Ameliorative effect of glabridin, a main component of *Glycyrrhiza glabra* L. roots in streptozotocin induced Type 1 diabetes in male albino rats

Eman A Abd El-Ghffar

Department of Zoology, Faculty of Science, Ain Shams University, Cairo, Egypt University, Khalifa El-Maamonst, Abbasiya sq., 11566, Cairo, Egypt

E-mail: eman_a@sci.asu.edu.eg

Received 01 January 2016, revised 19 January 2016

Glabridin (component of Licorice), a major flavonoid of *Glycyrrhiza glabra* L. (Fabaceae), is commonly used in the treatment of cardiovascular and central nervous system diseases. Also, it has been associated with a wide range of biological properties such as antioxidant, anti-inflammatory, estrogenic and anti-osteoporotic. Here, we investigated the antidiabetic effects of oral treatment with glabridin on streptozotocin (STZ)-induced diabetic rats. Type 1 diabetes was induced in male albino rats by a single intraperitoneal injection of STZ (60 mg/kg body weight). Diabetes appeared after 1-3 days after STZ injection. The animals were randomly divided into 6 groups (7 animals each) as follows; Group 1: Normal control group (negative control group); Group 2 & 3: glabridin-treated control groups (25&50 mg/kg body weight); Group 4:STZ diabetic group (positive control group); Group 5 & 6:glabridin treated diabetic groups(25&50 mg/kg body weight). In normal rats, no harmful effects were detected after orally administration of both doses of glabridin on all parameters measured. The anti-diabetic activity of glabridin (especially high dose) was mediated through significantly increased the body weight gain, enzymic/non-enzymic antioxidants and HDL-cholesterol, and significantly decreased relative organ weights, serum glucose, lipid profiles, lipid peroxidation (LPO), pro-inflammatory cytokine, liver and kidney functions. The present study indicated that the anti-inflammatory/anti-oxidant activity of glabridin (especially high dose) may have beneficial effects against complications shown in STZ diabetic rats.

**Keywords:** Licorice, Blood glucose, Liver enzymes, Lipid profile, Kidney function, Cytokine, Diabetic rats

**IPC Int. Cl.** A61K 36/00, A61B 5/15, C12N 9/00, C12N 7/42, A01D 13/37, A01D 13/00, A01D 16/02

Diabetes Mellitus (DM) is a major public health problem worldwide. It is characterized by hyperglycemia with alterations in metabolism of lipids, carbohydrates and proteins metabolism, as well as an increased risk of complications from vascular diseases. Uncontrolled hyperglycemia is responsible for causing dysfunction and failure of various organs which include neuropathy, nephropathy, cardiovascular and cerebrovascular diseases. There are two forms of DM: type 1 (insulin-dependent) and type 2 (non-insulin-dependent). Type 1 DM results from auto-immune and/or inflammatory processes that selectively disrupts insulin-producing pancreatic β-cell. Streptozotocin or Streptozocin or Iozostazin or Zanosar (STZ) is a synthetic antineoplastic agent that is an anti-tumor antibiotic (produced by *Streptomyces achromagens*) and chemically is related to other nitroso compounds used in cancer chemotherapy. STZ enters pancreatic beta cells through glucose transporter 2 channels in the plasma membrane and causes cellular toxicity and local immune responses that lead to hypo-insulinemia and hyperglycemia in animals. In some models, especially rats, a single dose of STZ is effective at inducing type 1 diabetes. In mice, however, multiple low doses (40 mg/kg) are the most effective at inducing type 1 diabetes. Using 60 mg/kg of STZ dose results in the toxicity of the Langerhans islets beta cells with emergence of clinical diabetes within 2-4 days. Insulin-dependent DM or type 1 diabetes induced with streptozotocin in rats display many complications seen in human subjects with uncontrolled DM due to its selective pancreatic beta-cell cytotoxicity caused by DNA alkylation and nitric oxide generation. There are a number of other reports suggested that diabetes is associated with the generation of reactive oxygen species (ROS) causing oxidative damage impaired pancreatic beta-cell insulin secretion by pancreatic beta cell lysis through DNA fragmentation. There are a number of different types of antidiabetic drug including insulin and other hyperglycemic agents such as biguanides and sulphonylure as control the blood
glucose level only when they are regularly administered. Because of adverse effects of antidiabetic drugs including hypoglycemia, obesity, retinopathy, neuropathy, vasculopathy and nephropathy, there is a great need to search for natural agents from medicinal plants with no side effects which would improve diabetic patient's health problems. Traditional Chinese medicine will no doubt play an important role in the therapy of DM and its complications. These compounds include polysaccharides, terpenoids, flavonoids, sterols and alkanoids. Glabridin is an isoflavane, a type an isoflavonoid, (major active constituent of Licorice root derived from the plant Glycyrrhiza glabra L.), which is believed to be partly responsible for anti-inflammatory, anti-hepatotoxic, anti-proliferative activity against human breast cancer cells, anti-oxidative activity, superoxide-scavenging activities in biological membranes, inhibit LPO and in prevention of low-density lipoprotein oxidation and protection of mitochondrial functions from oxidative stress. At this time, there are a restricted number of experimental studies on the effect of glabridin on DM at present. Thus, this study was carried out to elucidate the modulatory effects of glabridin extract at two different doses on experimentally induced hyperglycemia, hyperlipidemia and liver dysfunction by Streptozotocin (which destroys the beta cells). Furthermore, the present study investigated any side effects caused by the consumption of glabridin extract.

