Antihyperglycemic and antihyperlipidemic effect of bergenin on C57BL/6J mice with high fat-diet induced type 2 diabetes

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ABSTRACT

Background: There is a mounting evidence demonstrating causative links between hyperinsulinemia, hyperlipidemia and examined its effect on insulin resistance and oxidative stress. The objective of study is to investigate the effects of bergenin on insulin sensitivity, oxidative stress, hepatic markers, antioxidant and lipid abnormalities of the liver in high-fat diet (HFD) induced type 2 diabetes in C57BL/6J mice. Methods: Mice were segregated into two groups, one fed standard diet (NC) and the other fed high-fat diet (HFD) for 16 weeks. Mice were fed continuously with high fat diet for 16 weeks and subjected to intragastric administration of bergenin (40 mg/kg body weight (BW)), metformin (25 mg/kg BW) 9-16 weeks. After 16 weeks, assays were performed in plasma and liver. Results and discussion: HFD fed mice showed hyperglycemia, hyperinsulinemia, oxidative damage, lipid accumulation, elevated antioxidant and serum aminotransferases. The biological abnormalities associated with HFD feeding were significantly reduced by bergenin administration. Bergenin addition significantly improved insulin sensitivity index, reduced liver damage and oxidative changes, and brought back the antioxidants and lipids towards normal. The effects of bergenin were comparable with that of standard drug, metformin. Conclusion: These data suggest that bergenin affords hepatoprotection by its antioxidant and insulin-sensitizing activities. With additional studies, bergenin might be used as a functional drug or as an adjuvant in the management of insulin resistance and associated fatty liver disease.

KEYWORDS: high-fat diet, antioxidant, oxidative stress, bergenin, insulin sensitivity.

INTRODUCTION

Obesity has been identified as a prominent causative factor for the insulin resistance, hyperlipidemia and hyperglycemia associated with type 2 diabetes (T2DM). However, the recent increase in obesity is not to be thought due to specific congenital or hereditary defects in lipid metabolism, but to the inability of the body to cope with high energy food intake along with sedentary life style and lack of exercise. C57BL/6J mice when fed a high fat diet develop features of ectopic fat accumulation in various organs and in addition to including metabolic changes, fat exerts other deleterious effects such as generation of free radicals, oxidative stress, lipid peroxidation and antioxidant. Under these situations, the activation of endogenous antioxidants may play vital role in the early defense of the body from high-fat diet induced oxidative stress. During chronic consumption of fat, endogenous antioxidants may be overwhelmed to prevent ROS-induced damage and therefore diet-derived or supplemented antioxidants could be important to maintain health. The antioxidant property of phenolics is mainly due to their redox properties. They act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators.

Many oral medicines, including biguanide, thiazolidinedione, sulfonylurea and insulin were used for treatment of diabetes for several years. However, the usage of these agents has serious adverse effects. A large number of medicinal plants and their bioactive constituents have been used to treat diabetes. Among these the most important are flavonoids and polyphenolic compounds. They exhibit high antioxidant properties that terminate free-radical mediated reactions by donating hydrogen atom or an electron to the radicals. This aspect reinforces the idea that the dietary inclusion of natural antioxidants present in plant foods is an important disease obviating factor in humans. Bergenin is a polyphenol compound. It is dihydroisocoumarin derivative isolated from several medicinal plants such as Ficus racemosa, Mallotus japonicas, Bergenia crassifolia, Caesalpinia digyna, Astibe thunbergii, Ardisia japonica and also other genera. Bergenin contains five hydroxyl groups which are considered to be potentially active and it exhibits various biological activities such as antioxidant, hepatoprotective, antiarrhythmic, antimicrobial, antiviral and antiulcerogenic activities and anti-inflammatory. Bergenin is reported as insulin sensitiser and has potential antidiabetic effect.

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This study investigated the effects of bergenin supplementation on the following biomarkers of oxidative stress, lipid profiles and antioxidant enzymes activities in liver. Prior to this investigation, some researchers have also demonstrated that bergenin reduced blood glucose and lipid profile in the STZ-nicotinamide induced diabetic rats. Until now, however, little attention has been focus on the role of bergenin for treating T2DM. There no evidence that bergenin for treating HFD induced diabetes. To the best of our knowledge, this is the first report on bergenin ameliorates dyslipidemia in obese mice.

**MATERIALS AND METHODS**

**Chemicals**

Bergenin was purchased from Carbo synth (Compton, Berkshire, UK). All other chemicals used in this study were of analytical grade obtained from HIMEDIA, S.D. Fine chemicals and Sisco Research laboratories Pvt. Ltd (Mumbai, Maharashtra, India).

