

Hypoglycemic effects of glabridin, a polyphenolic flavonoid from licorice, in an animal model of diabetes mellitus

FEIHUA WU, ZHIGUI JIN and JIAN JIN

Department of Pharmacy, Ninth People's Hospital, School of Medicine, Shanghai Jiao Tong University, Huangpu, Shanghai 200011, P.R. China

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Abstract. The present study was designed to investigate the hypoglycemic effects of glabridin from licorice in an animal model of diabetes mellitus (DM). Male Kunming mice were used to induce DM using streptozotocin (STZ). After confirmation of the diabetic state, the mice were randomly divided into six groups of 10 animals each: normal control (NC), diabetic control (DC), diabetic + low-dose glabridin treatment (DLG) (glabridin, 10 mg/kg), diabetic + medium-dose glabridin treatment (DMG) (glabridin, 20 mg/kg), diabetic + high-dose glabridin treatment (DHG) (glabridin, 40 mg/kg) and diabetic + glyburide treatment group (DG) (glyburide, 4 mg/kg). Each treatment was continued daily for 28 days, and then body weight, fasting blood glucose (FBG), glucose tolerance, superoxide dismutase (SOD) and malondialdehyde (MDA) were measured. The data obtained showed that glabridin significantly increased body weight, glucose tolerance and SOD activities in the liver, kidney and pancreas, while decreasing FBG levels and MDA content in the liver, kidney and pancreas. These results demonstrated that glabridin possesses hypoglycemic effects.

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders with different underlying etiologies. It is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism. As a consequence of the metabolic derangements in diabetes, various complications develop including macro- and microvascular dysfunctions (1,2). The management of diabetes is

considered a global problem. Modern drugs, including insulin and other hyperglycemic agents such as biguanides and sulphonylureas control the blood glucose level only when they are regularly administered, although these treatments are laborious and have several disadvantages including hypoglycemia and obesity (3). Therefore the identification of efficacious agents with less severe side-effects is crucial. Over the past few decades, traditional Chinese medicine has played a key role in the therapy of DM and its complications (4,5). Based on a large number of chemical and pharmacological research studies, numerous bioactive compounds have been found in Chinese medicinal plants for the treatment of diabetes. These compounds include polysaccharides, terpenoids, flavonoids, sterols and alkanoids (6-10).

Licorice is the root and stolon of the *Glycyrrhiza* plant, which belongs to the family Leguminosae. This plant has been medicinally used for >4,000 years (11). It is a Chinese herb commonly used as an expectorant and to arrest coughing, reduce fever, comfort the stomach, alleviate urgency and potentiate the effects of various other herbs (12). Licorice has been reported to attenuate free radical-induced oxidative damage in the kidney, prevent carcinogenesis induced by toxicants or hormones and also has a significant hepatoprotective activity (13-15). Licorice contains flavonoids and triterpenoids (15). Glabridin (Fig. 1), a polyphenolic flavonoid, is a main active component in licorice, which has been reported to exhibit multiple pharmacological activities, such as cytotoxic, antimicrobial, anti-fatigue, estrogenic and anti-proliferative activity against human breast cancer cells (16). It also affects melanogenesis, inflammation, low-density lipoprotein oxidation and protection of mitochondrial functions from oxidative stress (17). However, there is a limited number of studies on the effect of glabridin on DM at present. Thus, the aim of this study was to investigate the hypoglycemic effects of glabridin from licorice in an animal model of DM.

Materials and methods

Reagents. Glabridin (purity >99% by HPLC analysis) was purchased from Shaanxi Langrun Biotechnology Co., Ltd. (Xi'an, China). Streptozotocin (STZ) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Glyburide (glibenclamide) was purchased from Shanxi Sanjin Pharmaceutical Co., Ltd. (Taiyuan, China). Glucose kit was purchased from

Correspondence to: Dr Zhigui Jin, Department of Pharmacy, Ninth People's Hospital, School of Medicine, Shanghai Jiao Tong University, 639 Zhizaoju Road, Huangpu, Shanghai 200011, P.R. China
E-mail: wufeihua1961@126.com; wufeihua1961@yahoo.com

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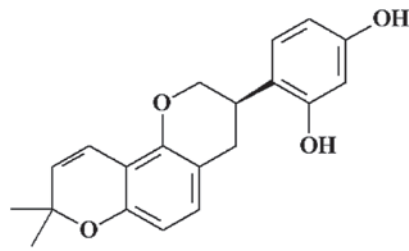


Figure 1. Chemical structure of glabridin.

