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Aster koraiensis extract lowers postprandial glucose in normoglycemic and high-fat-diet-induced obese mice

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Abstract The *Aster koraiensis* extract (ASKO) is a newly developed dietary herbal supplement. In this study, the potent blood glucose-lowering activity of ASKO in vitro and in vivo was investigated. In an in vitro glucose uptake assay, ASKO was found to enhance glucose transport in 3T3-L1 adipocytes. Oral administration of ASKO significantly reduced glucose levels in normoglycemic mice during oral glucose tolerance tests (OGTTs). In a long-term efficacy study, 4 weeks of oral ASKO treatment significantly attenuated blood glucose levels during OGTTs in diet-induced obese (DIO) mice. ASKO also enhanced plasma insulin levels after glucose loading, leading to a reduction in blood glucose levels. In addition, ASKO normalized glucose transporter-4 mRNA expression in the muscles of DIO mice. These results indicate that ASKO has postprandial glucose-lowering effects and could be beneficial in the management of prediabetes or type 2 diabetes mellitus.

Keywords *Aster koraiensis* · Glucose transporter · Postprandial glucose · Type 2 diabetes mellitus

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Introduction

Diabetes mellitus (DM) is an increasingly common metabolic disorder (Beagley et al., 2014; Guariguata et al., 2014), with type 2 DM (T2D) being more prevalent worldwide than type 1 DM (Hajiaghaalipour et al., 2015). DM is caused by a lack of insulin release from pancreatic β cells or by dysfunctional responses to insulin in various tissues (American Diabetes Association (ADA), 2010). Chronic hyperglycemia in patients with DM contributes to the development of macro- and micro-vascular complications (Yamagishi and Imaizumi, 2005).

Postprandial hyperglycemia is one of the early clinical signs in patients with T2D. Therefore, early intervention for hyperglycemia can inhibit or delay the development of various diabetes-related complications including nephropathy, retinopathy, and neuropathy (Ceriello, 2005). In addition, it has been reported that some food ingredients, including flavonoids, lower postprandial hyperglycemia, reducing the risk of T2D (Castro-Acosta et al., 2016; Hajiaghaalipour et al., 2015). In particular, dietary factors can reduce postprandial hyperglycemia by regulating glucose transporter (GLUT) (Hajiaghaalipour et al., 2015).

Aster koraiensis, described by Nakai Takenoshin in 1909, is a perennial native plant member of the Asteraceae family (Compositae) in Korea. It has been used as a food and as a medicinal plant to cure several diseases including pneumonia, chronic bronchitis, and pertussis (Ahn, 1998; Kim et al., 2017). Previous phytochemical investigations of *A. koraiensis* identified numerous secondary metabolites including polyacetylenes, benzofurans, and sesquiterpenoids (Lee et al., 2012). Recently, we showed that an extract of *A. koraiensis* reduced kidney podocyte apoptosis and protected retinal pericytes in diabetic rats (Kim et al., 2016). However, the exact details of the relationship

between *A. koraiensis* and diabetes have not yet been extensively investigated. Therefore, we studied the effects of an extract of *A. koraiensis* (ASKO) on hyperglycemia, and explored the mechanisms underlying its blood glucose-lowering activity.

Materials and methods

Preparation of the *A. koraiensis* extract and standard solution

A. koraiensis leaves were collected in Gongju, Korea, in September 2013, and identified by Prof. J.-H. Kim, Gachon University, Republic of Korea. A voucher specimen (KIOM-83A) has been deposited in the Herbarium of the Korea Institute of Oriental Medicine, Republic of Korea. The air-dried leaves of *A. koraiensis* were extracted with distilled water using a heat extractor. The extract solution was filtered and dried to produce ASKO powder. This was dissolved in distilled water prior to the experiments. The high-performance liquid chromatography fingerprint and levels of two major compounds in ASKO were described in a previous report (Kim et al., 2016).

