

Original Research Article

Daidzin decreases blood glucose and lipid in streptozotocin-induced diabetic mice

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Received: 4 January 2016

Revised accepted: 6 October 2016

Abstract

Purpose: To investigate the ameliorative effect of daidzin (DZ) on diabetes in streptozotocin (STZ)-induced diabetic Institute of Cancer Research (ICR) mice, with a view to determining its usefulness in the treatment of diabetes.

Methods: The effect of DZ (100, 200 and 400 mg/kg) on blood glucose was investigated in both normal and STZ-induced diabetic mice with glibenclamide (3 mg/kg) and metformin (400 mg/kg) as positive control, respectively. Serum or hepatic levels of lipid, proinflammatory factors, malondialdehyde (MDA) and superoxide dismutase (SOD) were measured. Glucosidase activity assay and glucose uptake by C2C12 myotubes were performed in vitro and the expression of glucose transporter 4 (GLUT4) in C2C12 cells was determined by western blot.

Results: DZ (200 and 400 mg/kg) did not decrease fasting blood glucose in normal mice but inhibited starch-induced postprandial glycemia. Oral administration of 400 mg/kg of DZ for 14 days significantly decreased mouse blood glucose ($p < 0.01$), as well as serum total cholesterol (TC, $p < 0.01$), triglycerides (TG, $p < 0.01$), low-density lipoprotein cholesterol (LDL-c, $p < 0.01$) levels in STZ-induced hyperglycemic mice and improved oral glucose tolerance. The serum and hepatic activity of SOD was enhanced ($p < 0.01$ and $p < 0.001$, respectively) while MDA level decreased ($p < 0.001$). Blood concentrations of interleukin-6 (IL-6, $p < 0.001$), tumor necrosis factor α (TNF- α , $p < 0.01$), monocyte chemoattractant protein 1 (MCP-1, $p < 0.01$) were also significantly reduced. In vitro glucosidase activity results showed that DZ inhibited α -glucosidase with IC50 values of 82, 98 and 389 $\mu\text{g/mL}$ for α -glucosidase from *S. cerevisiae*, *Rhizopus* sp. and rat intestines, respectively. It also stimulated glucose uptake and GLUT4 membrane translocation in C2C12 myotubes at 20 μM ($p < 0.05$).

Conclusion: Oral administration of DZ is effective in alleviating diabetic hyperglycemia, dyslipidemia and inflammation. Inhibition of α -glucosidase and stimulation of glucose consumption by muscles may account for its inhibitory effect on blood glucose.

Keywords: Daidzin, Diabetes, Inflammation, Superoxide dismutase (SOD), Malondialdehyde (MDA), Glucosidase, C2C12 myotubes, Glucose transporter

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Diabetes mellitus (DM) is becoming a most serious threat to human health with an incidence

of 8.7 % (about 387 million patients) throughout the world, imposing a heavy burden on national healthcare systems, particularly in developing countries [1,2]. In the DM state, insulin activity is

reduced, resulting in decreased insulin-stimulated glucose uptake. Simultaneously, abnormal metabolism of fuel nutrients [3], disordered activities of antioxidant enzymes [4] and increased inflammation [5] lead to a pronounced oxidative stress, which is characterized by increased malondialdehyde (MDA) level and decreased activities of antioxidant enzymes such as superoxide dismutase (SOD). In the current circumstances, a search for an antidiabetic herbal product with fewer side effects has gained considerable importance in the medical field [6]. Daidzin (DZ) is a major isoflavone glycoside obtained from *Puerariae radix*, a well-known Chinese herbal medicine for the treatment of diabetes and cardiovascular diseases [7-9]. Various pharmacological activities of DZ have been reported including antioxidant [10], estrogenic [11] and anti-inflammatory [12]. The effect of DZ on blood glucose remains controversial. Choi *et al* [13] reported the inhibitive effect of DZ on yeast α -glucosidase, suggesting that DZ may be an alternative to acarbose for the treatment of diabetes. However, Meezan *et al* [14] reported that intraperitoneal injection of DZ impaired glucose tolerance in ob/ob diabetic mice. Recently, Zang *et al* [15] demonstrated that the mixture of DZ and glycitin can efficiently decrease blood glucose and serum HbA1c in hyperglycemic mice. Although the mixture of DZ and glycitin were effective in the treatment of DM, the exact role of DZ was not distinguished in their report.