Biosino Biotechnology and Science, Inc. (Beijing, China). Superoxide dismutase (SOD) and malondialdehyde (MDA) kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China). Other chemicals were obtained from local sources and were of analytical grade.

Animals. Male Kunming mice (2 months old, 18–22-g body weight) were obtained from the Shanghai Center of Experimental Animals (Shanghai, China). The animals were housed under standard environmental conditions (22–25°C, humidity 60–70%, 12-h light/dark cycle) with free access to standard diet and water *ad libitum*. The mice used in this study were processed in accordance with the UK Animals (Scientific Procedures) Act 1986 and associated guidelines. The experimental protocol was approved by the Shanghai Jiao Tong University Animal Care and Use Committee (Shanghai, China).

Induction of diabetes mellitus. The mice were fasted for 16 h prior to the induction of DM. STZ was freshly prepared in 0.1-mol/l citrate buffer solution (pH 4.5) and was intraperitoneally injected into mice with a single dose of 60 mg/kg. DM was confirmed by the measurement of blood glucose from the tail vein 72 h after injection of STZ. Mice with a blood glucose level >11.0 mmol/l, as well as polydipsia, polyuria and polyphagia were selected for the experiment (18).

Experimental design. Following confirmation of the diabetic state, the mice were randomized into six groups of 10 animals each: i) normal control group (NC), non diabetic mice administered 0.5 ml of 0.5% Tween-80 solution; ii) diabetic control group (DC), diabetic mice administered 0.5 ml of 0.5% Tween-80 solution; iii) diabetic + low-dose glabridin treatment group (DLG), diabetic mice administered glabridin (10 mg/kg) in 0.5 ml 0.5% Tween-80 solution; iv) diabetic + medium-dose glabridin treatment group (DMG), diabetic mice administered glabridin (20 mg/kg) in 0.5 ml 0.5% Tween-80 solution; v) diabetic + high-dose glabridin treatment group (DHG), diabetic mice administered glabridin (40 mg/kg) in 0.5 ml 0.5% Tween-80 solution and vi) diabetic + glyburide treatment group (DG), diabetic mice administered 0.5 ml of 0.5% Tween-80 solution containing glyburide (4 mg/kg).

Each treatment was continued daily for 28 days. Fasting blood glucose (FBG) levels were measured once every week. Blood was collected from the tip of the tail vein (starting from 9:00 a.m.) after a 12- to 14-h overnight fast. At the same time, body weights of mice were measured. An oral glucose tolerance test (OGTT) was performed on the last day of treatment

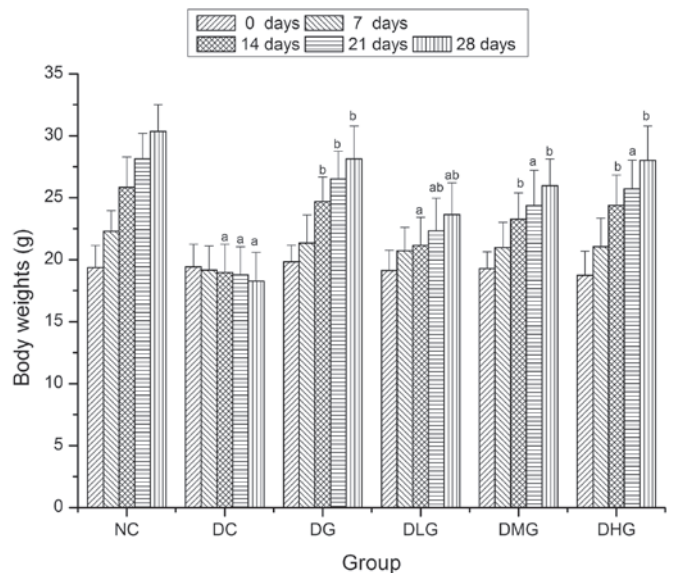


Figure 2. Effect of glabridin on the body weight of mice. Data are presented as the means \pm SD. ^a $P < 0.05$ when compared with the NC group; ^b $P < 0.05$ when compared with the DC group. NC, normal control; DC, diabetic control; DG, diabetic + glyburide treatment; DLG, diabetic + low-dose glabridin treatment; DMG, diabetic + medium-dose glabridin treatment; DHG, diabetic + high-dose glabridin treatment.