Methodology

Chemicals and glabridin preparation
Glabridin, (C20H20O4), [(R)-4-(3,4-dihydro-8,8-dimethyl-2H,8H-benzo[1,2-b:3,4-b’]dipyran-3yl)-1,3 benzenediol] extracted and purified (≥ 98%) from the root of Glycyrrhiza glabra L., was purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. Glabridin was dissolved in 1:4 dimethyl sulphoxide (DMSO): phosphate-buffered saline (PBS, pH 7.4). Streptozotocin, acetylcholine perchlorate and N-(1-Naphthyl) ethylene-diamine dihydrochloride (NEDD) were purchased from Sigma–Aldrich.

Animals
For this purpose, 42 albino male rats (Rattus norvegicus), weighing about 110-120 gm, were obtained from the Animal House Colony of the National Research Center (Dokki, Giza-Egypt). Animals were housed in polypropylene cages with wood dust and given standard rodent food pellets (Agricultural-Industrial Integration Company, Giza, Egypt) and tap water ad libitum. The animals were procured, maintained and used in accordance with WHO guideline for animal care and the study design was approved by the Ain Shams University Research Ethics Committee.

Induction of Diabetes
Hyperglycemia was induced by single intraperitoneal injection (i.p.) in volume of ~2 mL/kg of STZ in a dose of 60 mg/kg body weight (b.w.) dissolved in 0.1 mol/L citrate buffer, pH 4.5. Streptozotocin induces diabetes within 3 days by destroying the beta cells. After 72 hrs blood samples were withdrawn from the retro-orbital venous plexus under light diethyl ether anaesthesia and the plasma was separated by centrifugation for the determination of fasting blood glucose level. Only rats with plasma glucose levels more than 250 mg/dl were selected and considered as hyperglycemic rats and included in the study. Diabetic animals and non-diabetic control group were further grouped and were subjected to further experimentation.

Experimental design and treatment schedule
Experimental animals were randomly divided into 6 groups that included 7 animals each as follows: Group (1) was the negative control groups (or normal control group) in which received vehicle (1:4, DMSO : PBS) orally for 28 days. Group (2) & (3) included the animals which received orally (by gavage) and daily glabridin at 25 and 50 mg/kg body weight (b.w.), respectively. Group (4) was the positive control group (or diabetic control group) in which rats received a single inter peritoneal injection (i.p.) STZ in a dose level 60 mg/kg b.w. Group (5) & (6) hyperglycemic rats received orally and daily with glabridin at 25 and 50 mg/kg b.w., respectively. Treatments were begun at the onset of hyperglycemia. The doses of the glabridin used here were selected based on the effective dose of previous study by Hassanein.
without anticoagulant to separate serum by centrifugation in a cooling centrifuge (IEC centra-4R; International Equipment Co., Needham Heights, MA, USA) for 30 min at 3000 rpm and 4 °C. The serum was separated and divided into samples and preserved at -80 °C for further analysis. Immediately after killing the animals, the liver, kidney and spleen were immediately separated out of the body, cleaned and weighed.

