatty acids.27-28 Surprisingly all doses of UMB treatment effectively reduced the serum total cholesterol, triglyceride and NEFA levels.

There are marked differences in coumarin metabolism between susceptible rodent and other species. The major route of coumarin metabolism in murines is by a 3,4-epoxidation pathway, which results in the formation of toxic metabolites.29 These toxic metabolites might be a negative impact on glucose and lipid metabolisms in T2DM rat model. Although reports of adverse effects in humans resulting from coumarin administration are rare, it is acutely toxic to laboratory animals in chronic oral gavage administration at doses equal or higher of 200 mg/kg.30 Therefore, it is possible that the high dose of UMB (60 mg/kg) is less effective than other doses in regulating the hyperglycemic condition. However, in our study did not observe any signs of toxicity in all doses of umbelliferone-treated rats.

Hyperglycemia is the main cause of complications of diabetes because elevated glucose concentration directly injures cells and induces lipid peroxidation.31 Therefore, we further analyzed the effect of UMB on lipid peroxide alteration. Diabetic rats treated with UMB had a significantly lower MDA concentration in the serum and also in various tissues especially in the aortic tissue than that of non-treated diabetic rats (Figure 2A). The significantly increased MDA concentrations in serum and various tissues in chronic diabetic rats point to ROS-mediated cellular damage, denoting the presence of oxidative stress in chronic diabetes.

### Table 2 Effect of UMB on serum MDA, TNF-α, and MCP-1 in high-fat diet/streptozotocin-induced type 2 diabetic rats.

<table>
<thead>
<tr>
<th>Group of treatment</th>
<th>MDA (nmol/L)</th>
<th>TNF-α (pg/mL)</th>
<th>MCP-1 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>226.5 ± 3.2</td>
<td>14.9 ± 0.2</td>
<td>252.2 ± 5.0</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>310.0 ± 5.5</td>
<td>21.2 ± 0.3</td>
<td>430.4 ± 7.2</td>
</tr>
<tr>
<td>Diabetic + Pio 10</td>
<td>239.5 ± 7.4</td>
<td>17.0 ± 0.2</td>
<td>253.6 ± 2.6</td>
</tr>
<tr>
<td>Diabetic + UMB 10</td>
<td>246.0 ± 4.3</td>
<td>18.2 ± 0.3</td>
<td>269.6 ± 15.6</td>
</tr>
<tr>
<td>Diabetic + UMB 30</td>
<td>237.5 ± 2.0</td>
<td>17.8 ± 0.1</td>
<td>264.7 ± 7.8</td>
</tr>
<tr>
<td>Diabetic + UMB 60</td>
<td>253.6 ± 5.5</td>
<td>18.2 ± 0.3</td>
<td>280.0 ± 8.7</td>
</tr>
</tbody>
</table>

Values were mean ± S.E.M. (n = 6). *p < 0.05 when compared to the normal control group; #p < 0.05 when compared to the diabetic control group. Normal control: normal control rat treated with 5% Gum Arabic; Diabetic control: diabetic control rat treated with 5% Gum Arabic; DM + Pio 10: diabetic rat treated with pioglitazone 10 mg/kg; DM + UMB: diabetic rat treated with umbelliferone 10, 30, and 60 mg/kg
Thus, the treatment that aims at reducing oxidative stress will be beneficial in patients with T2DM.

It has been proposed that T2DM is a disease of the immune system, involving a cytokine-mediated acute-phase inflammatory response. A study in 3T3-L1 adipocytes culture, it has been shown that insulin resistance associated mediators, such as TNF-α, IL-6, and growth hormone have a significant stimulatory effect on MCP-1 secretion. Our results showed that UMB efficiently reduced TNF-α and MCP-1 level in diabetic rats, whereas it decreased the high blood glucose and improved glucose tolerance.

As high blood glucose level can cause increase in oxidative stress and inflammation. The hypoglycemic, hypolipidemic, antioxidant, and anti-inflammatory activities of UMB may be associated to each other. Therefore, administration of UMB could be helpful in not only reducing the high blood glucose but might delay the complication of diabetes by reducing the oxidative stress and inflammation.