Cell culture and differentiation

A mouse preadipocyte cell line (3T3-L1; American Type Culture Collection, Manassas, VA, USA) was cultured in Dulbecco's modified Eagle's medium containing 10% newborn calf serum. The cells were then incubated in a differentiation medium containing 10 μg/mL insulin (Sigma, St. Louis, MO, USA), 0.5 mM 3-isobutyl-1-methylxanthine (Sigma), and 0.25 μM dexamethasone (Sigma). After 2 days, the differentiation medium was replaced with medium containing 10 μg/mL insulin. After a further 2 days, this was replaced with fresh medium.

Cell viability assay

The viability of 3T3-L1 cells was examined using an MTS assay kit (Promega Corporation, Madison, WI, USA). The cells were plated with or without various concentrations of ASKO (0–100 μg/mL) and incubated for 24 h before determining viability. The MTS assay results were obtained by measuring the absorbance at 490 nm using a microplate reader (Tecan Group Ltd., Männedorf, Switzerland).

Measurement of glucose uptake

Glucose uptake was determined using a Glucose Uptake Cell-Based Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA). Briefly, confluent mature cells in culture plates were treated with ASKO (0, 12.5, 25, or 50 μg/mL) in glucose-free medium containing 150 μg/mL 2-[N-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino]-2-deoxy-D-glucose (NBDG) for 1 h. The amount of NBDG taken up by the cells was determined using a fluorescence microplate reader (Tecan Group Ltd.) at 466 nm/540 nm (excitation/emission).

Animals

The animal experiments were performed using 6-week-old male C57BL/6 mice (Orient Bio; Seoul, Korea). Four mice were housed per cage, under a 12-h light/dark cycle and at a controlled temperature (23 ± 1 °C), with water and food ad libitum. All procedures in the present study were approved by the Institutional Animal Care and Use Committee (IACUC) of the Korea Institute of Oriental Medicine (Daejeon, South Korea; IACUC approval no. 16-085).

Oral glucose tolerance test (OGTT) in normoglycemic mice

Seven-week-old mice were fasted for 16 h before glucose loading. Distilled water (vehicle) or ASKO (250 or 500 mg/kg) was orally administered to the mice 30 min before glucose loading (2 g/kg). The glucose levels in tail nick blood were determined 0, 30, 60, 90, and 120 min after glucose loading using Accu-chek Active (Roche Diagnostics GmbH, Mannheim, Germany). The area under the curve $(AUC)_{0–120\ min}$ for glucose was calculated using the trapezoidal rule.

OGTT in diet-induced obese (DIO) mice

To induce insulin resistance, DIO mice were maintained on a 60 kcal% fat diet from 7 weeks of age. At 18 weeks, the mice were randomly divided into the following 5 treatment groups of 10 mice: (1) control (normal chow diet); (2) DIO mice; (3) DIO mice treated with metformin (350 mg/kg); (4) DIO mice treated with 250 mg/kg ASKO; (5) DIO mice treated with 500 mg/kg ASKO. Metformin or ASKO were administered orally for 4 weeks. OGTT was conducted as described above for normoglycemic mice.

Plasma insulin detection

The levels of insulin in plasma obtained during OGTTs of normoglycemic or DIO mice were detected using an insulin radioimmunoassay kit (Linco Research, St. Charles, MO, USA). The concentration of insulin was determined by measuring the absorbance using a microplate reader.

Quantitative polymerase chain reaction

Total RNA was extracted from muscle tissue using the RNeasy Plus Mini Kit (QIAGEN, Hilden, Germany), and RNA was reverse-transcribed to cDNA. The cDNA was amplified using SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA, USA) with primers for glyceraldehyde 3-phosphate dehydrogenase (GAPDH; cat number: BR186qMmuCED0027497) and GLUT-4 (cat number: BR186qMmuCED0024734) purchased from Bio-Rad Laboratories, Inc. The results were normalized to those for GAPDH.

Statistical analysis

All data are expressed as the mean ± standard error of the mean (SEM). The analyses of data were performed by GraphPad Prism v.6.0 software (GraphPad Software, Inc., La Jolla, CA, USA) using one-way analyses of variance followed by Tukey's multiple comparison tests (> 2 groups) or Student's *t*-tests (2 groups); $p < 0.05$ was considered statistically significant.