**Measurements**

The body weight gain and relative organ weights (liver, kidney and spleen) were measured by using a Sartorius LP2200S balance (Gottingen, Germany). Relative organ weights (gm/100 gm b.w.) = [(organ weight in gm / body weight in gm)] x 100. The percentage of change of any parameter = ((T - C)/C) x100, where (T) = the mean value of the parameter in the treated group and (C) = the mean value of the parameter in the control group.

**Biochemical parameters**

All biochemical analyses [serum glucose, alanine & aspartate aminotransferases (ALAT & ASAT), urea, creatinine, total cholesterol, triglycerides, HDL-cholesterol, MDA, CAT and GSH] were manually done using commercial kits (Bio-Diagnostic, Dokki, Giza, Egypt). LDL-cholesterol = total cholesterol - (TAG/5) - HDL-cholesterol. Atherogenic indexes were calculated as follows: atherogenic index1= total cholesterol/HDL-cholesterol ratio; atherogenic index2=LDL-cholesterol/HDL-cholesterol ratio. Serum tumor necrosis factor (TNF) was measured by sandwich ELISA using a commercially available ELISA kit for anti-rat TNF-α antibody (R&D Systems, Minneapolis, MN) according to the manufacturer’s recommendations. The hematological parameters were evaluated with an automated hematological analyzer system (Hemat 8 analyzer, SEAC, Germany). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated as follows: MCV (fl/RBC) = (HCT x 10) / RBCs, MCH (pg/ RBC) = (Hb x 10) / RBCs and MCHC (gm/dL) = (Hb x 100) / HCT.

**Statistics**

Data were presented as mean with ± standard deviation (SE). Statistical analysis was performed with one-way ANOVA using GraphPad Prism version 4.03 for Windows (GraphPad Software Inc., San Diego, CA, USA). The difference between means was assessed by Turkey’s multiple comparison test, in which P values of <0.05, <0.01 and <0.001 were considered statistically significant, highly significant and very highly significant, respectively.

**Results**

**Modulatory effects of glabridin on body weight, relative organ weights and glucose level**

Healthy rats consumed either 25 or 50 mg/kg b.w. dose of glabridin induced a significant decrease (P<0.05-0.01) in liver relative weights (by -14.2% versus -17.3%, respectively) compared with the control group (Table 1). In addition, healthy rats consumed both doses of glabridin significantly decreased (P<0.001) in serum glucose level (by -22.3% versus -30.6%, respectively) compared with the control group. The body weight gain and

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Glabridin 25</th>
<th>Glabridin 50</th>
<th>STZ-only</th>
<th>STZ + Glabridin 25</th>
<th>STZ + Glabridin 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (gm)</td>
<td>130.6 ± 1.93</td>
<td>129.6 ± 1.72</td>
<td>130.7 ± 1.32</td>
<td>130.6 ± 1.07</td>
<td>129.4 ± 1.27</td>
<td>130.7 ± 0.68</td>
</tr>
<tr>
<td>Final body weight (gm)</td>
<td>162.1 ± 2.41</td>
<td>169.9 ± 3.52</td>
<td>171.7 ± 1.90</td>
<td>111.3 ± 2.20 ***</td>
<td>123.9 ± 1.75 *** ††</td>
<td>131.6 ± 2.44 *** †††</td>
</tr>
<tr>
<td>Body weight gain (gm)</td>
<td>31.57 ± 3.77</td>
<td>40.29 ± 3.53</td>
<td>41.0 ± 1.51</td>
<td>-19.29 ± 1.87 ***</td>
<td>0.86 ± 2.54 *** †† †††</td>
<td>151.90 ± 2.30 *** †††</td>
</tr>
<tr>
<td>Relative liver weight (gm/100 gm b.w.)</td>
<td>2.99 ± 0.09</td>
<td>2.57 ± 0.13 *</td>
<td>2.48 ± 0.11 **</td>
<td>4.97 ± 0.15 ***</td>
<td>4.41 ± 0.19 *** ††</td>
<td>3.78 ± 0.11 ** †††</td>
</tr>
<tr>
<td>Relative kidney weight (gm/100 gm b.w.)</td>
<td>0.303 ± 0.013</td>
<td>0.306 ± 0.020</td>
<td>0.303 ± 0.015</td>
<td>0.429 ± 0.017 ***</td>
<td>0.379 ± 0.007 **</td>
<td>0.364 ± 0.011 * †</td>
</tr>
<tr>
<td>Relative spleen weight (gm/100 gm b.w.)</td>
<td>0.221 ± 0.006</td>
<td>0.227 ± 0.007</td>
<td>0.223 ± 0.011</td>
<td>0.301 ± 0.009 ***</td>
<td>0.289 ± 0.011 ***</td>
<td>0.261 ± 0.007 * †</td>
</tr>
<tr>
<td>Glucose level (mg/dl)</td>
<td>74.88 ± 2.23</td>
<td>58.15 ± 3.30 ***</td>
<td>51.93 ± 2.27 ***</td>
<td>289.40 ± 9.19 ***</td>
<td>249.00 ± 12.30 *** †† †††</td>
<td>151.90 ± 2.30 *** †††</td>
</tr>
</tbody>
</table>

