Antihyperlipidemic effect of biochanin A on streptozotocin induced diabetic rats.

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Received on:10-11-2011; Revised: 15-12-2011; Accepted on:12-01-2012

ABSTRACT

Diabetes mellitus is a chronic metabolic disease with the highest rates of prevalence and mortality worldwide and hyperlipidemia has been ranked as one of the greatest risk factors contributing to coronary heart disease. This study was undertaken to evaluate the antiatherogenic and antihyperlipidemic effect of biochanin A on streptozotocin (STZ)-diabetic rats. Diabetes was induced in male albino Wistar rats by the administration of STZ (40 mg/kg BW) and biochanin A was administered post orally at a dose of 10 mg/kg BW for 45 days. The result showed an increase in plasma glucose, atherogenic index and also showed an elevation in total cholesterol (TC), triglycerides (TG), very low density lipoproteins-cholesterol (VLDL-C) and low density lipoprotein-cholesterol (LDL-C) and decrease in the level of plasma insulin and high density lipoprotein-cholesterol (HDL-C) in diabetic rats, whereas, diabetic rats treated with biochanin A shifted all these parameters towards normality. The effect of biochanin A was comparable with glibenclamide, a well known hypoglycemic drug. These findings suggest that biochanin A treatment has a therapeutic property by showing antiatherogenic and antihyperlipidemic effect on STZ-diabetic rats.

Key words: Diabetes mellitus; streptozotocin; biochanin A; atherogenic index; lipid profile; glibenclamide

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease with the highest rates of prevalence and mortality worldwide that is caused by an absolute or relative lack of insulin and/or reduced insulin activity, which results in hyperglycemia and abnormalities in carbohydrate, protein, and fat metabolism. It can be associated with serious complications and premature death, but people with diabetes should take steps to control the disease and lower the risk of complications. Diabetes mellitus is a major risk factor for the development of cardiovascular complications, and cardiovascular disease now accounts for 80% of all diabetic mortality. Atherosclerosis, a chronic disease of the vessel wall is associated with diabetes mellitus, which underlies the development of many acute cardiovascular disease events including myocardial infarction and stroke. During diabetes, a profound alteration in the concentration and composition of lipid occurs. Lipid-lowering therapy in diabetes is effective in reducing the risk of vascular complications, in spite of the use of many oral hypoglycemic agents such as sulphonylureas and biguanides. All of these pharmacological modalities also show limited efficacy and certain adverse effects such as liver toxicity, lactic acidosis, diarrhea and attenuation of response after prolonged use and are also expensive for developing countries. Comparatively very less side effects and low cost of phytochemicals from natural resources open new avenues for the treatment of various diseases including diabetes. Therefore, there is a need for phytochemicals that have antihyperglycemic and hypolipidemic potential, which are cost-effective and also safe without side effects on long-term usage.

Most recent studies on the treatment of type-2 diabetes have focused on the potential use of plant constituents with hypoglycemic and hypolipidemic effects. As such, there has been a growing interest in flavonoids, which are widely distributed in plants and ingested by humans, due to their antioxidant, mild estrogenic, and hypolipidemic activity. Earlier study reports that consumption of isoflavones is associated with protection against atherosclerosis. This observation is supported by experimental studies in diverse animal models of atherosclerosis showing that dietary isoflavone can inhibit the disease. Isoflavones are naturally occurring compounds in certain foods. Soy isoflavones are safe to ingest in amounts up to 100 mg/day. Salam et al., reported that out of a natural product library comprising 200 compounds, isoflavones regulates the fatty acid metabolism via the nuclear receptors PPARα and PPARγ. Thus the soy isoflavones could be used therapeutically with a low occurrence of unwanted side effects. Biochanin A is an O-methylated isoflavone. It is a natural organic compound in the class of phytochemicals known as flavonoids. Biochanin A can be found in red clover in soy, alfalfa sprouts, peanuts, chickpea (Cicer arietinum) and in other legumes. As a flavonoid, it also exhibits various pharmacological properties such as, anti-inflammatory, anti-carcinogenic, hypolipidemic effect.

The eventual objective of the present study is to evaluate the antiatherogenic and antihyperlipidemic effect of biochanin A on STZ-induced diabetic rats.