In the present work, we investigated the effects of oral administration of DZ on diabetes-related hyperglycemia, dyslipidemia, peroxidative state, inflammation in streptozotocin (STZ)-induced, to demonstrate the potential utility of DZ in the prevention and treatment of diabetes.

EXPERIMENTAL

Reagents

Daidzin (DZ), with a purity of 99.6 % as determined by HPLC, was purchased from Forever-biotech Co. Ltd (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM), glibenclamide, metformin, streptozotocin (STZ), 4-nitrophenyl- α -D-glucopyranoside (PNPG)-2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose(2-NBDG), α -glucosidase from *Saccharomyces cerevisiae*, rat intestinal acetone powder were procured from Sigma-Aldrich, Inc. (St Louis, USA). α -Glucosidase from *Rhizopus* sp. was sourced from Gold Wheat

Biotech, Inc. (Shanghai, China). Specific kits for glucose, total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), malondialdehyde (MDA), interleukin-6 (IL-6), tumor necrosis factor α (TNF- α), monocyte chemoattractant protein 1 (MCP-1), and activity of superoxide dismutase (SOD) were purchased from Jian Cheng Biotechnology Company (Nanjing, China). Antibodies against Glucose transporter type 4 (GLUT4) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were from Abcam, Inc. (Cambridge, USA).

Animal studies

Male Institute of Cancer Research (ICR) mice (20 - 22 g each) were obtained from the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences, Beijing, China. The study was carried out according to the "Principles of Laboratory Animal Care" [16] and approved by Animal Ethics Committee of Zhengzhou University (no. 2014090210). A standard pellet diet and water were given ad libitum. Animals were housed under a constant 12-h light and dark cycle and in an environment of 21 - 23 °C.

To test the effect of DZ in normal mice, the animals were randomly divided into five groups with eight mice in each group. Mice were daily treated with glibenclamide (3 mg/kg) or DZ (as indicated) orally for 14 days. Control group was given equal volume of distilled water. At the end of the experiment, animals were fasted for 4 hours and the fasting blood glucose levels were evaluated. For postprandial glycemia test, animals were fasted for 4 h then treated with the corresponding agents orally. After another 15 min, each mouse was given 4 g/kg of starch (p.o.) [17]. Blood glucose levels at 0, 1 and 2 h after starch administration were determined by a blood glucose meter (Roche, ACCU-CHEK Active). Area under the curve was calculated as previously reported [17].

Next, male ICR mice were rendered diabetic by intraperitoneal injection of STZ (80 mg/kg)[18] in citrate buffer, pH 4.5. Five days later, the animals were fasted for 8 h, blood samples taken from their tail vein and the plasma glucose concentration was measured. Animals with blood glucose concentration above 12 mM were selected for the study.

STZ-induced diabetic mice were divided into groups randomly with 8 mice in each group. Animals were daily treated with metformin (400 mg/kg) [19] or DZ (100, 200 and 400 mg/kg) orally for 14 days. Control group was given an

equal volume of distilled water. At the end of the experiments and after fasted for 4 h, animals were euthanized and the blood and liver samples was obtained for the estimation of various biochemical parameters by specific kits following the manufacturer's instructions.

For oral glucose tolerance test (OGTT), the animals were fasted for 4 h then treated with corresponding agents orally. After another 2 h, each mouse was given 4 g/kg of glucose (p.o.) [20]. Blood glucose levels were determined at 0, 0.5, 1, 1.5 and 2 h after glucose administration by a blood glucose meter.