b.w: body weight, STZ: Streptozotocin. Data are shown as mean ± SE of 7 animals; *P<0.05, **P<0.01, ***P<0.001: compared with the normal control group; †P<0.05, ††P<0.01, †††P<0.001: compared with the STZ-induced diabetic group.
serum glucose level were significantly decreased and increased ($P<0.001$) by -161.1% and 286.5%, respectively, in STZ-induced diabetic rats group compared with the control group. In addition, liver, kidney and spleen relative weights significantly increased ($P<0.05-0.001$) by 66%, 41.6% and 36.1%, respectively, in STZ-induced diabetic rats group compared with the control group (Table 1). The body weight gain and liver hypertrophy were significantly increased ($P<0.01-0.001$) and decreased ($P<0.05-0.001$), respectively in STZ-induced diabetic rats treated orally with either low or high dose glabridin (117.6% versus -97.3% and 47.3% versus 26.3%, respectively) compared with STZ-induced diabetic rats group. Kidney and spleen relative weights were significantly decreased ($P<0.05$) in diabetic rats treated orally with only high dose glabridin compared with STZ-induced diabetic group. The percentages of changes of the kidney and spleen relative weights, compared with the healthy control group, in diabetic rats treated with low and high dose of glabridin were 25.1% versus 20.2% and 30.4% versus 18.1%, respectively. Oral treatment of diabetic group with low and high dose of glabridin significantly decreased ($P<0.01-0.001$) serum glucose level compared with STZ-induced diabetic rats (by -22.3% versus -30.6%, respectively, compared with the control group).

Healthy rats consumed either 25 or 50 mg/kg b.w. dose of glabridin induced a significant decrease ($P<0.05-0.001$) in total cholesterol, LDL-cholesterol levels and atherogenic indexes by (-13.2% versus -15.5%, -58.8% versus -69%, -17.7% versus -20.2% and -61.4% versus -71.1%, respectively) compared with the control group (Table 2). Serum total cholesterol, triglyceride, LDL-cholesterol and atherogenic indices values were significantly increased ($P<0.001$) in STZ-induced diabetic rats group (by 55.9%, 46.1%, 285.5%, 142.6 and 483.7%, respectively) compared with the control group. On the other hand, serum HDL-cholesterol level was significantly decreased ($P<0.001$) in STZ-induced diabetic rats group (by -36.5%) compared with the control group. Hyperlipidemia has been reported to accompany hyperglycemia states, high levels of total cholesterol and triglycerides; importantly LDL-cholesterol is one of the major coronary risk factors which is the major cause of morbidity and deaths in diabetic subjects. The levels of serum lipids profiles are usually elevated in diabetes mellitus, similar increase was found in present study. The change of serum HDL-cholesterol level was completely modulated in diabetic rats treated with either low or high dose of glabridin ($P>0.05; -30.4$% versus -26.9%, respectively, compared with the control group). Oral treatment of diabetic rats with both doses of glabridin significantly alleviated ($P<0.05-0.001$) the increase in serum total cholesterol, triglyceride, LDL-cholesterol, atherogenic indices values induced by STZ. These modulatory effects of glabridin were dose dependent. The percentages of changes of all parameters measured, compared with the healthy control group, in diabetic rats treated with low and high dose of glabridin were 38% versus 33.2%, 32.4% versus 27%, 206.5% versus 182%, 98.8% versus 80.5% and 332.6% versus 272.1%, respectively.