Fig.1 Biochanin A

MATERIALS AND METHODS

Animals
Male albino (9 week-old) rats of Wistar strain with a body weight ranging from 180 to 200 g, were procured from Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, and were maintained in an air conditioned room (25 ± 1°C) with a 12 h light/12 h dark cycle. Feed and water approved by the Institutional Animals Ethics Committee of Rajah Muthiah Medical College and Hospital Reg No. 160/1999/CPCSEA, Proposal number: 738, Annamalai University, Annamalainagar.
**Chemicals**

Streptozotocin (STZ) and biochanin A were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). All other chemicals used in this study were of analytical grade obtained from E. Merck and HIMEDIA, Mumbai, India.

**Experimental induction of diabetes**

After an overnight fast, the animals were rendered diabetic by a single intraperitoneal injection of STZ (40 mg/kg BW) in freshly prepared citrate buffer (0.1 M pH 4.5). The STZ-injected animals were given 20% glucose solution for 24 h to prevent initial drug-induced hypoglycemic mortality. The STZ-injected animals exhibited hyperglycemia within a few days. Diabetes in STZ rats was confirmed by measuring the blood glucose (by glucose oxidase method) 96 h after injection with STZ. The animals with blood glucose above 230 mg/dL were considered diabetic and used for the experiments.

**Experimental design**

The animals were randomly divided into five groups of six animals each as given below. Biochanin A and glibenclamide were dissolved in 0.5% DMSO and administered orally once in a day in the morning for 45 days.

- Group I: Control (0.5% DMSO)
- Group II: Control + biochanin A (10 mg/kg BW/day)
- Group III: Diabetic control (0.5% DMSO)
- Group IV: Diabetic + biochanin A (10 mg/kg BW/day)
- Group V: Diabetic + glibenclamide (600 µg/kg BW/day).

After 45 days, the animals were anaesthetized using ketamine (24 mg/kg body weight, intramuscular injection), and sacrificed by cervical dislocation. Between 8:00 am and 9:00 am blood was collected in tubes with a mixture of potassium oxalate and sodium fluoride (1:3) to get plasma for various assays.

**Table 1. Effect of Biochanin A on and plasma glucose and insulin in STZ-diabetic rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 day Total glucose (mg/dL)</th>
<th>Plasma glucose (mg/dL) 45th day Change (%)</th>
<th>Insulin (µU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>90.85 ± 4.61</td>
<td>92.76 ± 4.56 (+) 2.29</td>
<td>14.98 ± 1.11*</td>
</tr>
<tr>
<td>Control + Biochanin A 10 mg/kg BW</td>
<td>88.45 ± 4.59</td>
<td>85.64 ± 5.86 (-) 3.17</td>
<td>15.08 ± 1.15+</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>242.02 ± 9.32</td>
<td>285.72 ± 11.33 (+) 15.08</td>
<td>7.55 ± 0.39*</td>
</tr>
<tr>
<td>Diabetic + Biochanin A 10 mg/kg BW</td>
<td>241.15 ± 9.21</td>
<td>121.24 ± 9.81 (-) 49.72</td>
<td>13.34 ± 0.65</td>
</tr>
<tr>
<td>Diabetic + glibenclamide 600 µg/kg BW</td>
<td>238.45 ± 9.34</td>
<td>98.24 ± 6.81 (-) 68.67</td>
<td>14.86 ± 1.23c</td>
</tr>
</tbody>
</table>

Values are given as means ± S.D for six rats in each group. Values not sharing a common superscript differ significantly at P < 0.05 (DMRT).

