Effect of mangiferin on hyperglycemia and atherogenicity in streptozotocin diabetic rats

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Received 8 March 2004; received in revised form 3 November 2004; accepted 7 December 2004

Abstract

In the present study, the effect of mangiferin (a xanthone glucoside, isolated from the leaves of Mangifera indica) on the atherogenic potential of streptozotocin (STZ)-diabetes was investigated. In addition, the effect of mangiferin on oral glucose tolerance in glucose-loaded normal rats was also determined. The chronic intraperitoneal (i.p.) administration of mangiferin (10 and 20 mg/kg) once daily (o.d.) for 28 days exhibited antidiabetic activity by significantly lowering fasting plasma glucose level at different time intervals in STZ-diabetic rats. Further, mangiferin (10 and 20 mg/kg, i.p.) showed significant antihyperlipidemic and antiatherogenic activities as evidenced by significant decrease in plasma total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C) levels coupled together with elevation of high-density lipoprotein cholesterol (HDL-C) level and diminution of atherogenic index in diabetic rats. In addition, the chronic administration of mangiferin (10 and 20 mg/kg, i.p.) for 14 days significantly as well as markedly improved oral glucose tolerance in glucose-loaded normal rats suggesting its potent antihyperglycemic activity. The accumulating evidences suggest that both pancreatic and extrapancreatic mechanisms might be involved in its antidiabetic or antihyperglycemic action. In conclusion, the present study demonstrates that mangiferin possesses significant antidiabetic, antihyperlipidemic and antiatherogenic properties thus suggesting its beneficial effect in the treatment of diabetes mellitus associated with hyperlipidemia and related cardiovascular complications.

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Keywords: Mangiferin; Streptozotocin; Atherogenic; Diabetic

1. Introduction

Mangiferin, a xanthone glucoside, is an active phytochemical present in various plants including Mangifera indica (Chopra et al., 1956). Mangiferin has been reported to possess antioxidant (Sanchez et al., 2000), antitumor (Guha et al., 1996), antiviral (Zheng and Lu, 1990; Zhu et al., 1993; Yoosook et al., 2000) and immunomodulatory activities (Guha et al., 1996; Leiro et al., 2004). The aqueous extract of Mangifera indica leaves has been reported to possess hypoglycemic activity in glucose-induced hyperglycemic rats as well as in mice (Aderibigbe et al., 1999, 2001). Furthermore, the active principle, mangiferin has recently been shown to have antidiabetic activity in KK/Ay mice, a genetic model of non-insulin-dependent diabetes mellitus (NIDDM) with hyperinsulinemia (Ichiki et al., 1998; Miura et al., 2001a, b). However, there is no experimental evidence presently available in the literature with regard to its effect on plasma glucose and lipid profiles (atherogenic value) in streptozotocin-induced diabetes mellitus in rats. Hence, the present study was carried out in an attempt to investigate the possible antidiabetic and antiatherogenic activities of mangiferin, isolated from the leaves of Mangifera indica in STZ-diabetic rats. In addition, the effect of mangiferin on
oral glucose tolerance in glucose-loaded normal rats was also studied.

2. Materials and methods

2.1. Mangiferin

The method of isolation and determination of purity of mangiferin can be found from our earlier report (Muruganandan et al., 2002). Briefly, shade-dried and powdered leaves of Mangifera indica were soxhleted for 12 h with petroleum ether (60–80 °C). The defatted plant material was extracted with ethanol under reflux for 16 h, which was further, defatted again with petroleum ether and crystallized repeatedly in aqueous ethylacetate into pale yellow needles. The purity was 95.56% mangiferin upon high performance liquid chromatographic (HPLC) analysis using PDA detector (254 nm) and a mobile phase of acetonitrile and 3% acetic acid (16:84) as reported earlier (Muruganandan et al., 2002).

2.2. Chemicals

Streptozotocin (Sigma Chemicals, St. Louis, MO, USA), heparin (SRL, Mumbai), insulin (highly purified bovine porcine insulin, Eli-Lilly Ranbaxy, New Delhi) were used in the study. Mangiferin was the isolated compound from Mangifera indica leaves as described above.

2.3. Experimental animals

Wistar male rats (100–125 g) were procured from the Laboratory Animal Resource Section of Indian Veterinary Research Institute (IVRI), Izatnagar, UP, India. The animals were maintained on standard ration and provided with clean drinking water ad lib. The animals were kept in air-conditioned room (temperature 22 ± 2 °C) and acclimatized for a period of 7 days.

2.4. Induction of experimental diabetes

Fasted male Wistar rats (100–125 g) were rendered diabetic by the single injection of STZ (55 mg/kg, i.v.) through tail vein. STZ was dissolved in ice-cold citrate buffer (pH 4.5) and injected immediately within few minutes to avoid degradation. The development of hyperglycemia in rats was confirmed by plasma glucose estimation 48 h post STZ injection. The rats with fasting plasma glucose level of above 250 mg/dl at 30 days post STZ injection were considered diabetic and only uniformly diabetic rats were included in the study.