Cell culture

C2C12 cells, originating from the American Type Culture Collection (ATCC) (Manassas, VA, USA), were obtained from the Peking Union Medical College. C2C12 myoblasts were cultured in DMEM supplemented with 10 % FBS at 37 °C and 5 % CO₂. To induce differentiation, media was replaced with DMEM containing 2 % horse serum (Sigma-Aldrich, Inc., St Louis, USA) whenever the cells reached confluence. Experiments were performed in differentiated C2C12 myotubes 5 days after differentiation was induced.

Glucose uptake assay

Glucose uptake assay was performed as previously reported [21]. Briefly, differentiated C2C12 myotubes were incubated with the serum-free DMEM containing the fluorescent glucose analog 2-NBDG (10 μM) and DZ (1, 5 or 10 μM) in the presence or absence of insulin (100 nM). After incubation for 12 h, medium was removed and cells were washed with phosphate-buffered saline (PBS) twice. Cells were scraped out in 1 mL of PBS and transferred into 5 mL polystyrene round-bottom tubes (BD Falcon) and kept at 4 °C. The amount of 2-NBDG taken up by the cells was measured by determining the fluorescence intensities at Ex/Em=475 nm/550 nm using a Tecan Infinite M1000Pro Microplate Reader (TECAN Group Ltd, Shanghai, China).

Western blot

GLUT4 membrane translocation in C2C12 myotubes was analyzed by western blot. Cell membrane preparation and western blot procedure were performed as previously reported [6]. GAPDH was used as internal reference. The immunoreactive bands were

quantified using ImageJ 4.1 software (NIH, Bethesda, USA).

α-Glucosidase inhibition assay

Inhibition of α-glucosidase and α-amylase activities were performed according to the chromogenic method described previously [22], using PNPG as substrate. Acarbose was used as positive control. The inhibitory activity was calculated as in Eq 1.

$$\text{Inhibition} = \{(Ac - At)/Ac\}100 \dots\dots\dots (1)$$

where Ac and At are the absorbance of control and test samples, respectively.

Statistical analysis

The results are expressed as mean ± standard error of the mean (SEM). The data were analyzed by one-way analysis of variance (ANOVA) using SPSS 13.0 software. Significant difference was set at $p < 0.05$.

RESULTS

Effect of daidzin in normal mice

The effect of daidzin (DZ) on blood glucose was first assessed in normal mice. As shown in Figure 1A, DZ did not decrease fasting blood glucose levels in normal mice. This indicates that DZ may not stimulate insulin secretion and hence has a low risk of inducing hypoglycemia. In contrast, treatment with DZ (200 and 400 mg/kg) significantly inhibited the increase of serum glucose levels after oral administration of starch ($p < 0.05$) (Figure 1B and 1C), suggesting that DZ is effective in preventing postprandial glycemia.

Effect of daidzin in diabetic mice

We further evaluated the antihyperglycemic effect of DZ in streptozotocin (STZ)-induced diabetic mice. Oral administration of DZ (200 and 400 mg/kg) decreased fasting blood glucose ($p < 0.05$ and $p < 0.01$, respectively) (Figure 2A) and improved glucose tolerance in oral glucose tolerance test (Figure 2B and C). The potency of DZ at 400 mg/kg was significant but weaker than metformin (400 mg/kg), a popular antidiabetic medicine. These results suggested that DZ can decrease blood glucose levels and improve glucose tolerance in diabetic animals.