after overnight fasting. Blood was collected at 0, 30, 60 and 120 min after an oral glucose load of 3.0 g/kg of body weight. Following completion of the experiment, the mice were sacrificed by cervical decapitation. The liver, kidney and pancreas were dissected out, washed in ice-cold saline, and homogenized in Tris-HCl buffer. Supernatant fractions of liver homogenate were used to measure SOD activity and MDA content.

Statistical analysis. Results were presented as the means \pm standard deviation (SD). The data were evaluated by means of an analysis of variance (ANOVA:MANOVA), using the Newman-Keuls test. Differences with a value of $P < 0.05$ were considered to indicate a statistically significant difference.

Results

Effect of glabridin on the body weight of mice. Prior to the experiment, the body weights were not significantly different among all the groups ($P > 0.05$) (Fig. 2). After 14 days, the body weights of the mice in the DG, DMG and DHG groups were significantly increased when compared with the DC group ($P < 0.05$), while the body weights of mice in the DHG group were increased, although not significantly ($P > 0.05$). After 28 days, the body weights of the mice in the DG, DLG, DMG and DHG groups were significantly increased when compared with the DC group ($P < 0.05$), while the body weights of the mice in the DLG group remained significantly decreased when compared with the NC group ($P < 0.05$).

Effect of glabridin on FBG levels of mice. As shown in Fig. 3, FBG levels in the NC group remained constant and were significantly decreased as compared with the diabetic groups (DC, DG, DLG, DMG and DHG) during the experimental period ($P < 0.05$). After 7 days, FBG levels in the diabetic treat-

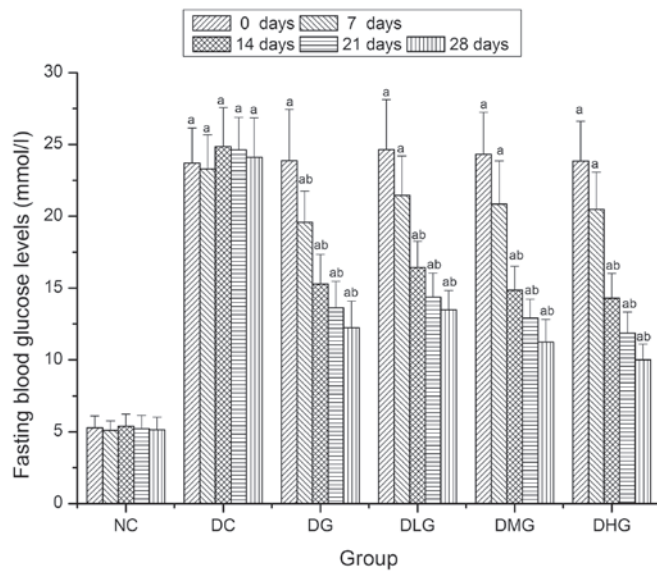


Figure 3. Effect of glabridin on fasting blood glucose (FBS) levels of mice. Data are presented as the means \pm SD. ^a $P < 0.05$ when compared with the NC group; ^b $P < 0.05$ when compared with the DC group. NC, normal control; DC, diabetic control; DG, diabetic + glyburide treatment; DLG, diabetic + low-dose glabridin treatment; DMG, diabetic + medium-dose glabridin treatment; DHG, diabetic + high-dose glabridin treatment.