2.5. Experimental design

The experiment was designed to determine the effect of mangiferin on hyperglycemia and atherogenesis in streptozotocin-induced uniformly diabetic rats.
2.7. Biochemical analysis

The plasma was separated from heparinized blood samples immediately after collection and analyzed for glucose, total cholesterol, HDL-C and triglycerides using standard enzymatic colorimetric kits obtained from M/s Qualigens Diagnostics, Mumbai, India. Plasma glucose was estimated by O-toluidine method. Total cholesterol and triglycerides were estimated by enzymatic methods of CHOD-PAP and GPO-PAP, respectively. LDL-C was calculated by using the following formula (Noda et al., 2000):

\[
LDL-C = \text{total cholesterol} - \left( \frac{\text{triglycerides}}{5} \right) \text{HDL-C}
\]

Atherogenic index was calculated by using the following formula (Kayamori and Igarashi, 1994):

\[
\text{atherogenic index} = \left( \frac{\text{total cholesterol} - \text{HDL-C}}{\text{LDL-C}} \right)
\]

2.8. Statistical analysis

The results were expressed as mean ± S.E.M. All the data were analyzed by one way analysis of variance followed by multiple comparison test (Tukey's test) at the 5% level of significance. A value of \( P < 0.05 \) was considered statistically significant.

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Days</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>0</td>
<td>75.92 ± 2.86</td>
<td>320.84 ± 28.24</td>
<td>320.97 ± 32.78</td>
<td>323.05 ± 23.41</td>
<td>305.21 ± 19.92</td>
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<tr>
<td></td>
<td>14</td>
<td>76.01 ± 9.81</td>
<td>324.58 ± 19.85</td>
<td>112.49 ± 5.67</td>
<td>180.03 ± 13.81</td>
<td>160.67 ± 12.62</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>72.72 ± 3.90</td>
<td>329.79 ± 14.19</td>
<td>107.04 ± 7.89</td>
<td>165.66 ± 16.18</td>
<td>158.65 ± 8.45</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>0</td>
<td>76.51 ± 11.87</td>
<td>127.61 ± 13.34</td>
<td>118.70 ± 20.12</td>
<td>126.50 ± 13.60</td>
<td>131.11 ± 16.96</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>75.51 ± 4.59</td>
<td>110.5 ± 11.40</td>
<td>98.19 ± 5.70</td>
<td>93.70 ± 7.73</td>
<td>86.57 ± 9.25</td>
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<tr>
<td></td>
<td>28</td>
<td>85.3 ± 5.78</td>
<td>131.5 ± 8.39</td>
<td>68.58 ± 4.25</td>
<td>80.88 ± 4.00</td>
<td>82.07 ± 3.27</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>0</td>
<td>122.49 ± 2.06</td>
<td>207.92 ± 7.15</td>
<td>206.53 ± 11.59</td>
<td>203.46 ± 4.96</td>
<td>215.40 ± 9.13</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>138.52 ± 13.23</td>
<td>226.78 ± 8.58</td>
<td>136.34 ± 9.45</td>
<td>144.98 ± 9.99</td>
<td>123.22 ± 6.64</td>
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<tr>
<td></td>
<td>28</td>
<td>124.00 ± 6.80</td>
<td>296.91 ± 23.60</td>
<td>115.80 ± 13.31</td>
<td>128.50 ± 7.45</td>
<td>128.50 ± 9.57</td>
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<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>0</td>
<td>57.06 ± 10.12</td>
<td>141.05 ± 3.15</td>
<td>142.75 ± 17.13</td>
<td>142.40 ± 10.34</td>
<td>143.17 ± 3.71</td>
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<td></td>
<td>14</td>
<td>71.68 ± 13.83</td>
<td>160.19 ± 7.04</td>
<td>80.28 ± 9.02</td>
<td>89.86 ± 12.19</td>
<td>57.94 ± 8.99</td>
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<td></td>
<td>28</td>
<td>52.79 ± 5.61</td>
<td>230.28 ± 16.60</td>
<td>57.80 ± 14.51</td>
<td>69.09 ± 15.44</td>
<td>64.45 ± 8.36</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>0</td>
<td>49.99 ± 0.95</td>
<td>41.44 ± 1.91</td>
<td>39.48 ± 1.26</td>
<td>41.65 ± 0.73</td>
<td>42.19 ± 1.21</td>
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<tr>
<td></td>
<td>14</td>
<td>54.94 ± 2.11</td>
<td>43.57 ± 2.87</td>
<td>44.76 ± 2.19</td>
<td>46.19 ± 3.59</td>
<td>53.36 ± 3.63</td>
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<tr>
<td></td>
<td>28</td>
<td>53.86 ± 3.48</td>
<td>37.43 ± 1.06</td>
<td>44.93 ± 1.67</td>
<td>46.61 ± 1.15</td>
<td>48.90 ± 0.84</td>
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<tr>
<td>Atherogenic index (units)</td>
<td>0</td>
<td>1.59 ± 0.23</td>
<td>4.03 ± 0.28</td>
<td>4.16 ± 0.26</td>
<td>3.88 ± 0.09</td>
<td>4.05 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>1.5 ± 0.10</td>
<td>4.27 ± 0.26</td>
<td>2.09 ± 0.28</td>
<td>2.13 ± 0.46</td>
<td>2.46 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>1.26 ± 0.12</td>
<td>4.82 ± 0.70</td>
<td>1.67 ± 0.18</td>
<td>1.74 ± 0.09</td>
<td>1.70 ± 0.10</td>
</tr>
</tbody>
</table>