### Modulatory effects of glabridin on hematological parameter

RBC count, Hb content, HCT level, blood indices (MCV, MCH and MCHC) were significantly decreased ($P<0.05-0.001$) in STZ-induced diabetic group (by -11.4%, -50.8%, -25%, -14.9%, -43.9% and

<table>
<thead>
<tr>
<th>Table 2—The modulatory effect of glabridin on serum lipid profile and atherogenic indexes in healthy and diabetic rats groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
</tr>
<tr>
<td>Cholesterol level (mg/dl)</td>
</tr>
<tr>
<td>Triglyceride level (mg/dl)</td>
</tr>
<tr>
<td>HDL-Cholesterol level (mg/dl)</td>
</tr>
<tr>
<td>LDL-Cholesterol level (mg/dl)</td>
</tr>
<tr>
<td>Atherogenic Index</td>
</tr>
<tr>
<td>Atherogenic Index²</td>
</tr>
</tbody>
</table>

Atherogenic index (1) = total cholesterol : HDL-cholesterol ratio, atherogenic index (2) = LDL-cholesterol : HDL-cholesterol ratio, b.w.: body weight, STZ: Streptozotocin; Data are shown as mean ± SE of 7 animals; *$P<0.05$, **$P<0.01$, ***$P<0.001$: compared with the normal control group; †$P<0.05$, ††$P<0.01$, †††$P<0.001$: compared with the STZ-induced diabetic group.
Modulatory effects of glabridin on liver and kidney functions

Healthy rats consumed both doses of glabridin induced a significant decrease \((P<0.001)\) in serum ALAT and ASAT activities (by -27.8\% versus -30.3\% and-18.2\% versus -19.9\%, respectively) compared with the control group (Fig. 1). Serum ALAT, ASAT, urea and creatinine levels were significantly increased \((P<0.05-0.001)\) in STZ-induced diabetic group (by 155.1\%, 185.1\%, 46.9\% and 64.3\%, respectively) compared with the healthy control group (Fig. 1). Oral treatment of diabetic rats with both doses of glabridin significantly decreased and \(\) modulated \((P<0.05-0.001)\) serum ALAT and ASAT activities (by -27.8\% and -19.9\%, respectively) compared with the control group. Oral treatment of diabetic rats with either low or high dose of glabridin significantly decreased and \(\) increased \((P<0.05-0.001)\) serum CAT activity and GSH concentration compared with STZ-induced diabetic rats. The percentages of changes of these parameters measured (MDA, CAT and GSH), compared with the healthy control group, in diabetic rats treated with low and high dose of glabridin were 99.5\% versus 74.6\%, -33.1\% versus -24.3\% and -41.7\% versus -29.5\%, respectively.