Results and discussion

Prediabetes (previously known as impaired glucose tolerance) is a health condition defined by abnormal fasting blood glucose levels (Cowie et al., 2009). Most patients with prediabetes progress to develop impaired glucose tolerance, and finally show symptoms of overt T2D (Morris et al., 2013). Dietary interventions that manage blood glucose levels in patients with prediabetes can delay or prevent the development of T2D (Perreault et al., 2012). Therefore, dietary interventions that improve glycemic control in prediabetic individuals provide a therapeutic option and could help to reduce the prevalence of T2D.

Numerous plants have been used within traditional medicine for therapeutic purposes. In our previous studies, we reported that an ethanolic extract of *A. koraiensis* inhibited the formation of advanced glycation end products and reduced their cross-linking with proteins in vitro (Kim et al., 2016). This extract prevented podocyte apoptosis in renal tissues (Lee et al., 2012; Sohn et al., 2010) and in retinal pericytes (Kim et al., 2016) of streptozotocin-induced diabetic rats. However, this extract failed to improve hyperglycemia in streptozotocin-induced diabetic rats. To the best of our knowledge, the present study is the first to demonstrate that ASKO shows for the first time blood glucose-lowering activity in vitro and in vivo.

To investigate the cytotoxic effects of ASKO on 3T3-L1 adipocytes, MTS assay was performed at various concentrations of ASKO (1–100 μg/mL). The viability of these cells was not affected by exposure to up to 100 μg/mL ASKO (Fig. 1A). In vitro glucose transport assay can be employed in myocytes, adipocytes, and hepatocytes. Among them, we evaluated glucose uptake by 3T3-L1 adipocytes. We found that ASKO treatment for 1 h enhanced glucose uptake by these adipocytes in vitro (Fig. 1B). In an animal model, a single administration of ASKO was shown to significantly reduce the glycemic response, as compared to control. OGTTs found that the blood glucose levels of the control group reached 251.4 mg/dL 30 min after glucose loading. After 60 min, this decreased to 176.0 mg/dL and continued to drop, reaching an average of 163.2 mg/dL 120 min post loading. However, ASKO significantly suppressed blood glucose levels throughout the course of the experiment, when compared to the glucose levels in control mice (Fig. 2A). The glucose AUC values for both ASKO-treated groups (ASKO-250, 630.4 ± 45.16 mg min/dL; and ASKO-500, 623.2 ± 48.67 mg min/dL) were significantly lower than that of the control group (721.4 ± 36.46 mg min/dL) (Fig. 2B). There was no difference between the AUC of glucose in the two ASKO-treated groups. In addition, mice treated with ASKO showed significantly increased plasma

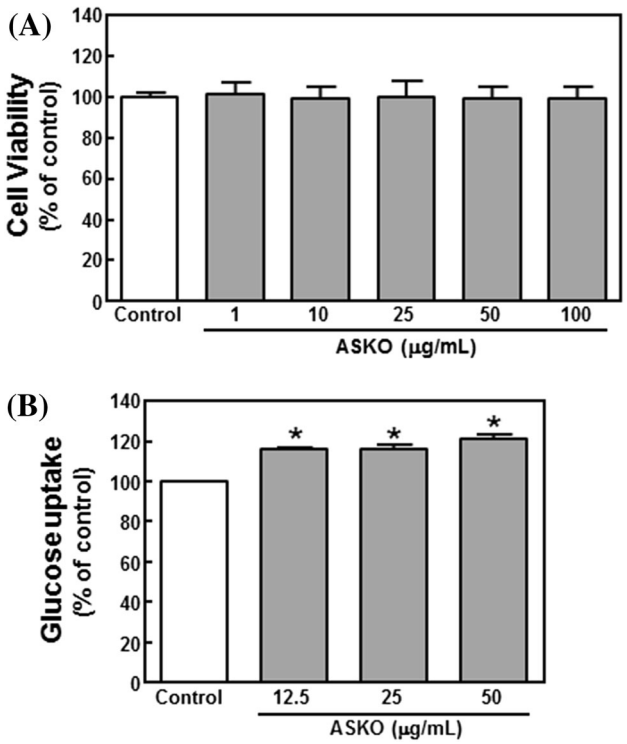

Fig. 1 Effects of an extract of *Aster koraiensis* (ASKO) on the viability of 3T3-L1 adipocytes and glucose uptake. (**A**) Cell viability. Confluent mature adipocytes were treated with ASKO for 24 h. (**B**) Glucose uptake. Confluent mature adipocytes were treated with ASKO for 1 h. Results are calculated as a percentage of the values in the control group. Data are expressed as the mean ± SEM, n = 4; *$p < 0.05$ versus control

