Angelica keiskei Extract Improves Insulin Resistance and Hypertriglyceridemia in Rats Fed a High-Fructose Drink

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Angelica keiskei is a traditional herb peculiar to Japan and abundantly contains vitamins, dietary fiber and such polyphenols as chalcone. We investigated in the present study the effect of A. keiskei on insulin resistance and hypertriglyceridemia in fructose-drinking rats as a model for the metabolic syndrome. Male Wistar rats were given a 15% fructose solution as drinking water for 11 weeks. Fructose significantly increased the levels of serum insulin and triglyceride (TG) compared with the control level. Treatment with AE enhanced the expression of the genes related to fatty acid β-oxidation and high-density lipoprotein (HDL) production. Treatment with AE enhanced the expression of the acyl-CoA oxidase 1 (ACO1), medium-chain acyl-CoA dehydrogenase (MCAD), ATP-binding membrane cassette transporter A1 (ABC1), and apolipoprotein A1 (Apo-A1) genes. These results suggest that AE improved the insulin resistance and hypertriglyceridemia of the fructose-drinking rats.

Key words: Angelica keiskei; insulin resistance; hypertriglyceridemia; fructose-drinking rat

Insulin resistance is a metabolic disorder characterized by reduced insulin sensitivity in the target tissues, including skeletal muscle, liver and adipose tissue. Insulin resistance is closely associated with type II diabetes mellitus (T2DM), dyslipidemia and hypertension. The insulin resistance syndrome, also known as the metabolic syndrome, increases the risk for cardiovascular disease (CVD).1,2 According to the national health and nutrition survey by Ministry of Health, Labor and Welfare of Japan, 50% of men and 20% of women aged 40–74 years are patients with or candidates for the metabolic syndrome. CVD is a major cause of mortality, accounting for almost 25% of all deaths in Japan. It is well known that dietary intervention and moderate exercise are effective for preventing or treating insulin resistance. However, these lifestyle modifications are frequently neglected. Recent studies have shown that such food materials as herbs,3–6 spices,7 vegetables,8 green tea9 and wine10 improved insulin resistance in animal models.

Angelica keiskei Koidzumi (“Asahita” in Japanese) is an edible plant grown along the Pacific Coast of Japan. This traditional herb was also used as a folk medicine in the Izu islands for a long time. Fresh leaves of A. keiskei are nowadays widely used for green juice and health-promoting food in Japan. A. keiskei uniquely contains chalcones as polyphenols in its leaves, roots and yellow exudates from the stem. Xanthoangelol (XA) and 4-hydroxyderricin (4HD) are two major prenylated chalcones in A. keiskei.11 It has also been reported that about twenty minor chalcones were isolated from A. keiskei.12–15 These chalcones have shown anti-tumor promoting,16 anti-cancer,17,18 anti-bacterial,19 anti-ulcer20 and artery relaxation21 actions. Our previous studies indicated that XA and 4HD markedly induced adipogenic differentiation of 3T3-L1 preadipocytes and enhanced glucose uptake in 3T3-L1 adipocytes.22,23 It has also been reported that XA and 4HD enhanced the glucose transporter type 4 (GLUT4)-dependent glucose uptake in skeletal muscle cells.24 Moreover, the chalcones from A. keiskei have exhibited anti-diabetic, anti-dyslipidemic and anti-hypertensive actions in animal models. We have reported that oral administered XA and 4HD prevented hyperglycemia in KK-Ay type II diabetic mice.25 Ogawa et al. have reported that XA and 4HD also improved hypertension and hyperlipidemia in spontaneously hypertensive rats.26,27 These reports suggested that A. keiskei could be useful for preventing insulin resistance and the metabolic syndrome. However, little is known about the effects of chalcones from A. keiskei on insulin resistance.