**Experimental animals**

Male C57BL/6J mice 3 weeks of age were obtained from NIN Hyderabad and housed in polypropylene cages. Animals were maintained under standard conditions with a 12h light/dark cycle. The animals received a standard pellet diet (Karnataka State Agro Corporation Ltd., Agro feeds division, Bangalore, India) and water ad libitum. After acclimatization for period of 1 week, mice were randomly divided into six groups. The animals used in the present study were cared as per the principles and guidelines of the Institutional Animal Ethical Committee (IAEC), Annamalai Nagar. The study protocols were approved by the Institutional Animal Ethics Committee of Rajah Muthiah Medical College and Hospital, Annamalainagar (Reg No. 160/1999/CPCSEA, Proposal number: 913).

**Experimental induction of diabetes**

The type 2 diabetes was induced through HFD. The standard diet which is commercially obtained from Sai Enterprises, Chennai, had a fat composition of 4.2%. The beef tallow based high fat diet was composed of protein -17.7 g, fat-35.2 g, carbohydrate-34.5 g, fibre-3.4 g, minerals - 6.8 g and vitamins- 1.8g. Mice (6 nos.) from normal control group (group I) were fed standard diet for a period of 16 weeks. Mice from rest of the groups (group II-VI) were fed high fat diet for a period of 16 weeks. At the end of 8th week, the mice from all the groups were tested for blood glucose levels. Mice with blood glucose level of 220 mg/dL and above were considered to have developed insulin resistance and were subjected to intragastric administration of various doses of bergenin and metformin (as mentioned in the experimental design) during 8th to 16th weeks.

**Diet and experimental design**

The experimental design consisted six groups (n=6) of mice. Group I: Normal control (NC) mice fed with a standard diet for 8 weeks. Group II: HFD diabetic mice fed with high fat diet for a period of 16 weeks. Group III: HFD mice fed with standard diet for 16 weeks and administered with bergenin (10 mg/kg BW) by gavage for the last 8 weeks. Group IV: HFD diabetic mice administered with bergenin (20 mg/kg BW) by gavage for the last 8 weeks. Group V: HFD diabetic mice administered with bergenin (40 mg/kg BW) by gavage for the last 8 weeks. Group VI: HFD diabetic mice administered with metformin (25mg/kg BW) by gavage for the last 8 weeks. At the end of experimental period, mice were fasted overnight and sacrificed by cervical dislocation. Blood was collected by cutting the jugular vein in the heparinized glass tubes and plasma was separated and stored at 4°C. Liver tissue was excised immediately from the mice and washed in ice-cold isotonic saline and blotted with a filter paper. A portion of the tissue was weighed, homogenized in 0.1 M Tris–HCl buffer (pH 7.4) and the homogenate was used for lipid estimations.

**Measurement of oral glucose tolerance test and insulin sensitivity index**

Oral glucose tolerance test (OGTT) was carried out on the 55th day after an overnight fast (12 hours) as described elsewhere. For this, animals were administered glucose (2 g/kg b.w, oral) after collecting fasting blood samples. Additional blood samples were drawn after one hour and two hours by sinoocular puncture in heparinized test tubes and centrifuged at 3000 x g for 10 minutes to separate plasma. Glucose was measured using a kit (Agappe Diagnostics Pvt, Ltd., Kerala, India). Plasma insulin was assayed using an enzyme-linked immunosorbent assay kit (Accubind, Monobind Chemicals, Ltd, Lake Forest, CA). Insulin sensitivity was assessed by computing insulin sensitivity index ($ISI_{0,120}$).

$$\text{ISI}_{0,120} = \frac{\text{MCR}}{\log \text{MSI}}$$

Where, MCR is Metabolic Clearance Rate, MPG = mean plasma glucose, the mean of 0 and 120 min glucose values

$$\text{MSI} = \text{Mean serum insulin (mU/L)} \text{ calculated as the mean of the 0 and 120 min insulin values.}$$

$$m = (75000\text{mg} + (0 \text{min glucose} – 120 \text{min glucose} \times 0.19 \times \text{BW} / 120 \text{min})$$

**Estimation of lipid profile**

Lipids in liver were extracted by the method of Folch and colleagues.
Total lipids, extracted with chloroform-methanol mixture (2:1 (v/v)) from liver, were evaporated to dryness and used for the estimation. Estimation of cholesterol, TG and FFAs, in liver was carried out as following procedures described earlier21.