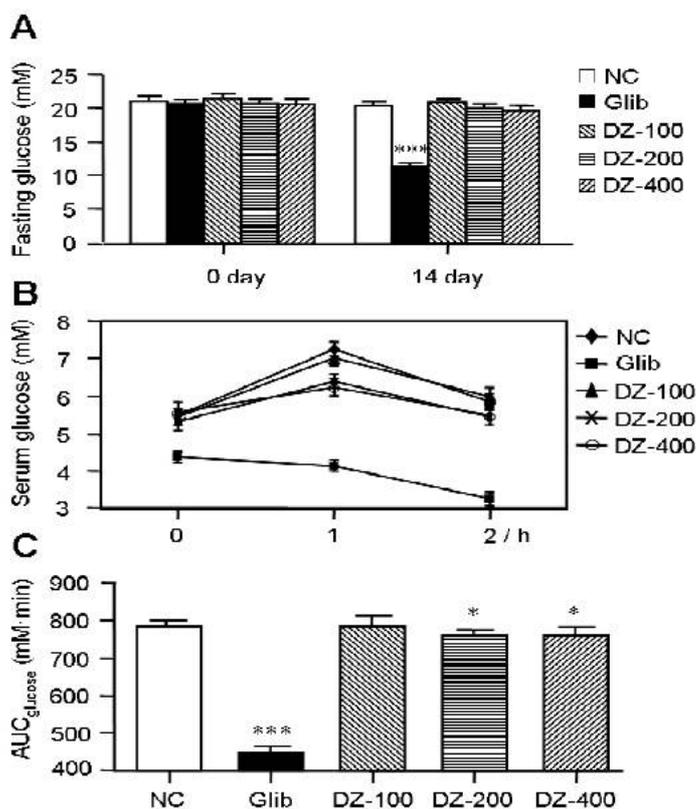


Figure 1: Effect of daidzin (DZ) on blood glucose levels in normal mice. (A) Fasting blood glucose. (B) Postprandial glycemia. (C) Area under the curve of postprandial blood glucose concentration. Values represent mean \pm SEM (n = 8); * p < 0.05, *** p < 0.001 versus NC group. NC, normal control; Glib, glibenclamide; DZ, daidzin

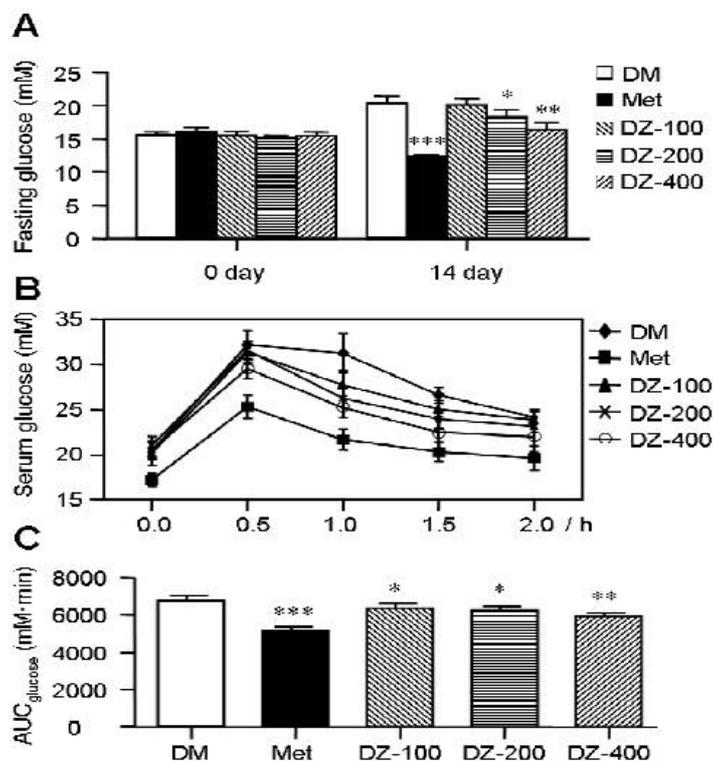


Figure 2: Effect of daidzin (DZ) on blood glucose levels in diabetic mice. (A) Fasting blood glucose. (B) Oral glucose tolerance test (OGTT). (C) Area under the curve of OGTT. Values represent mean \pm SEM (n = 8); * p < 0.05, ** p < 0.01, *** p < 0.001 versus DM group. NM, diabetic control; Met, metformin; DZ, daidzin

Daidzin effect on blood lipid of diabetic mice

As shown in Figure 3, treatment with DZ dose-dependently reduced the levels of serum TC, TG and LDL-c. At the optimal dose (400 mg/kg), DZ decreased serum concentration of TC, TG and LDL-c by 23.81, 29.84 and 21.43 %, respectively, which is lesser than the effect of metformin (400 mg/kg). The level of serum HDL-c was elevated by metformin but not affected by DZ (Figure 3).