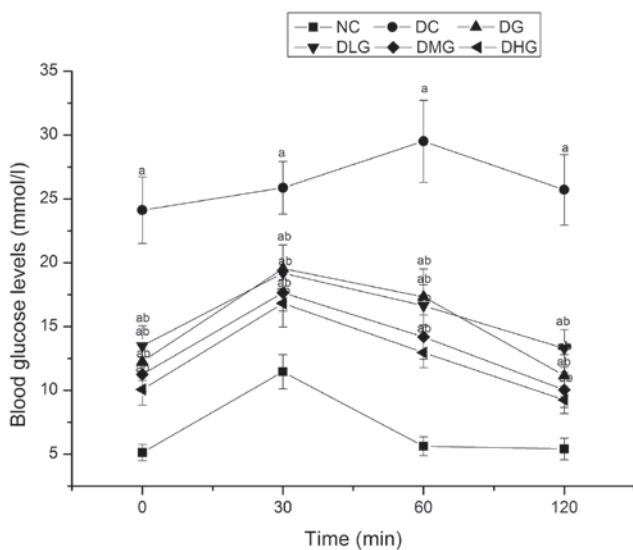


Figure 4. Effect of glabridin on glucose tolerance of mice. Data are presented as the means \pm SD. ^a $P < 0.05$ when compared with the NC group; ^b $P < 0.05$ when compared with the DC group. NC, normal control; DC, diabetic control; DG, diabetic + glyburide treatment; DLG, diabetic + low-dose glabridin treatment; DMG, diabetic + medium-dose glabridin treatment; DHG, diabetic + high-dose glabridin treatment.

ment groups (DG, DLG, DMG and DHG) showed a decreasing trend. After 28 days, FBG levels in the DG, DLG, DMG and DHG groups were significantly decreased as compared with the DC group ($P < 0.05$), being 196.9, 178.8, 214.6 and 240.9% lower, respectively.

Effect of glabridin on glucose tolerance of mice. Blood glucose levels in the DG, DLG, DMG and DHG groups were significantly decreased when compared with the DC groups at

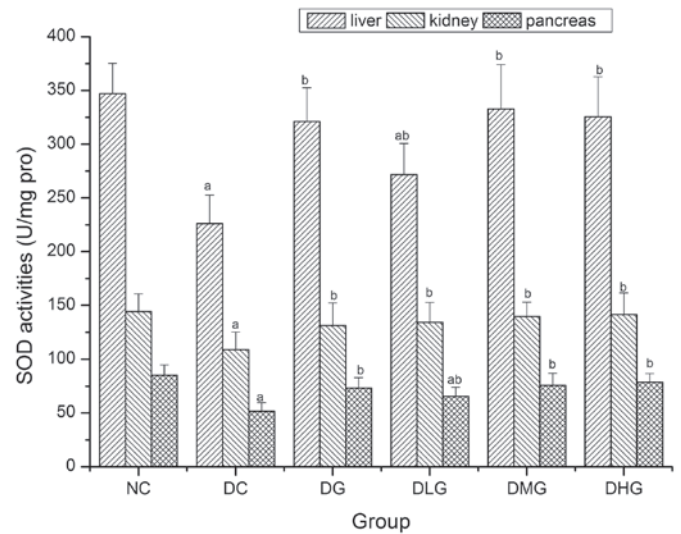


Figure 5. Effect of glabridin on superoxide dismutase (SOD) activities in the liver, kidney and pancreas of mice. Data are presented as the means \pm SD. ^a $P < 0.05$ when compared with the NC group; ^b $P < 0.05$ when compared with the DC group. NC, normal control; DC, diabetic control; DG, diabetic + glyburide treatment; DLG, diabetic + low-dose glabridin treatment; DMG, diabetic + medium-dose glabridin treatment; DHG, diabetic + high-dose glabridin treatment.