Measurement of liver injury and oxidative stress marker
To assess the liver injury, activities of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel22, plasma alkaline phosphatase (ALP) was estimated by Kind and Kings method.23 Lipid peroxidation was evidenced by measuring the formation of thiobarbituric acid re-active substances (TBARS) lipid hydroperoxides (LHP) content in liver samples following the method of Niehaus and Samuelsson24, Jiang et al.25 respectively.

Measurement of antioxidant
Superoxide dismutase (SOD) in the liver was assayed by the method of Kakkar et al., 198426. The activity of catalase (CAT) in the liver was determined by the method of Sinha27. The activity of glutathione peroxidise (GPx) in the liver was measured by the method of Rotruck et al28. The antioxidant status in plasma and liver was evaluated by assaying the levels of non-enzymatic antioxidants such as reduced glutathione (GSH) by the method of Ellman29, vitamin E by that of Baker et al.30 and vitamin C by that of Roe and Kuether31.

Statistical analysis
The results were expressed as mean ± standard deviation (SD) for 6 mice in each group. Data were analysed by one way analysis of variance followed by Duncan’s multiple range test (DMRT) using SPSS version 16 (SPSS, Chicago,IL). Post hoc testing was performed for inter-group comparisons using the least significance difference (LSD) test; p values ≤ 0.05 were considered as significant.

RESULT

Effect of bergenin on oral glucose tolerance test and insulin sensitivity index
OGTT results (Fig. 1.) showed a rise in glucose levels in HFD-induced mice, plasma samples were collected at 0, 60 and 120 min respectively after an oral glucose challenge compared to normal control mice. All the three treatments showed improved tolerance to glucose. The level of plasma glucose is significantly (P < 0.05) increased in HFD-induced diabetic mice compared to normal control mice and those levels were improved towards near normal on treatment with bergenin (10, 20, 40 mg/kg BW). Metformin (25 mg/kg BW) treatment showed significantly decreased plasma glucose. ISI_{0-120} (Fig. 2.), a measure of insulin sensitivity, assessed using the OGTT values, was significantly decreased in HFD-induced diabetic mice compared to normal control mice. Administration of bergenin and metformin improved insulin sensitivity and showed additive improvement in ISI_{0-120} values compared to HFD. The dosage of the bergenin was selected from the dose fixation study using three different doses (10, 20, 40 mg/kg BW/day). From the results of the study (fig.1.), we found that 40mg/kg BW to be effective in lowering glucose and insulin levels among three doses.

Fig. 1. Effect of bergenin on oral glucose tolerant test (OGTT) in HFD-fed C57BL/6J mice.

Fig. 2. Effect of bergenin on insulin sensitivity index (ISI_{0-120}) in HFD-fed C57BL/6J mice.

Effect of bergenin on liver injury and oxidative stress marker
Table 1 showed increased plasma activities of AST, ALT and ALP were found in HFD induced mice, indicating damage to liver cells. Treatment of HFD induced diabetic mice with bergenin resulted in significantly (P < 0.05) lower AST, ALT, and ALP activity. These values were compared with metformin activity. Table 2 lists TBARS and LHP levels in the different groups. Significantly (P < 0.05) higher TBARS and LHP levels were found in HFD induced diabetic mice compared with control mice. In bergenin treated HFD-induced dia-
Table 1. Effect of Bergenin on AST, ALP and ALT in HFD-fed C57BL/6J mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal control</th>
<th>HFD</th>
<th>HFD+BGN (10 mg/kg BW)</th>
<th>HFD+MET (25 mg/kg BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (IU/L)</td>
<td>77.98±4.22²</td>
<td>72.48±4.22²</td>
<td>37.12±2.24⁴</td>
<td>31.66±2.31⁴</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>145.45±8.87⁴</td>
<td>130.57±8.01¹</td>
<td>85.69±5.7⁶</td>
<td>83.66±5.9⁶</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>126.33±10.8²</td>
<td>130.57±10.8²</td>
<td>68.47±10.8²</td>
<td>66.33±10.8²</td>
</tr>
</tbody>
</table>

Values are means ± SD for six samples from 6 mice in each group. Values not sharing a common superscript differ significantly at p < 0.05. Duncan’s Multiple Range Test (DMRT).