**Table 2. Effect of Biochanin A on total cholesterol, triglycerides, high density lipoprotein-C, very low density lipoprotein-C, low density lipoprotein-C and atherogenic index in the plasma of STZ-diabetic rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mg/dL)</th>
<th>TG (mg/dL)</th>
<th>HDL-C (mg/dl)</th>
<th>VLDL-C (mg/dL)</th>
<th>LDL-C (mg/dL)</th>
<th>Atherogenic Index (TC/HDL-C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>707.3 ± 346*</td>
<td>60.17 ± 2.75*</td>
<td>49.17 ± 2.35*</td>
<td>12.03 ± 0.55*</td>
<td>18.06 ± 0.55*</td>
<td>1.61 ± 0.01*</td>
</tr>
<tr>
<td>Control + Biochanin A 10 mg/kg BW</td>
<td>74.39 ± 2.76*</td>
<td>58.53 ± 2.62*</td>
<td>47.24 ± 2.62*</td>
<td>11.70 ± 0.52*</td>
<td>15.44 ± 0.38*</td>
<td>1.57 ± 0.02*</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>155.07 ± 11.08*</td>
<td>159.27 ± 10.43*</td>
<td>24.89 ± 1.29*</td>
<td>31.56 ± 2.07*</td>
<td>98.38 ± 7.75*</td>
<td>6.22 ± 0.12*</td>
</tr>
<tr>
<td>Diabetic + Biochanin A 10 mg/kg BW</td>
<td>92.42 ± 8.88*</td>
<td>79.46 ± 4.56*</td>
<td>41.82 ± 2.73*</td>
<td>15.89 ± 0.91*</td>
<td>34.70 ± 1.23*</td>
<td>2.22 ± 0.04*</td>
</tr>
<tr>
<td>Diabetic + glibenclamide 600 µg/kg BW</td>
<td>84.28 ± 3.73*</td>
<td>70.24 ± 3.55*</td>
<td>44.19 ± 3.46*</td>
<td>14.04 ± 0.70*</td>
<td>26.05 ± 0.43*</td>
<td>1.90 ± 0.06*</td>
</tr>
</tbody>
</table>

Values are given as means ± S.D for six rats in each group. Values not sharing a common superscript differ significantly at P < 0.05 (DMRT).

Tissues (liver and kidney) were collected and stored at 4°C for the measurement of various parameters.

**Biochemical estimations**

Glucose was estimated by the method of Trinder using a reagent kit (Trinder, 1969)[9]. The insulin in the rat plasma was measured by the method of Burgi et al. (1988)[10]. Total lipids were extracted from the liver and kidney tissues according to the method of Folch et al.,[11]. Total cholesterol was estimated by the method of Allain et al.,[10]. HDL-C was estimated by the method of Izzo et al.,[11]. Atherogenic index was calculated by the method of Malspina et al.[12]. VLDL-C and LDL-C were calculated by the method of Friedewald et al.[13]. Triglycerides were estimated by the method of McGowan et al.[14]. Free fatty acid content was estimated by the method of Falholt et al.[15]. Phospholipid was estimated by the method of Silversmit and Davis[14].

**Statistical analysis**

Values are given as means ± S.D. for six rats in each group. Data were analyzed by one-way analysis of variance followed by Duncan’s Multiple Range Test (DMRT) using SPSS version 10 (SPSS, Chicago, IL). The limit of statistical significance was set at P = 0.05.

**RESULTS**

Table 1 shows the effect of oral administration of biochanin A on the level of plasma glucose and insulin in control and STZ-diabetic rats. Diabetic rats showed increased plasma glucose and decreased insulin level. Administration of biochanin A to diabetic rats significantly decreased the plasma glucose and increased the insulin level.

Table 2 shows that the TC, TG, VLDL-C, LDL-C and atherogenic index increased and HDL-C decreased in diabetic rats, and treatment with biochanin A shifted all these parameters toward normal levels.

Table 3 shows the effect of biochanin A on free fatty acids and phospholipids in control and STZ-induced diabetic rats. Diabetic rats showed increased level of FFA and PL, and treatment with biochanin A significantly decreased the levels of FFA and PL.

Tables 4, 5 and 6 show the effect of biochanin A on total cholesterol, triglycerides, free fatty acids and phospholipids in the liver, kidney and heart on control and STZ-diabetic rats. Diabetic rats showed increased TC, TG, FFA and PL levels and treatment with biochanin A brought these changes to normal levels.
**Table 3. Effect of Biochanin A on free fatty acids and phospholipids in the plasma of STZ-diabetic rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>FFA (mg/dL)</th>
<th>PL (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>60.57 ± 5.23</td>
<td>81.54 ± 7.54</td>
</tr>
<tr>
<td>Control + Biochanin A</td>
<td>58.12 ± 5.14</td>
<td>80.29 ± 6.23</td>
</tr>
<tr>
<td>10 mg/kg BW</td>
<td>113.14 ± 10.26</td>
<td>149.68 ± 11.15</td>
</tr>
<tr>
<td>Diabetic</td>
<td>70.21 ± 6.84</td>
<td>101.28 ± 9.47</td>
</tr>
<tr>
<td>Diabetic + glibenclamide</td>
<td>63.45 ± 5.26</td>
<td>99.84 ± 7.72</td>
</tr>
</tbody>
</table>

Values are given as means ± S.D for six rats in each group.