In conclusion, UMB is a proven beneficial compound for diabetic state. UMB not only reduces the blood glucose but also reduces the oxidative stress and inflammation processes. Eventually, UMB can be administered as a complementary therapeutic regimen.

Acknowledgement
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References


บทคัดย่อ
ฤทธิ์ลดระดับน้ำตาลในเลือด, ฤทธิ์ต้านออกซิเดชั่น, และฤทธิ์ต้านกำรอักเสบของสารอัมเบลิเฟอโรนในหนูขาวเบาหวานชนิดที่ 2 ที่เหนี่ยวนำด้วยอาหารไขมันสูงร่วมกับฉีดสเตร็ปโตโซโตซิน
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บทนำ:
ภาวะเครียดออกซิเดชั่น และการอักเสบเรื้อรัง เป็นกระบวนการทางพยาธิสภาพที่สำคัญ ซึ่งก่อให้เกิดภาวะตื่นตัว ต่ออินซูลิน และการเกิดโรคเบาหวานชนิดที่ 2 รวมถึงภาวะแทรกซ้อนจากโรคนี้ สำหรับการศึกษาที่นี้ มีวัตถุประสงค์เพื่อประเมินฤทธิ์ของสารอัมเบลิเฟอโรน ต่อภาวะควบคุมระดับน้ำตาลในเลือด, ภาวะไขมันในเลือดสูง, ภาวะเครียดออกซิเดชั่น และกระบวนการอักเสบเรื้อรัง ในหนูขาวเบาหวานชนิดที่ 2

วิธีการศึกษา:
นำหนูขาวเพศผู้สายพันธุ์ Wistar เนื้อหนา 60 กรัม ให้เป็นเบาหวานชนิดที่ 2 โดยการให้หนูได้รับอาหารไขมันสูงนาน 3 สัปดาห์ จากนั้นให้ฉีดสารสเตร็ปโตโซโตซิน (ขนาด 35 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัม) เพื่อทำลายตับอ่อน วิธีการดังกล่าวทำให้หนูที่มีระดับน้ำตาลในเลือดสูงกว่า 126 มิลลิกรัม/dl จะได้รับสารอัมเบลิเฟอโรนนาน 6 สัปดาห์ เพื่อตรวจสอบระดับน้ำตาลในเลือด, ค่าไขมัน, ค่า malondialdehyde (MDA) และสารอักเสบ tumor necrosis factor-α (TNF-α) และ monocyte chemoattractant protein-1 (MCP-1)

ผลการศึกษา:
การเหนี่ยวนำหนูขาวเบาหวานชนิดที่ 2 ด้วยวิธีนี้ทำให้หนูมีระดับน้ำตาลในเลือดสูง การทำงานของอินซูลิน บกพร่อง มีการเพิ่มขึ้นของระดับ โคเลสเตอรอล, ไตรกลีเซอไรด์ และกรดไขมินอิสระในเลือด ระดับ MDA และสารอักเสบ TNF-α และ MCP-1 ในเลือดสูงขึ้น ผลการทดสอบพบว่า สารอัมเบลิเฟอโรนสามารถลดระดับน้ำตาลในเลือด, เพิ่มการทำงานของอินซูลิน และลดระดับไขมันในเลือด นอกจากนี้สารอัมเบลิเฟอโรนช่วยลดระดับ MDA ในเลือดและในเนื้อเยื่อต่างๆ ตลอดจนช่วยลดการทำงานของ TNF-α และ MCP-1 ดีกว่า

วิจารณ์และ
ผลการทดสอบนี้แสดงให้เห็นว่าสารอัมเบลิเฟอโรนสามารถลดระดับน้ำตาลในเลือด, ลดระดับไขมันในเลือด

สรุปผลการศึกษา:
ลดภาวะเครียดออกซิเดชั่น, และลดการอักเสบในหนูขาวเบาหวานชนิดที่ 2 ได้

ค่าสำคัญ: สารอัมเบลิเฟอโรน, หนูขาวเบาหวานชนิดที่ 2, ภาวะเครียดออกซิเดชั่น, การอักเสบ