Daidzin improves peroxidative states in diabetic mice

Treatment with DZ (100, 200 and 400 mg/kg) dose-dependently increased SOD activity and decreased the concentration of MDA in serum and liver (Figure 4). The antioxidative effect of

DZ at 400 mg/kg was more pronounced than that of metformin (400 mg/kg).

Daidzin alleviates inflammation in diabetic mice

Treatment with DZ significantly decreased serum levels of interleukin-6 (IL-6), tumor necrosis factor α (TNF- α) and monocyte chemotactic protein 1 (MCP-1) (Figure 5), displaying potent anti-inflammatory effects. This observation is in accordance with previous reports [12,23]. The efficacy of DZ (200 and 400 mg/kg) in decreasing serum IL-6, TNF- α and MCP-1 was comparable or slightly higher than that of metformin (400 mg/kg).

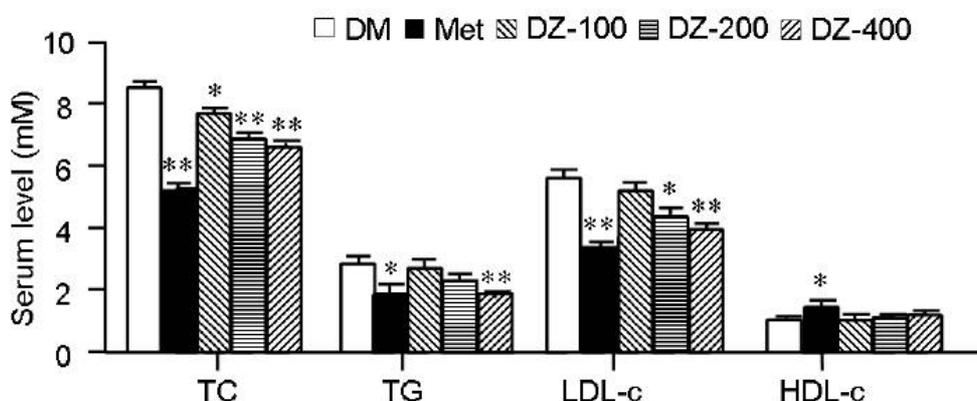


Figure 3: Effect of daidzin (DZ) on serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol in diabetic mice. Values represent mean \pm SEM (n = 8). * p < 0.05, ** p < 0.01 versus DM group. NM, diabetic control; Met, metformin; DZ, daidzin