Table 2. Effect of Bergenin on TBARS and LHP in the liver of HFD-fed C57BL/6J mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal control</th>
<th>HFD</th>
<th>HFD+BGN (10 mg/kg BW)</th>
<th>HFD+MET (25 mg/kg BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS</td>
<td>0.96±0.06⁶</td>
<td>3.25±0.18⁴</td>
<td>1.66±0.09⁶</td>
<td>1.21±0.07⁶</td>
</tr>
<tr>
<td>LHP</td>
<td>79.33±4.78⁸</td>
<td>1414.21±9.11³</td>
<td>93.53±7.03³</td>
<td>82.51±5.55³</td>
</tr>
</tbody>
</table>

Values are means ± SD for six samples from 6 mice in each group. Values not sharing a common superscript differ significantly at p < 0.05. Duncan’s Multiple Range Test (DMRT).

Effect of bergenin on antioxidant levels

Table 3 depicted the activities of enzymatic and non-enzymatic antioxidants in the liver of the different groups. The activities of enzymatic antioxidants SOD, CAT, GPx were significantly (P < 0.05) lowered in HFD induced diabetic mice. The GSH activities, as well as vitamin C and E levels, were significantly (P < 0.05) decreased in HFD-induced diabetic mice compared with controls. In bergenin treated HFD-induced diabetic mice, these parameters returned to normal levels.

Table 3. Effect of Bergenin on the activities of enzymatic and non enzymatic antioxidant in the liver of HFD-fed C57BL/6J mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal control</th>
<th>HFD</th>
<th>HFD+BGN (40 mg/kg BW)</th>
<th>HFD+MET (25 mg/kg BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD(U*/mg protein)</td>
<td>9.33±0.78⁸</td>
<td>8.15±0.36³</td>
<td>7.04±0.36³</td>
<td>6.92±0.36³</td>
</tr>
<tr>
<td>CAT(U*/mg protein)</td>
<td>82.24±4.86⁶</td>
<td>36.97±2.43⁶</td>
<td>35.16±2.43⁶</td>
<td>33.97±2.43⁶</td>
</tr>
<tr>
<td>GPX(U*/mg protein)</td>
<td>2.15±0.66³</td>
<td>4.01±0.34⁴</td>
<td>3.94±0.34⁴</td>
<td>3.87±0.34⁴</td>
</tr>
<tr>
<td>GSH (µg/mg protein)</td>
<td>12.43±0.88⁸</td>
<td>7.48±0.44⁴</td>
<td>9.23±0.56⁶</td>
<td>10.64±0.69⁶</td>
</tr>
<tr>
<td>Vitamin C (µg/mg protein)</td>
<td>1.59±0.09⁹</td>
<td>0.63±0.02⁴</td>
<td>0.92±0.05⁴</td>
<td>1.01±0.07⁴</td>
</tr>
<tr>
<td>Vitamin E (µg/mg protein)</td>
<td>7.23±0.57⁷</td>
<td>5.29±0.31⁷</td>
<td>5.29±0.31⁷</td>
<td>5.29±0.31⁷</td>
</tr>
</tbody>
</table>

Effect on liver lipid profiles

Hepatic cholesterol, TG and FFA levels are given in table 4 respectively. HFD-induced diabetic mice showed increased cholesterol, TG, and FFA. These were reduced effectively and significantly (P< 0.05) after treatment with bergenin in liver compared to HFD-induced diabetic mice. These values were comparable to those of metformin (25 mg/kg BW).

Table 4. Effect of Bergenin on Lipid profiles in the liver of HFD-fed C57BL/6J mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal control</th>
<th>HFD</th>
<th>HFD+BGN (40 mg/kg BW)</th>
<th>HFD+MET (25 mg/kg BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/g wet tissue)</td>
<td>11.53±0.68³</td>
<td>26.61±1.87⁴</td>
<td>19.27±1.04³</td>
<td>15.83±0.98³</td>
</tr>
<tr>
<td>TG (mg/g wet tissue)</td>
<td>10.6±0.9³</td>
<td>22.78±1.7⁴</td>
<td>15.16±0.9⁴</td>
<td>11.29±0.82⁴</td>
</tr>
<tr>
<td>FFA (mg/g wet tissue)</td>
<td>12.2±0.7²</td>
<td>26.19±1.5⁶</td>
<td>20.96±1.6⁶</td>
<td>18.61±1.0⁶</td>
</tr>
</tbody>
</table>

Values are means ± S.D. of 6 mice from each group. Values not sharing a common superscript differ significantly at p < 0.05 (DMRT)