**Table 4. Effect of Biochanin A on total cholesterol, triglycerides, free fatty acids and phospholipids in the liver of streptozotocin-diabetic rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mg/dL)</th>
<th>TG (mg/dL)</th>
<th>FFA (mg/dL)</th>
<th>PL (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.83 ± 0.27</td>
<td>3.79 ± 0.38</td>
<td>4.53 ± 0.38</td>
<td>17.12 ± 1.49</td>
</tr>
<tr>
<td>Control + Biochanin A</td>
<td>4.72 ± 0.34</td>
<td>3.54 ± 0.27</td>
<td>4.44 ± 0.32</td>
<td>17.27 ± 1.39</td>
</tr>
<tr>
<td>10 mg/kg BW</td>
<td>7.24 ± 0.49</td>
<td>7.24 ± 0.52</td>
<td>10.78 ± 0.58</td>
<td>34.32 ± 2.93</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>5.73 ± 0.29</td>
<td>6.46 ± 0.43</td>
<td>6.16 ± 0.25</td>
<td>22.34 ± 1.97</td>
</tr>
<tr>
<td>Diabetic + Biochanin A</td>
<td>4.24 ± 0.41</td>
<td>4.24 ± 0.25</td>
<td>5.42 ± 0.40</td>
<td>21.23 ± 1.72</td>
</tr>
</tbody>
</table>

Values are given as means ± S.D for six rats in each group.

**Table 5. Effect of Biochanin A on total cholesterol, triglycerides, free fatty acids and phospholipids in the kidney of STZ-diabetic rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mg/dL)</th>
<th>TG (mg/dL)</th>
<th>FFA (mg/dL)</th>
<th>PL (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.67 ± 0.19</td>
<td>4.29 ± 0.25</td>
<td>5.42 ± 0.35</td>
<td>12.12 ± 1.12</td>
</tr>
<tr>
<td>Control + Biochanin A</td>
<td>2.48 ± 0.18</td>
<td>4.57 ± 0.29</td>
<td>5.27 ± 0.32</td>
<td>12.74 ± 0.82</td>
</tr>
<tr>
<td>10 mg/kg BW</td>
<td>5.34 ± 0.29</td>
<td>7.22 ± 0.40</td>
<td>15.14 ± 1.43</td>
<td>25.37 ± 1.87</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>2.91 ± 0.13</td>
<td>4.73 ± 0.59</td>
<td>8.45 ± 0.23</td>
<td>18.32 ± 1.24</td>
</tr>
<tr>
<td>Diabetic + Biochanin A</td>
<td>2.79 ± 0.13</td>
<td>4.12 ± 0.32</td>
<td>6.11 ± 0.32</td>
<td>14.53 ± 1.10</td>
</tr>
</tbody>
</table>

Values are given as means ± S.D for six rats in each group.

**DISCUSSION**

Streptozotocin is well known for its selective pancreatic islet β-cell cytoxicity and has been extensively used to induce diabetes mellitus in animals [17]. In the present study, we observed an increase in the level of plasma glucose and decrease in the level of insulin in STZ-diabetic rats. The diabetic rats treated with biochanin A decreases the plasma glucose and increases the insulin level which might be due to elevated secretion of insulin from the existing β-cells, which, in turn, increases the utilization of glucose by the tissue. However the standard drug glibenclamide showed a better reduction of blood glucose that biochanin A.