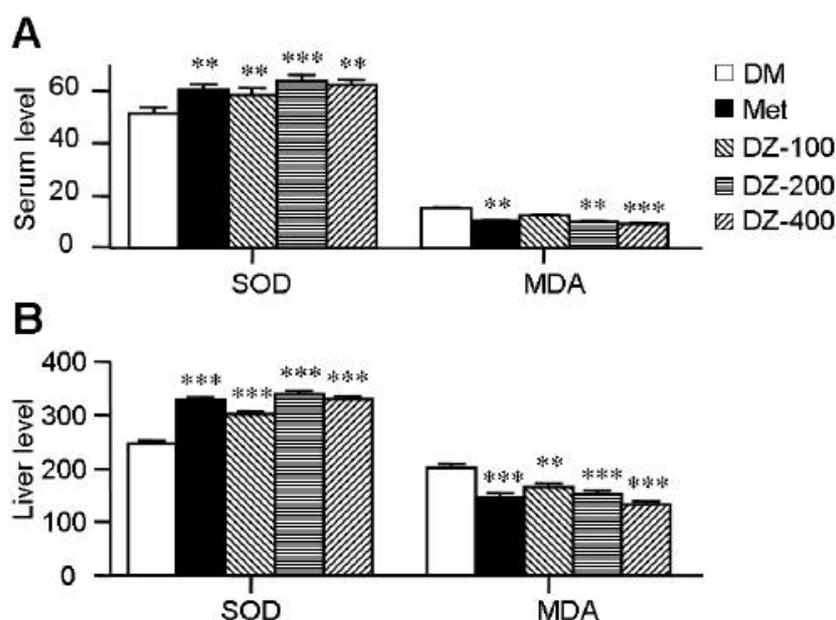


Figure 4: Effect of daidzin (DZ) on SOD activity and MDA concentration in diabetic mice. (A) Serum level (in U/mL for SOD, nmol/mL for MDA). (B) Liver level (in U/mg protein for SOD, nmol/mg protein for MDA). Values represent mean \pm SEM (n = 8). ** p < 0.01, *** p < 0.001 versus DM group. NM, diabetic control; Met, metformin; DZ, daidzin

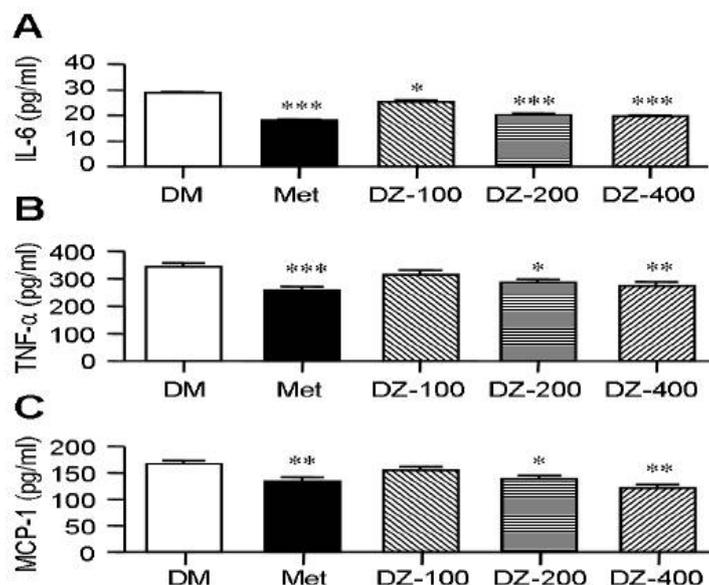


Figure 5: Effect of daidzin (DZ) on serum levels of IL-6 (A), TNF-α (B) and MCP-1 (C). Values represent mean ± SEM (n = 8). **p < 0.01, ***p < 0.001 versus DM group. NM, diabetic control; Met, metformin; DZ, daidzin

Daidzin stimulates glucose uptake and increases GLUT4 translocation in C2C12 myotubes

We also found that DZ activated glucose consumption in a dose-dependent manner in C2C12 myotubes. The effect of DZ on glucose uptake was assessed by NBD-glucose (2-NBDG) uptake assay. Treatment with DZ for 12 h

increased 2-NBDG uptake by C2C12 myotubes in a dose-dependent manner (Figure 6A), suggesting a potent activity of DZ in stimulating glucose uptake by myotubes. In accordance with this finding, DZ largely increased GLUT4 membrane translocation without significantly enhancing its total expression level (Figure 6B and 6C).