Modulatory effects of glabridin on pro-inflammatory cytokine

Serum TNF-\(\alpha\) level significantly increased \((P<0.001)\) in STZ-induced diabetic group (by 39.6\%) compared with the control group (Fig. 3). Only diabetic rats treated with the high dose of glabridin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Glabridin 25</th>
<th>Glabridin 50</th>
<th>STZ-only</th>
<th>STZ + Glabridin 25</th>
<th>STZ + Glabridin 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC ((10^3\text{ cell/mm}^3))</td>
<td>9.61 ± 0.87</td>
<td>9.48 ± 0.59</td>
<td>9.56 ± 0.53</td>
<td>12.71 ± 0.74 *</td>
<td>12.41 ± 0.33 *</td>
<td>11.94 ± 0.50</td>
</tr>
<tr>
<td>RBC ((10^6\text{ cell/mm}^3))</td>
<td>5.71 ± 0.12</td>
<td>6.07 ± 0.19</td>
<td>6.19 ± 0.12</td>
<td>5.06 ± 0.15 **</td>
<td>5.17 ± 0.06 *</td>
<td>5.11 ± 0.09 *</td>
</tr>
<tr>
<td>Hb ((\text{gm/dL}))</td>
<td>12.49 ± 0.47</td>
<td>12.94 ± 0.46</td>
<td>13.44 ± 0.64</td>
<td>6.14 ± 0.27 ***</td>
<td>7.47 ± 0.66 ***</td>
<td>8.40 ± 0.56 *** †</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>40.11 ± 1.21</td>
<td>40.59 ± 0.87</td>
<td>43.07 ± 1.10</td>
<td>30.10 ± 0.97 ***</td>
<td>31.44 ± 0.86 ***</td>
<td>32.16 ± 0.73 ***</td>
</tr>
<tr>
<td>MCV (fL/RBC)</td>
<td>70.34 ± 2.30</td>
<td>67.14 ± 1.82</td>
<td>69.72 ± 2.33</td>
<td>59.83 ± 2.58 **</td>
<td>60.84 ± 1.94 *</td>
<td>62.87 ± 0.66</td>
</tr>
<tr>
<td>MCH (pg/RBC)</td>
<td>21.86 ± 0.75</td>
<td>21.42 ± 0.82</td>
<td>21.77 ± 1.17</td>
<td>12.27 ± 0.76 ***</td>
<td>14.43 ± 1.24 ***</td>
<td>16.48 ± 1.17 ** †</td>
</tr>
<tr>
<td>MCHC (gm/dL)</td>
<td>31.16 ± 0.94</td>
<td>32.01 ± 1.41</td>
<td>31.23 ± 1.36</td>
<td>20.51 ± 1.02 ***</td>
<td>23.99 ± 2.46 *</td>
<td>26.24 ± 1.90</td>
</tr>
<tr>
<td>Platelets ((x10^{11}\text{ mm}^3))</td>
<td>570.3 ± 26.1</td>
<td>517.1 ± 50.9</td>
<td>499.1 ± 33.4</td>
<td>741.4 ± 4.5 **</td>
<td>718.6 ± 14.8 **</td>
<td>709.3 ± 10.5 *</td>
</tr>
</tbody>
</table>

b.w.: body weight; Hb: hemoglobin, HCT: hematocrit, MCH: mean corpuscular Hb, MCHC: mean corpuscular, Hb concentration, MCV: mean corpuscular volume, RBCs: red blood corpuscles, STZ: Streptozotocin; Data are shown as mean ± SE of 7 animals; *\(P<0.05\), **\(P<0.01\), ***\(P<0.001\): compared with the normal control group; †\(P<0.05\): compared with the STZ-induced diabetic group.
Fig. 1—Serum aminotransferases (a&b), urea (c) and creatinine (d) levels in healthy and diabetic rats groups with or without glabridin. Values are means, with standard errors represented by vertical bars. STZ: Streptozotocin. *P<0.05, **P<0.01, ***P<0.001: compared with the normal control group; †P<0.05, ††P<0.01, †††P<0.001: compared with the STZ-induced diabetic group.

Fig. 2—Serum catalase activity (a), GSH (b) and MDA (c) concentrations in healthy and diabetic rats groups with or without glabridin. Values are means, with standard errors represented by vertical bars. CAT: Catalase, GSH: Reduced glutathione, MDA: Malondialdehyde, STZ: Streptozotocin. *P<0.05, **P<0.01, ***P<0.001: compared with the normal control group; †P<0.05, ††P<0.001: compared with the STZ-induced diabetic group.
Fig. 3—Serum TNF-α level in healthy and diabetic rats groups with or without glabridin. Values are means, with standard errors represented by vertical bars. STZ: Streptozotocin, TNF: Tumor necrosis factor. *P<0.05, **P<0.01, ***P<0.001: compared with the normal control group; †P<0.05: compared with the STZ-induced diabetic group.

significantly decreased (P<0.05) serum TNF-α level compared with STZ-induced diabetic rats. The percentages of changes of serum TNF-α level, compared with the healthy control group, in diabetic rats treated with low and high dose of glabridin were 26.7% versus 19.7%, respectively.

Discussion

DM, a metabolic disorder, is characterized by weight loss, hyperglycemia and hyperlipidemia as well as impairment of liver and kidney functions as seen in STZ-treated animals. Several studies have shown an association between hyperglycemia and decreased body weight of diabetic animals. The reduction of body weight might be due to the low utilization of uptake blood sugar in cell. Also, the loss of body weight gain may be due to the injurious effects of STZ which caused alkylation of DNA and necrotic lesions. The reduction of body weight might be due to the low utilization of uptake blood sugar in cell. Also, the loss of body weight gain may be due to the injurious effects of STZ which caused alkylation of DNA and necrotic lesions. The reduction of body weight might be due to the low utilization of uptake blood sugar in cell. Also, the loss of body weight gain may be due to the injurious effects of STZ which caused alkylation of DNA and necrotic lesions. The reduction of body weight might be due to the low utilization of uptake blood sugar in cell. Also, the loss of body weight gain may be due to the injurious effects of STZ which caused alkylation of DNA and necrotic lesions.