Abbreviations: ABC1, ATP-binding membrane cassette transporter A1; ACO1, acyl-CoA oxidase 1; AE, ethanol extract of Angelica keiskei; Apo-A1, apolipoprotein A1; CPT-1, carnitine palmityltransferase-1; CVD, cardiovascular disease; ELISA, enzyme-linked immunosorbent assay; FFA, free fatty acid; GLUT4, glucose transporter type 4; HBA1C, hemoglobin A1c; 4HD, 4-hydroxyderricin; HDL, high-density lipoprotein; HOMA-R, homeostasis model assessment insulin resistance; MCAD, medium-chain acyl-CoA dehydrogenase; PPAR, peroxisome proliferator-activated receptor; T2DM, type II diabetes mellitus; TG, triglyceride; TZD, thiazolidinedione; XA, xanthoangelol
The excess ingestion of fructose has generally induced insulin resistance in humans and rodents.\(^{27,28}\)
Fructose-drinking or -fed rats are used as a model for the metabolic syndrome. We investigate in this study the effect of an ethanol extract of Angelica keiskei (AE) containing chalcones on the insulin resistance and hypertriglyceridemia in fructose-drinking rats.

**Materials and Methods**

**Preparation of AE.** Angelica keiskei was cultivated and harvested in Kagoshima Prefecture in Japan. Dried leaves and stems (10 kg) were washed with hot water, and the washed residue was extracted twice with 90% ethanol (20 L) for 2 h. The resulting extract was concentrated under reduced pressure and dried. Powdered AE contained XA (7.30 g per kg) and 4HD (3.45 g per kg) which constituted 97% of the total chalcones.

**Animals and experimental design.** Male Wistar rats (5 weeks old, Japan SLC, Shizuoka, Japan) were housed in standard cages (2 rats per cage) at 23 ± 2°C and 55 ± 10% relative humidity with a 12 h light/12 h dark cycle (lights on at 06.00 h). After a 1-week acclimatization period, the rats were assigned to three groups of 8 rats each by body weight. The Fructose (F) group and Fructose-AE (F-AE) group were given 15% fructose water for 11 weeks. The normal (N) group was given tap water and CE-2 for 11 weeks. The animals were fed ad libitum with a powdered CE-2 diet (Clea, Tokyo, Japan) with or without 3% w/w of AE. The Normal (N) group was given tap water and CE-2 for 11 weeks. The fructose-drinking groups (F and F-AE) were given 15% fructose water for 11 weeks. The F-AE group was also fed a diet containing 3%/w AE. The normal group (N) was given tap water. The body weight, food intake, and fluid intake were measured once a week. The liver weight and liver lipid contents were measured after 11 weeks of feeding.

**Biological measurements.** At the end of the feeding period, blood samples were collected from the caudal vena cava of anesthetized rats. Each sample was centrifuged at 1,500 g for 10 min at 4°C, and aliquots of the serum were stored at −80°C until being analyzed. The blood glucose level was measured by a Glucose CII-Test kit (Wako Pure Chemicals, Osaka, Japan). The serum insulin level was measured by a rat insulin ELISA kit (Morinaga Institute of Biological Science, Kanagawa, Japan). The serum triglyceride (TG), free fatty acid (FFA), total cholesterol and high-density lipoprotein (HDL)-cholesterol levels were measured with commercial assay kits (Triglyceride E-test, NEFA C-test, Total Cholesterol E-test and HDL-Cholesterol E-test, Wako Pure Chemicals). Serum adiponectin was measured by an ELISA kit (Assaypro, Saint Charles, MO, USA). HOMA-R, an index of insulin resistance, was calculated by using the formula, blood glucose (mmol/L) × insulin (μU/mL)/22.5. The liver was excised, weighed and divided into several pieces for storage at −80°C (for the lipid analysis) or in an RNAlater solution (Ambion, Austin, TX, USA) (for the gene expression analysis). Lipids from the liver were extracted by hexane/isopropanol (3:2) and measured by using the assay kits just described.