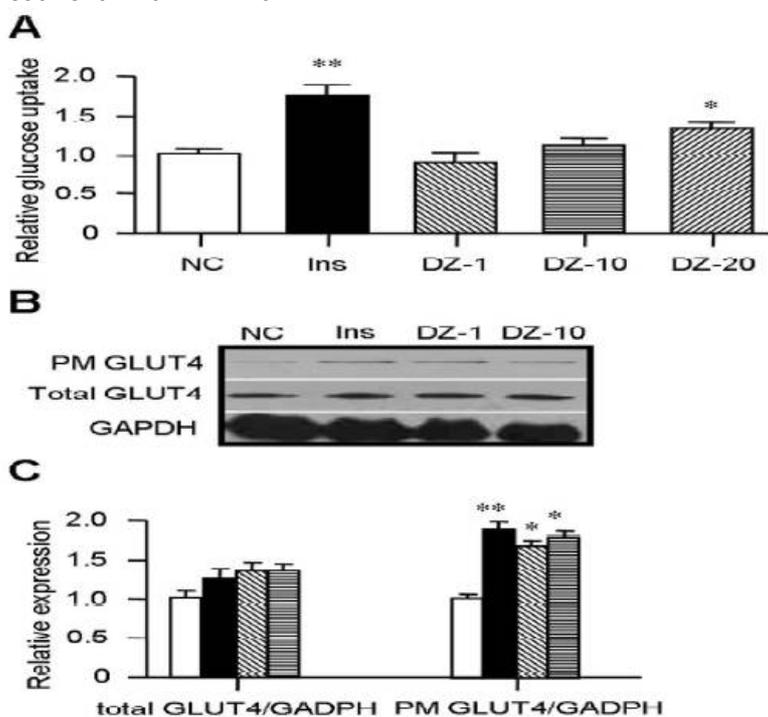


Figure 6: Effect of daidzin (DZ) on glucose uptake (A) and glucose transporter 4 (GLUT4) translocation in C2C12 myotubes. (B) Western blot for total GLUT4, plasma membrane (PM) GLUT4 and GAPDH. (C) Quantitative results of B analysis by ImageJ 4.1 software. Values represent mean ± SEM. NBD-glucose (2-NBDG) was used as indicator. Insulin (100 nM) was used as positive control. The concentration of DZ was 1, 10 and 20 μM. Results are representative of 3 different experiments with n = 3 in each group. *p < 0.05, **p < 0.01. NC: negative control; Ins: insulin; DZ: daidzin; PM, plasma membrane

Daidzin inhibits the activity of α -glucosidase *in vitro*

We further investigated the inhibitive effect of DZ on the activity of α -glucosidase *in vitro*. As shown in Figure 7, DZ inhibited the activity of α -glucosidase in a dose-dependent manner. The IC_{50} values of DZ on α -glucosidase from *S. cerevisiae*, *Rhizopus sp.* and rat intestines were 82 μ g/mL, 98 μ g/mL and 389 μ g/mL, respectively. The *in vitro* effect of DZ on glucosidase was in good accordance to its inhibitive action on postprandial glycaemia in ICR mice (Figure 1B and C). These results suggest that DZ is an effective inhibitor of α -glucosidase *in vitro* and *in vivo*.

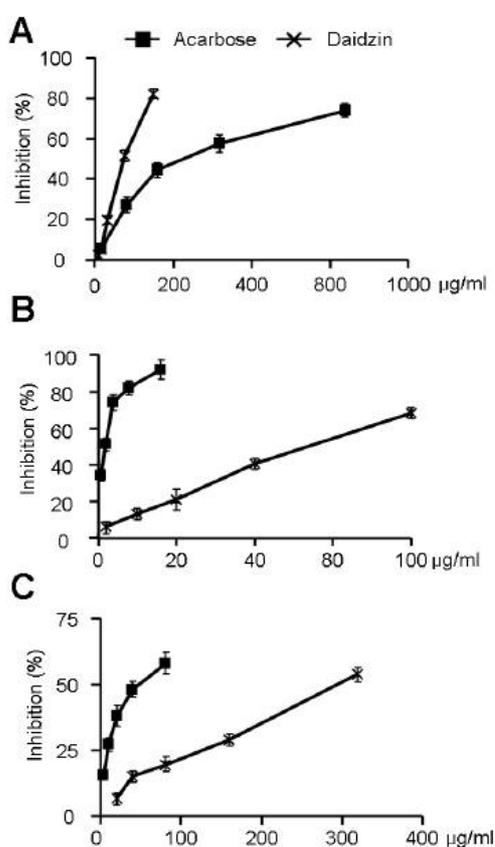


Figure 7: Inhibitory activity of daidzin (DZ) on α -glucosidase from *S. cerevisiae* (A), *Rhizopus sp.* (B) and rat intestines (C). 4-Nitrophenyl- α -glucopyranoside (PNPG) was used as substrate. Acarbose was used as positive control. Data were plotted and the IC_{50} values were calculated by GraphPad Prism 4 software. Each value is expressed as mean \pm SEM of triplicate experiments