Weight loss as seen in the present study in STZ-treated animals may be due lipolysis and gluconeogenesis during diabetes. Also, the reduction in body weight resulted mainly from increase/decrease in the LPO/antioxidant system and by increasing pro-inflammatory/pyrogenic cytokines (TNF-α). In addition, hyperglycemia induced by STZ, is due to an absolute or relative deficiency of circulating insulin levels by the destruction of pancreatic beta-cells, similar increase was found in present study. Also, hyperglycemia results in the generation of free radicals which can exhaust antioxidant defenses (such as CAT and GSH levels), thus leading to the disruption of cellular functions, oxidative damage to membranes and enhanced susceptibility to LPO as seen in the present study. Several studies described that the organ weights including liver, kidney and spleen increase in diabetic animals, similar results were found in present study. Hypertrophy of liver could be attributed to increase triglyceride accumulation leading to enlarged liver which could be due to the increased influx of fatty acids into the liver induced by hypoinsulinemia and the low capacity of excretion of lipoprotein secretion from liver resulting from a deficiency of apo-lipoprotein B synthesis. Present finding of this study are in agreement with other previous studies. Ichinose et al. proposed that increase in kidney weight is associated with the increase in renal expression of angiogenic factors such as vascular endothelial growth factor–A, angiopoietin–2 and fibrogenic factor transforming growth factor–β-1 induced by high glucose. The improvement of body weight gain was concomitant with the improvement of cellular anti-oxidant defense system. Also, this finding may be due to the ability of glabridin to reduce hyperglycemia. In addition, the modulatory effects of high dose of glabridin extracts on the increase in relative organ weights shown in STZ-diabetic rats resulted mainly from decreasing the blood glucose and lipid levels by improving lipid metabolism and from decreasing LPO by improving the antioxidant defence system. The present study showed that low and high dose of glabridin significantly alleviated hyperglycemia induced by STZ in rats. The possible mechanism of hypoglycemic action of glabridin may be through potentiation of pancreatic secretion of insulin from beta cells of islets or due to enhanced transport of blood glucose to the peripheral tissue or due to decrease oxidative stress by its antioxidant property, thus preserving pancreatic beta cell integrity. Glabridin constituted the major flavonoid in the licorice root extract. The flavonoid exert its hypoglycemic effect by either promoting the entry of glucose into cells, stimulation of glycolytic enzymes and glycogenic enzymes, depression of gluconeogenic enzymes or inhibiting the glucose-6-phosphatase in the liver, subsequently reducing the release of glucose in the blood. These modulatory effects of glabridin on the previous parameters were dose dependent.
Also, treatment of diabetic rats with glabridin (specially high dose) significantly alleviated most signs of the metabolic syndrome including hyperlipidemia induced by STZ. In addition, Fuhrman et al. reported that polyphenols glabridin (derived from licorice) inhibited LDL oxidation in a dose-dependent manner. The hypolipidaemic effect and the marked decrease in atherogenic indexes shown in the present study by glabridin (specially high dose) in STZ-treated rats may reduce the incidence of atherosclerosis in diabetic patients. There is a strong positive correlation between hyperglycemia and overproduction of ROS and impairment of antioxidant enzymes that lead to increased oxidative stress. ROS are believed to be responsible for hematological complications develop in diabetes consist mainly of abnormalities in the function, morphology and metabolism of RBC, WBC and platelets. The significant decrease in RBC, Hb and HCT in diabetic rats may be as a result of the cellular damage on the erythrocyte membrane as a result of oxidative stress induced by STZ. Anemia have been reported in DM is due to the increase glycation of proteins such as Hb and non-enzymic oxidation of lipids, proteins and DNA peroxidation and finally programmed cell death. The decrease in Hb content in the diabetic rats may have been due to a reduction in protein synthesis in all tissues and absolute or relative insulin deficiency. Significant decrease in the WBC counts in diabetic rats may be possible immune-modulatory effects of glabridin. In addition, significant increase in Hb and MCH which may be due to its antioxidant property as well as hypoglycemic effects. ROS are able to destroy membrane lipids and proteins as well as DNA which led to severe damage. The elevation biomarkers of liver and kidney enzymes such as