**Gene expression analysis.** Hepatic mRNA was isolated by RNA Iso Plus (Takara Bio, Shiga, Japan). Total RNA was subjected to real-time RT-PCR by using a SYBR PrimeScript RT-PCR kit (perfect real time).

**Statistical analysis.** All data are expressed as the mean ± SEM. A one-way analysis of variance (ANOVA) was used to compare means among the groups. Post hoc analyses were performed by the least significant difference (LSD) test if the ANOVA data were significant.

**Results**

**Body weight, food intake and fluid intake**

There was no significant difference in body weight among all groups by the end of the experiment (Table 2). The daily food intake by the F group and F-AE group was significantly lower than that by the N group (\(p < 0.001\) and \(p < 0.001\), respectively), while the daily fluid intake by the F group and F-AE group was significantly higher than that by the N group (\(p < 0.01\) and \(p < 0.05\), respectively). There was no appreciable difference in the food and fluid intake between the F group and F-AE group.

**Liver weight and liver lipids**

The absolute and relative liver weights of the F group were significantly higher than those of the N group (\(p < 0.001\) and \(p < 0.001\), respectively). Table 2 shows

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**Table 1.** Primers Used for Real-Time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACO1</td>
<td>TTCAAGAAGAAACGGTCGCAA</td>
<td>TGGCTCCCTCAAGAAAGATGCC</td>
</tr>
<tr>
<td>MCAD</td>
<td>ACCGGAGCTTGCGGATAGG</td>
<td>CATTGGTGCTTCGTCATC</td>
</tr>
<tr>
<td>PPARα</td>
<td>CGGCTCATCTCGGAAAGAA</td>
<td>AAGGCTTCTCTGACGCTAG</td>
</tr>
<tr>
<td>CPT-1</td>
<td>GCATCCCCAGGAAAGAGACAA</td>
<td>CGGCCCTCATAGGGCAGA</td>
</tr>
<tr>
<td>Apo-A1</td>
<td>TAAAGGTGTGCGCGGAGGAG</td>
<td>GCTACGGTGAAGTGGTTC</td>
</tr>
<tr>
<td>ABCA1</td>
<td>AGGAACCCCATTCACCAAACA</td>
<td>CTGCTACACTGOCAGCAAGG</td>
</tr>
<tr>
<td>LDLR</td>
<td>AGCCATCTTCAAGGCGCAACC</td>
<td>TGCCCTCACAGTTCACC</td>
</tr>
<tr>
<td>β-Actin</td>
<td>TAAAGGCAACCCGTGAAAGA</td>
<td>CCAGGGCACATAGGACGAA</td>
</tr>
</tbody>
</table>

**Table 2.** Effects of AE on the Body Weight, Food Intake, Fluid Intake, Liver Weight and Liver Lipid Contents in Fructose-Drinking Rats

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>F</th>
<th>F-AE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>320 ± 8</td>
<td>332 ± 7</td>
<td>323 ± 6</td>
</tr>
<tr>
<td>Food intake (/g/d)</td>
<td>18.7 ± 0.2</td>
<td>13.8 ± 0.3***</td>
<td>14.5 ± 0.4***</td>
</tr>
<tr>
<td>Fluid intake (/mL/d)</td>
<td>23.9 ± 0.3</td>
<td>27.9 ± 0.9***</td>
<td>26.6 ± 1.1*</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>10.2 ± 0.3</td>
<td>12.1 ± 0.3***</td>
<td>10.6 ± 0.3***</td>
</tr>
<tr>
<td>Liver total cholesterol (mg/g of liver)</td>
<td>3.18 ± 0.03</td>
<td>3.64 ± 0.03***</td>
<td>3.30 ± 0.04***</td>
</tr>
<tr>
<td>Liver TG (mg/g of liver)</td>
<td>20.2 ± 1.0</td>
<td>20.0 ± 1.1</td>
<td>19.8 ± 0.8</td>
</tr>
</tbody>
</table>

(Takara Bio) according to the manufacturer’s protocol and a Thermal Cycler Dice real time system (Takara Bio). The following PCR conditions were applied: 1 cycle of 95°C for 10 s, 45 cycles of 95°C for 5 s and 60°C for 30 s, this being followed by 1 cycle of 95°C for 15 s, 60°C for 30 s and 95°C for 15 s, β-Actin was used as the internal control gene. The primer pairs used in the present study are listed in Table 1.