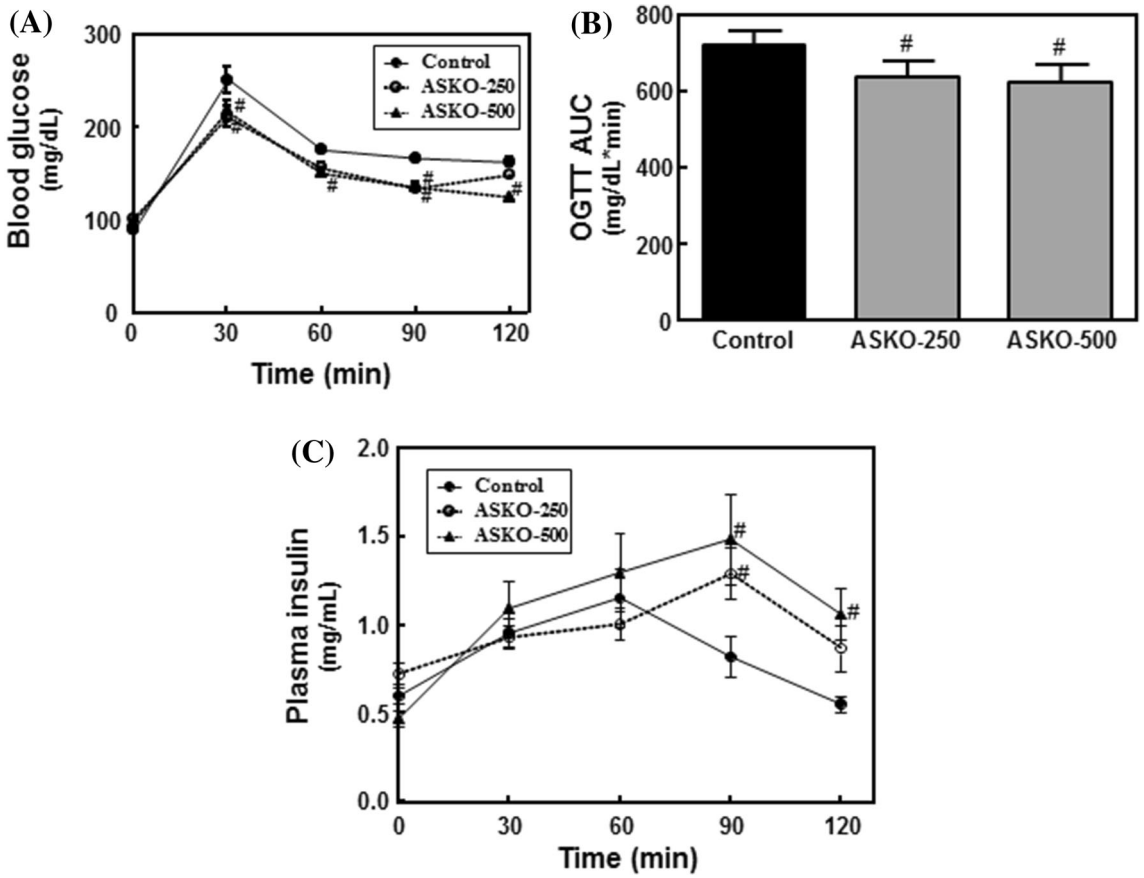

Fig. 2 Effects of a single administration of an extract of *Aster koraiensis* (ASKO) on glucose tolerance in normoglycemic mice. (**A**) Levels of glucose in the blood. ASKO was orally administered 30 min before glucose loading (2 g/kg). Oral glucose tolerance tests (OGTTs) were conducted, and changes in blood glucose were noted over time. (**B**) Change in area under the curve $(AUC)_{0–120\ min}$ for blood glucose during OGTTs. (**C**) Changes in plasma insulin over time during OGTTs. Data are expressed as the mean ± SEM, n = 6; #$p < 0.05$ versus control

insulin levels 90 and 120 min after glucose loading (Fig. 2C).