DISCUSSION

Daidzin (DZ) is a major isoflavone glycoside from *Puerariae radix*, a herb widely used in Chinese medicinal formulae for the treatment of diabetes

[7-9]. Many pharmacological studies have been made on DZ but its effect on blood glucose remains controversial. In the present work, we demonstrated that oral administration of DZ is adequate in alleviating diabetes mellitus in STZ-induced hyperglycemic mice.

Treatment with DZ did not decrease fasting blood glucose in normal mice, suggesting that it has minimal effect on insulin secretion and thus possesses less risk for hypoglycemia. However, oral administration of DZ significantly decreased blood glucose and improved oral glucose tolerance in diabetic mice, showing an adequate effect on hyperglycemia. It also significantly decreased serum levels of TC, TG and LDL-c and slightly increased HDL-c levels. Simultaneously, diabetes-related inflammation and peroxidative state were largely alleviated by DZ. These results demonstrated that oral administration of DZ is beneficial for the treatment of hyperglycemia and diabetes-related syndromes.

Meezan *et al* [14] found that intraperitoneal injection (i.p.) of DZ was detrimental for glucose homeostasis. This contradiction may be due to the different route by which DZ is administered. As is well-known that isoflavones including DZ will undergo intensive metabolism in the gut of animals by gut microbiota after oral administration. Therefore, the actual bioactive compounds after DZ oral administration may likely be its metabolites other than DZ itself which will require further investigation. A recent study by Zang *et al* [15] demonstrated the positive role of the mixture of DZ and glycytin in decreasing blood glucose and serum HbA1c, which partly supported our findings.

There are many approaches to efficiently decrease blood glucose level such as promoting insulin secretion [24], inhibiting glucose absorption [25], stimulating glucose uptake by tissues [26] and increasing insulin sensitivity [27]. Insulinotropic agents such as glibenclamide, stimulate insulin secretion and dramatically decrease blood glucose level in both normal and diabetic animals. Treatment with DZ showed no effects on blood glucose in normal mice. It is thus unlikely to promote insulin secretion. However, it significantly inhibited postprandial glycemia induced by starch, implying that DZ may be effective in inhibiting α -glucosidases and slowing glucose ingestion.

α -Glucosidase data showed that DZ inhibited the activities of α -glucosidase of various origins in a dose-dependent manner, which was in good accordance with its *in vivo* action on postprandial

glycaemia, suggesting that DZ may inhibit hyperglycemia through at least in part inhibition of α -glucosidase. Promoting glucose uptake by muscles is another effective way to decrease blood glucose [8], in which GLUT4 plays a key role. Glucose uptake assay in C2C12 myotubes showed that DZ significantly promoted glucose consumption by myotubes and enhanced GLUT4 membrane translocation as well, indicating that stimulating glucose uptake by muscles and enhancing GLUT4 membrane translocation may involve in the blood glucose-lowering action of DZ.

CONCLUSION

The findings of this work demonstrate that daidzin (DZ) can effectively decrease blood glucose and lipid, improve peroxidation and alleviate inflammation in diabetic animals. Stimulation of glucose consumption and inhibition of glucosidase may be involved in the blood glucose modulating effect of DZ. These results suggest that DZ is an effective regulator of glucose consumption in mice and may find application in the treatment of type 2 diabetes in human.

DECLARATIONS

Acknowledgement

This work was financially supported by International Science and Technology Cooperation Project of Henan Province (no. 152101120048).

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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