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**Gene Forward primer Reverse primer**

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<thead>
<tr>
<th>Gene</th>
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<th>Reverse primer</th>
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<td>TGGCTCCCTCAAGAAAGATGCC</td>
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</tr>
<tr>
<td>Apo-A1</td>
<td>TAAAGGTGTGCGCGGAGGAG</td>
<td>GCTACGGTGAAGTGGTTC</td>
</tr>
<tr>
<td>ABCA1</td>
<td>AGGAACCCCATTCACCAAACA</td>
<td>CTGCTACACTGOCAGCAAGG</td>
</tr>
<tr>
<td>LDLR</td>
<td>AGCCATCTTCAAGGCGCAACC</td>
<td>TGCCCTCACAGTTCACC</td>
</tr>
<tr>
<td>β-Actin</td>
<td>TAAAGGCAACCCGTGAAAGA</td>
<td>CCAGGGCACATAGGACGAA</td>
</tr>
</tbody>
</table>

Data are shown as the mean ± SEM (\(n = 8\)). *\(p < 0.05\), **\(p < 0.01\), ***\(p < 0.001\) (vs. the N group) **\(p < 0.01\), ***\(p < 0.001\) (vs. the F group)
The blood glucose (A) and insulin (B) levels were measured and HOMA-R (C) was calculated after 11 weeks of feeding as described in the Materials and Methods section. Data are shown as the mean ± SEM (n = 8). *p < 0.05, **p < 0.01, ***p < 0.001 (vs. the N group). ###p < 0.001 (vs. the F group).

Fig. 1. Effects of AE on the Levels of Blood Glucose, Insulin and HOMA-R in Fructose-Drinking Rats.

Blood glucose and insulin

Figure 1 shows that blood glucose in the F group was much higher than that in the N group (174 ± 9 mg/dL, 151 ± 7 mg/dL, p < 0.05). Treating with AE significantly reduced the blood glucose level (−16.5%, p < 0.01). The serum insulin level in the F group was 2.2-fold higher than that in the N group (5.22 ± 0.51 μg/mL, 2.37 ± 0.19 μg/mL, p < 0.001). The HOMA-R level in the F group was 2.6-fold higher than that in the N group (64.3 ± 8.4 vs. 25.1 ± 2.3, p < 0.001). AE markedly suppressed the fructose-induced increase in insulin level (−47.3%, p < 0.001) and HOMA-R level (−56.4%, p < 0.001).

Serum lipids (TG, FFA and cholesterol)

Figure 2 shows the level of serum lipids at the end of the experiment. The serum TG level in the F group was 5.22-fold higher than that in the N group (2.6-fold higher than that in the N group). Treating with AE lowered the serum TG level (−37%, p < 0.001; −25%, p < 0.05 vs. the N group). The serum FFA level in the F group was appreciably higher than that in the N group (p < 0.05). Treating with AE significantly reduced the serum FFA level (p < 0.05). The serum total and HDL-cholesterol levels in the F-AE group (89.5 ± 2.7 mg/dL and 48.6 ± 1.6 mg/dL, respectively) were much higher than those in the N group (62.4 ± 1.9 mg/dL and 33.7 ± 1.2 mg/dL, respectively) and F group (68.6 ± 2.9 mg/dL and 33.9 ± 1.1 mg/dL, respectively).