DISCUSSION

In the present study, bergenin restored metabolic parameters, improved insulin sensitivity, reduced oxidative stress, lipid accumulation and enhanced antioxidative potential when feeding mice with HFD diet. Increased fat consumption resulted in significant elevation of blood glucose, hyperinsulinemia and the altered values ISI<sub>0,120</sub> suggest decline in whole body insulin sensitivity in HFD-fed mice in the present study. Oral administration with bergenin showed antihyperglycemic and antihyperinsulinimic effects in diabetic mice comparable to those of metformin, an antidiabetic drug. Kumar et al.,<sup>17</sup> also reported that bergenin has an antidiabetic activity in streptozotocin – nicotinamide induced diabetic rats. HFD administration was coupled with microvesicular steatosis and hepatocellular damage. The amplified activities of marker enzymes, AST, ALP and ALT are reminiscent of liver injury. Treatment with bergenin notably prevented the elevation of these enzymes to an extent that was comparable to the standard drug metformin. Bergenin prevents liver cell damage and preserves cell integrity possibly leading to survival of the functionally active cells. These results are in streak with the findings reported by Lim et al.,<sup>10</sup> who observed the hepatoprotective action of bergenin in CCl<sub>4</sub>-treated rats. HFD diet induced the changes in liver function parameters reported hepatocellular damage and reduced synthetic capacity of the liver. Liver damage was confirmed by increased levels of lipid peroxidative markers<sup>12</sup>. HFD diet is associated with increased ROS production and enhanced markers of oxidative stress in several tissues. Elevated levels of TBARS and LHP in
the liver of HFD mice revealed a clear manifestation of the excessive formation of free radicals and the activation of the lipid peroxidation system. Hydroxyl radicals, upon reaction with the polyunsaturated fatty acids of the biomembranes, yield LHPs, which can initiate a chain of reaction resulting in oxidative damage to tissue lipids. Marked increase in these substances in HFD mice is consistent with a previous report that HFD induces oxidative damage in the liver. The association between fat consumption and oxidative stress is not simple, because it involved in the catabolism of fatty acids with a number of biochemical mechanisms which yield H₂O₂ as a byproduct. In addition, increased oxygen consumption will generate other oxidant molecules. The main enzymes responsible for the antioxidant response are catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) which acts in synchrony to protect cells against oxidant molecules. Therefore, modulation of these enzymes in the cellular antioxidant system may protect against oxidative stress. SOD induces dismutation of the superoxide radical to hydrogen peroxide, and catalase/GSH-GPx converts hydrogen peroxide into water. Non-enzymatic antioxidants such as GSH, vitamins C and E are closely interlinked to each other and participate to form an antioxidant network. This helps to regenerate one another from their oxidized forms there by playing an excellent role in protecting the cells from oxidative damage. GSH is one of the most important intracellular antioxidants which present in high concentration and it reflects the detoxification potential in the liver. Depletion of enzymatic and nonenzymatic antioxidants in the liver tissue of HFD induced mice has been reported earlier. The reduction could be attributed to the increased utilization of these antioxidants by hepatocytes in an attempt to counteract the increased formation of lipid peroxides. The administration of bergenin increased the antioxidant potential and also reduced the formation of lipid peroxidation end products. The medicinal effects of bergenin, a polyphenolic antioxidants against oxidative stress-mediated disorders are mostly ascribed to their free radical scavenging action, chelation of redox active metal ions. Nazir et al., have reported that bergenin has strong antioxidant properties by scavenging the free radicals. Excessive fat accumulation leads to hyperlipidemia and other obesity-related metabolic disorders. Hyperlipidemia is a disorder characterized by increase in blood lipoprotein or cholesterol levels. Abnormalities in circulating plasma lipoproteins account for the increased depots of TG in liver. Accumulation of TG, a chief matrix of the lipid droplets in hepatocytes, is accelerated under conditions such as insulin resistance, if left without appropriate treatment. It is well known that under normal situation insulin activates the enzyme lipoprotein lipase which hydrolyses the TG. Insulin deficiency results in the failure to activate the enzymes thereby resulting in hypertriglyceridemia. Increased retention of lipids in the hepatocytes, mostly in the form of TC, TG and FFA, is known to be the common early trait of fatty liver. Levels of TC, TG, FFA and phospholipids were significantly elevated in liver in the HFD fed mice. In the present study, treatment with bergenin to HFD induced mice significantly decreased the plasma and liver lipid levels. Bergenin has favorable effects on the lipoprotein profile. In previous study, Jahromi et al., reported that extracted bergenin from Flueggea microcarpa has also possess lipid lowering activity.

CONCLUSION

In HFD induced diabetic mice, bergenin has the potential to decrease oxidative stress by declining lipid peroxidation levels through its antihyperglycemic, antihyperlipidemic and antioxidant properties. Further investigations on the molecular signals underlying the mechanism of action of bergenin are yet to be explored.

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