The effects of repeated ASKO administration on long-term glycemic control were investigated in DIO mice. Consistent with the OGTT results obtained after a single administration of ASKO, repeated oral administration of ASKO for 4 weeks also significantly decreased blood glucose levels (Fig. 3A). The AUC values for glucose in the ASKO-treated groups (ASKO-250, 1002 ± 238.7 mg min/dL; and ASKO-500, 1051 ± 102.7 mg min/dL) were significantly lower than that observed in the vehicle-treated DIO group (1313 ± 296.9 mg·min/dL) (Fig. 3B). The blood glucose-lowering activity of ASKO was lower than that of metformin. These findings reveal that long-term repeated administration of ASKO produced similar blood glucose-lowering effects during OGTTs in DIO mice. In addition, ASKO increased the postprandial insulin levels at 90 and 120 min after glucose loading (Fig. 3C). Interestingly, these findings indicated that ASKO may enhance plasma insulin levels after glucose loading, leading to a decrease in blood glucose levels.

Glycogen is the stored form of glucose in mammals. In humans, the majority of glycogen is stored in skeletal muscle (Jensen et al., 2011). Insulin promotes glucose removal from the blood in skeletal muscle by increasing GLUT-4 translocation and stimulating glucose uptake. Therefore, we evaluated the effects of ASKO on GLUT-4 mRNA levels in muscle tissue. As shown in Fig. 3(D), the DIO group showed lower GLUT-4 mRNA expression in muscle tissue (36 ± 9.9%), as compared to the control group. GLUT-4 expression in DIO mice was significantly increased by ASKO at a dosage of 500 mg/kg (111 ± 24.0%). Metformin also slightly increased GLUT-4 expression (58 ± 14.0%). This suggests that ASKO could lower postprandial hyperglycemia by normalizing the muscle mRNA expression of GLUT-4. In the present study, 250 mg/kg was an effective dose of ASKO in mice. For an average adult human body weight of 60 kg (Manimaran et al., 2010), the equivalent dose is 1.2 g/day. This