n = 6/group. Values are mean ± S.E.M. Group 1: normal control (normal saline 1 ml/kg); Group 2: diabetic control; Group 3: positive control (diabetic + insulin 6U/kg); Group 4: diabetic + mangiferin (10mg/kg); Group 5: diabetic + mangiferin (20mg/kg).

* \( P < 0.05 \), Group 1 vs. Group 2.

^ \( P < 0.05 \), treated diabetic groups vs. diabetic control group.

3. Results

Streptozotocin treatment caused significant weight reduction in rats as compared to the vehicle treated normal rats at day 30 of injection (163.33 ± 10.54 g versus 206.67 ± 13.18 g). However, the chronic treatment of mangiferin (10 and 20 mg/kg, i.p.) for 28 days significantly (\( P < 0.05 \)) restored the body weight loss as compared to the vehicle treated diabetic control rats observed at the end of 28 days of treatment period (191.67 ± 15.35 g and 200 ± 10.72 g versus 130 ± 5.16 g, respectively). However, the standard drug insulin (6 U/kg, i.p.) also exhibited significant improvement in body weight loss of the diabetic animals following 28 days of treatment (196.67 ± 8.37 versus 130 ± 5.16 g). Streptozotocin treatment resulted in significant elevation of plasma glucose, triglycerides, total cholesterol, LDL-C and reduction in HDL-C levels as compared to the normal control rats as noted at different periods of the study (Table 1).

The chronic administration of mangiferin (10 and 20 mg/kg, i.p.) resulted in significant (\( P < 0.05 \)) reduction in plasma glucose level at different periods in the experimental duration of 28 days in STZ-diabetic rats with the maximum percent reduction of plasma glucose being 49.77 and 51.89, respectively, on 28th day of treatment. However, the standard drug insulin (6 U/kg, i.p.) exhibited significant and more potent antidiabetic activity with maximum percent reduction of plasma glucose 67.54 on 28th day as compared to the diabetic control group (Table 1).

There was a significant reduction in plasma triglycerides, total cholesterol and LDL-C levels of diabetic rats treated...
with mangiferin (10 and 20 mg/kg, i.p.) as comparable to insulin (6 U/kg, i.p.) at various time intervals (Table 1). However, there was a significant ($P < 0.05$) elevation in the HDL-C level in mangiferin (10 and 20 mg/kg, i.p.) treated diabetic rats on 28th day as compared to the diabetic control group ($P < 0.05$) elevation in the HDL-C level in mangiferin (10 and 20 mg/kg, i.p.) treated diabetic rats indicating its potent antidiabetic activity. In our previous experiment as compared to the vehicle treated diabetic control group, we showed that the chronic treatment of mangiferin (10 and 20 mg/kg, i.p.) caused significant as well as moderate reduction in the glycosylated hemoglobin levels in STZ-diabetic rats further substantiating its potential in the long term glycemic control of diabetes mellitus (Muruganandan et al., 2002). Since STZ (55 mg/kg, i.v.) effectively destroys pancreatic $\beta$ cells and causes persistent hyperglycemia, the mechanism of antidiabetic action of mangiferin might involve actions other than pancreatic $\beta$ cells insulin release/secrection (insulinotropic effect), i.e. possibly through other extrapancreatic actions in these STZ-diabetic rats (Bwititi et al., 2000; Jouad et al., 2004). The extrapancreatic actions perhaps might include the stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic processes with concomitant decrease in glycogenolysis and gluconeogenesis (Saxena and Vikram, 2004). Furthermore, it is also likely that it might reduce blood glucose level by inhibiting the glucose absorption from the intestine. The latter hypothesis could be supported by the recent findings that mangiferin inhibits $\alpha$-glucosidase enzymes (sucrase, isomaltase, maltase) (Yoshikawa et al., 2001) which are involved in the digestion of carbohydrate into simple sugars in the gut leading to delay or inhibition of carbohydrate breakdown and subsequent glucose absorption from the intestine (Emelien et al., 1999).