Serum adiponectin

Figure 3 shows that the serum adiponectin concentration in the F group was much lower than that in the N group (301 ± 21 mg/dL vs. 143 ± 14 mg/dL, p < 0.001). Treating with AE markedly suppressed the fructose-induced increase in serum TG (−24.2%, p < 0.01). The serum FFA level in the F group was appreciably higher than that in the N group (p < 0.05). AE significantly reduced the serum FFA level (p < 0.05). The serum total and HDL-cholesterol levels in the F-AE group (89.5 ± 2.7 mg/dL and 48.6 ± 1.6 mg/dL, respectively) were much higher than those in both the N group (62.4 ± 1.9 mg/dL and 33.7 ± 1.2 mg/dL, respectively) and F group (68.6 ± 2.9 mg/dL and 33.9 ± 1.1 mg/dL, respectively).

Hepatic gene expression

The hepatic mRNA expression levels of the genes involved in lipid and glucose metabolism are shown in Table 3. The mRNA levels of acyl-CoA oxidase 1 (ACO1), medium-chain acyl-CoA dehydrogenase (MCAD), apoLipoprotein-A1 (Apo-A1), and ABCA1 in the F group were much lower than those in the N group, while there was no appreciable difference in the mRNA levels of peroxisome proliferator-activated receptor-α (PPAR-α), carnitine palmitoyltransferase-1 (CPT-1) and the low-density lipoprotein receptor (LDLR) between the F group and N group. The mRNA levels of ACO1, MCAD, CPT-1, Apo-A1 and ABCA1 at 11 weeks were appreciably more up-regulated in the F-AE group than in the F group.
Drinking Rats

Kakuda et al. reported that XA and 4HD, the major substances in AE, reduced the serum insulin, blood glucose and HOMA-R levels. These results clearly suggest that AE reduced the serum insulin, blood glucose and HOMA-R levels by 2.2-fold and 2.6-fold. Treating with AE for 11 weeks reduced the serum insulin, blood glucose and HOMA-R levels. These results indicate that AE could possibly reduce the blood glucose level by enhancing fatty acid β-oxidation in the liver.

It has recently been proposed that insulin resistance and the metabolic syndrome are caused by the reduced productivity of adiponectin, an adipocytokine secreted from adipose tissue. Adiponectin activates AMP-activated protein kinase (AMPK), enhances fatty acid β-oxidation and improves insulin resistance via adiponectin receptors in the liver and skeletal muscle. Thiazolidinediones (TZDs), PPAR-γ agonists, have been widely used for treating T2DM as an insulin sensitizer. TZDs have improved the insulin resistance and increased the plasma adiponectin level in T2DM patients, high fat/sucrose-fed rats and ZDF rats. It has been reported that TZDs enhanced adipogenic differentiation in 3T3-L1 cells by PPAR-γ activation, and increased the number of small adipocytes which highly expressed adiponectin in obese animal models.

We have already reported that AE, XA and 4HD also induced differentiation in 3T3-L1 adipocytes, while they exhibited no PAR-γ activation in the GAL4-PPAR-γ ligand-binding domain chimeric system. Moreover, we have confirmed that 3T3-L1 adipocytes differentiated by XA or 4HD were capable of producing adiponectin (Ohnogi, H. and Kudo, Y., unpublished data).