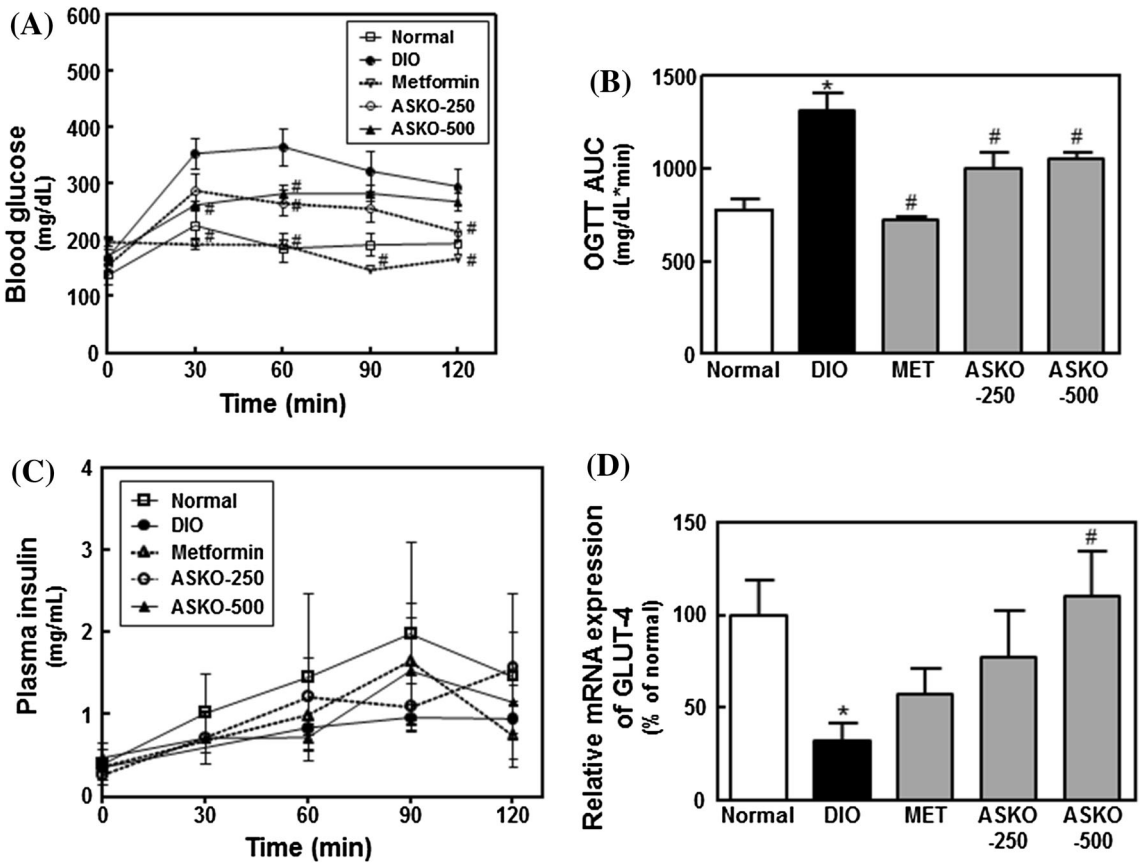

Fig. 3 Effects of repeated administration of an extract of *Aster koraiensis* (ASKO) on glucose tolerance and on the mRNA expression of *GLUT-4* in diet-induced obese (DIO) mice. DIO mice were maintained on a 60 kcal % fat diet from 7 weeks of age. After 10 weeks on the high-fat diet, DIO mice were orally administered ASKO once a day for 4 weeks. (**A**) Changes in blood glucose over time during oral glucose tolerance tests (OGTTs). (**B**) Change in area under the curve $(AUC)_{0-120\ min}$ for blood glucose during OGTTs. (**C**) Changes in plasma insulin over time during OGTTs. (**D**) Relative mRNA expression of *GLUT-4* in muscle. The results were expressed as a percentage of GAPDH expression. Data are expressed as the mean ± SEM, n ≥ 8; $*p < 0.05$ versus control; $\#p < 0.05$ versus DIO mice

daily amount of ASKO could theoretically be produced from about 5.7 g raw herb. Interestingly, this dosage is feasible for human application because traditional Korean practice usually employs an *A. koraiensis* dose of about 5–10 g raw herb per day.

Our previous study identified two major compounds (Chlorogenic acid (CA) and 3,5-di-O-caffeoylquinic acid (CQA)) in ASKO (Kim et al., 2016). These compounds have potent anti-diabetic effects (Escandon-Rivera et al., 2012; Meng et al., 2013). CA has a potent insulin-sensitizing activity that is similar to that of metformin (McCarty, 2005). Bassoli et al. reported that CA significantly decreased the peak blood glucose level during OGTT by inhibiting intestinal glucose absorption (Bassoli et al., 2008). CA also enhanced the expression of GLUT-4 and increased glucose uptake (Ong et al., 2012). CQA has potent α-glucosidase inhibitory activity (Hwang et al., 2016). Although the present study did not provide direct evidence that these two major compounds were responsible for the anti-diabetic effects observed, we hypothesize that the insulin-sensitizing and glucose uptake-enhancing activities of ASKO may be due to these bioactive molecules.

In conclusion, this is the first study to provide evidence that ASKO has postprandial glucose-lowering effects in vivo and promotes glucose uptake by adipocytes in vitro. Further studies may be required to identify the complete phytochemical profile of ASKO, to determine the feasibility of using ASKO for the treatment of patients with prediabetes or T2D, and to elucidate whether ASKO can enhance glucose uptake by myocytes and hepatocytes.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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