ALAT, ASAT, urea and creatinine in serum with hyperglycemia are used as indicator for the identification of hepatic and renal damage in STZ-induced diabetic rats. The increase in aminotransferases levels and kidney markers, as seen in the present study, may be due to the cellular damage in the liver and kidney caused by STZ led to liver injury; resulted mainly from excessive release of free radicals and lipid peroxidation. Pro-inflammatory cytokines such as TNF-α are potent inducers of free radicals and inflammatory mediators, which increased in diabetes. Data of the present study showed that treatment of diabetic rats with glabridin was able to significantly alleviated hepatic and renal damage as evident by lowering their biomarker enzymes through scavenging the free radicals (ROS and RNS) and terminating the membrane LPO by improving the cellular anti-oxidant defense system. The nephroprotective activity of licorice or glabridin, notably glabridin was previously reported studies. Also, the increased activities of antioxidant enzymes by glabridin treatment may act as an added compensation mechanism to maintain the cell integrity and protection against free radical damage by decreasing pro-inflammatory cytokine. The highest suppressive effect on these parameters of diabetic rats was induced by the high dose of glabridin. GSH, the first line defense against LPO, is an essential electron donor to glutathione peroxidase in the reduction of hydro-peroxides and serves as a nucleophilic co-substrate to glutathione-S-transferases in the detoxification of xenobiotes. Hyperglycemia results in the generation of free radicals which can exhaust non-enzymic/ enzymic antioxidant defenses system via the production of several reducing sugars (through glycolysis and the polyol pathway) thus leading to the disruption of cellular functions, oxidative damage to membranes and enhanced susceptibility to LPO. Also, the mRNA transcription levels of the antioxidant enzymes were down-regulated by diabetes. The increased LPO such as MDA in diabetes may be due to the insufficient antioxidant system. Treatment with glabridin induced amelioration of oxidative stress biomarkers of STZ-induced diabetic rats by improving GSH and CAT, and inhibition of MDA levels. The antioxidant activities of glabridin agreed with other previous studies. In addition, flavonoids are able to indirectly participate in the reduction of oxidative stress in diabetic patients by improving glycemic control and/or are able to exert antioxidant activity. On the other hand, hyperglycemia induced pathways get together to elevate the level of NF-κB, a pro-inflammatory master key, which activates pro-inflammatory cytokines gene expressions and apoptosis cascade leading to programmed cell death of islet beta cells and increase oxidative stress. Several inflammatory mediators such pro-inflammatory cytokines (TNF-α) increased in diabetic (as seen in the present study) and these mediators have been considered to be the link between inflammation and insulin resistance. This reduction in serum TNF-α level may be
because of glabridin showed its antioxidant and anti-inflammatory properties. The anti-inflammatory properties of glabridin may be attributed to inhibition of NF-κB, therefore, blocking the release of pro-inflammatory cytokines and amplification of inflammation via inhibition of downstream inflammatory mediators. The mechanism of glabridin on inflammation requires further research. All modulatory effects obtained in the present study were increased by increasing the dose of glabridin. Another interesting finding of the present study was that the only deleterious effect of glabridin in healthy rats was the significant reduction in liver relative weight which is mainly due to hypoglycemia and hypocholesterolemia in rats that received the both doses of glabridin compared with the control animals (Tables 1 & 2). All other parameters measured in this study did not significantly alter (P>0.05) in healthy rats that received both doses of glabridin compared with the healthy control animals (as data shown). In conclusion, the present study supports the hypothesis that glabridin may has beneficial effects against hyperglycemia and its related complications in STZ diabetic rat model. The modulatory effect of glabridin was partially but significant, and dose dependent. Where, the high dose of glabridin (50 mg/kg b.w.) exerted a better effect when compared with the low dose (25 mg/kg b.w.). This study suggests that glabridin may be considered as beneficial natural agents for the treatment of DM. The further study showed that the effect of glabridin in human volunteers and diabetic patients.

Acknowledgement
This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. The authors have no potential financial conflict of interest.

References