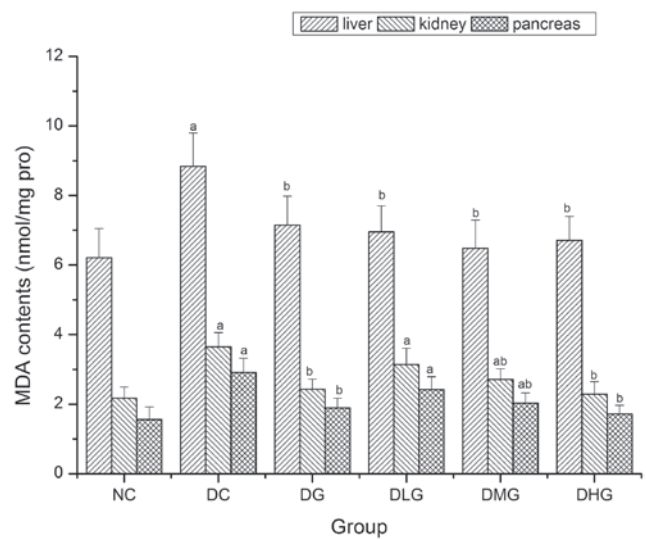


Figure 6. Effect of glabridin on malondialdehyde (MDA) contents in the liver, kidney and pancreas of mice. Data are presented as the means \pm SD. ^a $P < 0.05$ when compared with the NC group; ^b $P < 0.05$ when compared with the DC group. NC, normal control; DC, diabetic control; DG, diabetic + glyburide treatment; DLG, diabetic + low-dose glabridin treatment; DMG, diabetic + medium-dose glabridin treatment; DHG, diabetic + high-dose glabridin treatment.

different time intervals (0, 30, 60 and 120 min) ($P < 0.05$), while they remained significantly increased when compared with the NC group ($P < 0.05$) (Fig. 4).

Effect of glabridin on SOD activities in the liver, kidney and pancreas of mice. As shown in Fig. 5, when compared with the DC group, SOD activities in the liver, kidney and pancreas were significantly increased in the DG, DLG, DMG and DHG groups ($P < 0.05$), while SOD activities in the liver and

pancreas in the DLG group were still significantly decreased when compared with the NC group ($P < 0.05$).

Effect of glabridin on MDA contents in the liver, kidney and pancreas of mice. MDA contents in the liver, kidney and pancreas were significantly decreased in the DG, DMG and DHG groups when compared with the DC group ($P < 0.05$) (Fig. 6). In the DLG group, MDA content in the liver was significantly decreased ($P < 0.05$) when compared with the DC group, and MDA contents in the kidney and pancreas were decreased, although not significantly ($P < 0.05$).

Discussion

STZ (N-nitroso derivative of glucosamine) is a broad-spectrum antibiotic extracted from *Streptomyces achromogenes*. It is a pancreatic β -cell toxin that induces rapid and irreversible necrosis of β -cells and is widely used to induce DM in experimental animal models (19,20). STZ-induced DM is characterized by severe loss in body weight, which may be due to degradation of structural proteins since they are known to contribute to body weight (21). In this study, a significant body weight loss was observed in the DC group and significant improvement of body weight was observed in the glabridin treatment groups (DLP, DMP and DHP). This finding may be due to the ability of glabridin to reduce hyperglycemia.

DM is a serious chronic disease. Effective blood glucose control is the key to preventing or reversing diabetic complications and improving quality of life in patients with diabetes (22,23). In the present study, STZ-induced diabetic mice presented obvious hyperglycemic symptoms, while glabridin produced a significant decrease in FBG levels in diabetic mice. In addition, glucose tolerance also improved significantly after glabridin treatment. These results indicated that glabridin possesses hypoglycemic effects and that the 40-mg/kg dose of the glabridin exerted a better effect when compared to doses of 10 or 20 mg.

Previous studies have shown that reactive oxygen species (ROS) and lipid peroxidation are important in the pathogenesis of DM and its complications (24-26). An imbalance between ROS generation and the reduced activity of antioxidant defenses or both of these phenomena lead to oxidative stress. Hyperglycemia is a cause of oxidative stress in diabetic patients and reduces the capacity of the endogenous antioxidant defense system via the production of several reducing sugars (through glycolysis and the polyol pathway) (26). In the present study, glabridin significantly increased SOD activities, while decreasing MDA contents in the liver, kidney and pancreas. Therefore, it may be concluded that the antioxidative activities of glabridin in STZ-induced diabetic mice, at least in part, may be related to hypoglycemic effects.

In summary, the present study has demonstrated that glabridin possesses hypoglycemic effects. The 40-mg/kg dose of glabridin exerted a better effect when compared to doses of 10 or 20 mg. Further pharmacological and biochemical investigations would clearly elucidate the mechanism of action and would be beneficial in investigating the role of glabridin as a therapeutic target in diabetes treatment research.

Acknowledgements

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