Lipids play an important role in the pathogenesis of diabetes mellitus. Hyperlipidemia is a recognized consequence of diabetes mellitus demonstrated by the elevated levels of tissue cholesterol, phospholipids and free fatty acids [18,19]. Lowering the plasma lipid levels through dietary or drug therapy appears to be associated with a decrease in the risk of vascular disease. STZ-diabetic rats showed increase in the plasma cholesterol and triglyceride concentrations [20]. Further, the abnormal high concentration of serum lipids in the diabetic subjects is mainly due to increase in the mobilization of free fatty acids from fat depots. The marked hyperlipidemia that characterizes the diabetic state may be regarded as a consequence of uninhibited actions of lipolytic hormones on fat depots [21]. Insulin is required for the inhibition of hormone sensitive lipase and on the other hand, glucagons and other hormones enhance lipolysis. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides [22]. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia. Diabetic rats treated with biochanin A and glibenclamide significantly decreased TC and TG towards normal level which could be due to an increase in insulin secretion which, in turn, inhibits hormone sensitive lipase and increases the utilization of glucose thereby decreasing the mobilization of free fatty acids from fat depots. Tauseef Siddiqui reported that two isoflavones, biochanin A and formononetin isolated from *gram Cicer arietinum*, have been shown to possess hypolipidemic properties for Triton WR-1339 induced hyperlipidemia in male albino rats, when administered as a crude extract or as individual compounds [23]. Isoflavones have mechanism of action, that potentially involving in the regulation of fatty acid metabolism via the nuclear receptor PPARα and PPARβ. It indicates that Biochanin A is more useful in the treatment of diabetes as it has hypolipidemic effect. Moreover, its hypolipidemic effect could represent a protective mechanism against the development of atherosclerosis, which is usually associated with diabetes.

The concentration of LDL-C is one of the most important predictors of atherosclerosis and coronary heart disease [24], and reduction in its level reduces the morbidity and mortality of patients with CHD. Normally circulating LDL-C undergoes reuptake in the liver via specific receptors and gets cleared from the circulation [25]. This increased LDL-C concentration in the plasma of diabetic rats might be due to the defect in LDL-C receptor either through failure in its production (or) function. HDL-C is protective by reversing cholesterol transport, inhibiting the oxidation of LDL-C and by neutralizing the atherogenic effects of oxidized LDL-C. A greater increase of LDL-C and VLDL-C may also cause a greater decrease of HDL-C as there is a reciprocal relationship between the concentration of VLDL-C and HDL-C. Decreased HDL-C may also be due to diminished lecithin cholesterol acyl transferase activity. In our study, the diabetic rats treated with biochanin A showed an elevation in HDL-C and reduction in LDL-C and VLDL-C. Thus, biochanin A could alleviate the risk of cardiovascular diseases.

Isoflavones prevent the formation of atherosclerosis by scavenging the reactive oxygen species and thereby preventing oxidative damage. Moreover it inhibits the oxidation of low density lipoprotein, which plays a central role in the formation of atherogenesis [26]. In our study atherogenic index was increased in diabetic rats, and treatment with biochanin A showed decrease which might be due to consumption of the isoflavone, biochanin A which is associated with the protection against atherosclerosis.

Phospholipids are vital components of biomembrane and play an important role in the transport of triglycerides [27]. In STZ-diabetic rats, the elevated level of phospholipids may be due to the elevated levels of FFA and TC.
which can promote the synthesis of phospholipids.[25] Diabetic rats treated with biochanin A showed a decrease in the FFA which in turn decreased the phospholipids level. Thus, our findings demonstrate that biochanin A is having good antihyperglycemic and antihyperlipidemic effects, which is evidenced by the decreased levels of glucose, TC, TG, LDL-C, VLDL-C, FFA and PL, and elevated level of insulin, HDL-C in diabetic rats.

In conclusion, our study shows that biochanin A is having antihyperglycemic, antihyperlipidemic and antiatherogenic effect on STZ induced diabetic rats. The effect of biochanin A is comparable with standard drug, glibenclamide. Though the dosage of biochanin A is higher than the standard drug, due to the nontoxic nature of biochanin A, a study on the combined therapy of biochanin A and glibenclamide would be beneficial. Further study on the metabolism and pharmacokinetics of biochanin A is warranted.

ACKNOWLEDGMENT

The financial assistance to Ms. R. Harini as Senior Research Fellowship from the Indian Council of Medical Research, New Delhi is gratefully acknowledged.

REFERENCES