Table 3. Effect of AE on the Hepatic mRNA Expression in Fructose-Drinking Rats

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>F</th>
<th>F-AE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACO1</td>
<td>1.00 ± 0.05</td>
<td>0.519 ± 0.038***</td>
<td>1.02 ± 0.05***</td>
</tr>
<tr>
<td>MCAD</td>
<td>1.00 ± 0.08</td>
<td>0.319 ± 0.033***</td>
<td>0.764 ± 0.035***</td>
</tr>
<tr>
<td>PPAR-α</td>
<td>1.00 ± 0.15</td>
<td>0.745 ± 0.090</td>
<td>0.937 ± 0.118</td>
</tr>
<tr>
<td>CPT-1</td>
<td>1.00 ± 0.11</td>
<td>0.828 ± 0.092</td>
<td>1.12 ± 0.089</td>
</tr>
<tr>
<td>Apo-A1</td>
<td>1.00 ± 0.08</td>
<td>0.570 ± 0.080***</td>
<td>0.837 ± 0.068#</td>
</tr>
<tr>
<td>ABCA1</td>
<td>1.00 ± 0.03</td>
<td>0.839 ± 0.054*</td>
<td>0.998 ± 0.044#</td>
</tr>
<tr>
<td>LDLR</td>
<td>1.00 ± 0.07</td>
<td>0.887 ± 0.065</td>
<td>1.02 ± 0.03</td>
</tr>
</tbody>
</table>

mRNA was isolated from the liver after 11 weeks of feeding. The mRNA levels were analyzed by real-time RT-PCR and normalized to β-actin. The gene expression levels are indicated as relative values compared to those of the Normal group (N).

Data are shown as the mean ± SEM (n = 8).

*p < 0.05, **p < 0.01, ***p < 0.001 (vs. the N group)

Discussion

We evaluated in the present study the effect of AE on insulin resistance and hypertriglyceridemia in fructose-drinking rats. It is well known that dietary fructose induces insulin resistance, hypertension, dyslipidemia and the metabolic syndrome in humans and animals. We confirmed that fructose drinking definitely induced hyperinsulinemia, insulin resistance and hypertriglyceridemia in this study. Figure 2 shows that fructose increased the respective serum insulin and HOMA-R levels by 2.2-fold and 2.6-fold. Treating with AE for 11 weeks reduced the serum insulin, blood glucose and HOMA-R levels. These results clearly suggest that AE improved the insulin resistance. We have previously reported that XA and 4HD, the major substances in AE, enhanced the glucose uptake in matured 3T3-L1 adipocytes and suppressed hyperglycemia and polydipsia in KK-A1 diabetic mice. Kawabata et al. have reported that XA and 4HD enhanced the glucose uptake in cultured skeletal muscle cells by inducing translocation of GLUT4, the insulin-responsive glucose transporter, to the plasma membrane, and that an acetic acid extract of Angelica keiskei improved acute hyperglycemia in an oral glucose tolerance test on ICR mice. These reports suggest that the anti-insulinemic and anti-hyperglycemic effects of AE were possibly due to the direct insulin-like activity of chalcones from A. keiskei.

Insulin resistance is not only a major incidence of T2DM, but is also deeply related to dyslipidemia. AE markedly reduced the serum TG level caused by excess ingestion of fructose in this study. To clarify the mechanism for the anti-hypertriglyceridemic action of AE, we analyzed the expression levels of the genes related to fatty acid β-oxidation in the liver. The expression of the ACO1 and MCAD genes in the F group was lower than that in the N group. ACO1 and MCAD are the key enzymes respectively involved in fatty acid β-oxidation in peroxisomes and mitochondria. Our results suggest that ingesting fructose suppressed fatty acid β-oxidation activity in the liver and then elevated the serum TG level. AE improved the expression of the ACO1 and MCAD genes that had been reduced by fructose. Moreover, AE enhanced the gene expression of CPT-1, the key enzyme that transports long-chain fatty acids into mitochondria. These observations indicate that AE could possibly reduce the blood TG level by enhancing fatty acid β-oxidation in the liver.

In conclusion, AE improved insulin resistance and hypertriglyceridemia in fructose-drinking rats which were used as the metabolic syndrome model. The genetic analysis showed that AE enhanced the expres-
sion of the genes related to fatty acid β-oxidation and HDL production in the liver. Although further studies are needed to clarify these effects, *A. keiskei* and its chalcones would be useful for preventing the metabolic syndrome.

**Acknowledgment**

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**References**