Although, in the present study mangiferin was administered intraperitoneally, the inhibition of $\alpha$-glucosidase enzymes by the mangiferin excreted through bile into gut, i.e. through enterohepatic circulation cannot be ruled out. Also, in our preliminary investigation, we found that both the single and chronic administration of mangiferin did not have any significant effect on the basal fasting plasma glucose level in normal rats (data not shown). Nevertheless, chronic administration of mangiferin (10 and 20 mg/kg, i.p.) significantly improved oral glucose tolerance in glucose-loaded normal rats indicating its potent antihyperglycemic activity. This result is in accordance with the previous results conducted with the aqueous extract of Mangifera indica leaves further suggesting that the active principle, mangiferin might be responsible for the glucose lowering action on oral glucose tolerance test (Aderibigbe et al., 1999, 2001). These evidences tempt us to speculate that apart from the aforementioned probable insulin independent extrapancreatic actions, the other possible pancreatic mechanism, i.e. stimulating insulin release from the pancreatic $\beta$ cells might contribute in improving oral glucose tolerance in the glucose-loaded normal rats. Taken together, it can be summarized as mangiferin might possess both pancreatic and extrapancreatic mechanisms in its antidiabetic action and such apparent dual pancreatic and extrapancreatic actions of mangiferin would be more advantageous to the existing oral antidiabetic monotherapy.

In our study, STZ (55 mg/kg, i.p.) treated diabetic rats exhibited clear-cut abnormalities in lipid metabolism as evidenced from the significant elevation of plasma total cholesterol, triglycerides, LDL-C, atherogenic index and reduction of HDL-C levels. Treatment with mangiferin (10 and 20 mg/kg, i.p.) for 28 days significantly and greatly reduced study, we showed that the chronic treatment of mangiferin (10 and 20 mg/kg, i.p.) caused significant as well as moderate reduction in the glycosylated hemoglobin levels in STZ-diabetic rats further substantiating its potential in the long term glycemic control of diabetes mellitus (Muruganandan et al., 2002). Since STZ (55 mg/kg, i.v.) effectively destroys pancreatic $\beta$ cells and causes persistent hyperglycemia, the mechanism of antidiabetic action of mangiferin might involve actions other than pancreatic $\beta$ cells insulin release/secrection (insulinotropic effect), i.e. possibly through other extrapancreatic actions in these STZ-diabetic rats (Bwititi et al., 2000; Jouad et al., 2004). The extrapancreatic actions perhaps might include the stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic processes with concomitant decrease in glycogenolysis and gluconeogenesis (Saxena and Vikram, 2004). Furthermore, it is also likely that it might reduce blood glucose level by inhibiting the glucose absorption from the intestine. The latter hypothesis could be supported by the recent findings that mangiferin inhibits $\alpha$-glucosidase enzymes (sucrase, isomaltase, maltase) (Yoshikawa et al., 2001) which are involved in the digestion of carbohydrate into simple sugars in the gut leading to delay or inhibition of carbohydrate breakdown and subsequent glucose absorption from the intestine (Emelien et al., 1999).

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plasma total cholesterol, TG and LDL-C associated with concomitant significant increase in HDL-C levels and decrease in atherogenic index in diabetic rats indicating its potent anti-hyperperlipidemic and antithrombogenic activity. The glucose lowering activity of the mangiferin can be due to the consequence of an improved lipid metabolism apart from the direct interaction of glucose homoeostasis. The triglycerides lowering property of mangiferin could indirectly contribute to the over all antihyperglycemic activity through a mechanism of so-called glucose–fatty acid cycle (Randle et al., 1963). According to the Randle’s glucose–fatty acid cycle increased supply of plasma triglycerides per se could constitute a source of increased free fatty acid (FFA) availability and oxidation that can impair insulin action, glucose metabolism and utilization leading to development of hyperglycemia. Therefore, the reduction of triglycerides following treatment with mangiferin would also facilitate the glucose oxidation and utilization and subsequently the reduction of hyperglycemia. In our earlier study, we found that mangiferin significantly reduced malondialdehyde (MDA) level, a marker of lipid peroxidation in different organs viz., heart, liver and kidney by ameliorating changes in the antioxidant enzymes indicating its possible antioxidant activity which is advantageous in treatment of diabetic complications (Muruganandan et al., 2002).

In conclusion, the present study demonstrates that mangiferin (10 and 20 mg/kg, i.p.) at the dose levels tested exhibits potent antidiabetic, antihyperlipidemic and antithrombogenic activities in STZ-diabetic rats and also shows the improvement in oral glucose tolerance in glucose-loaded normal rats without inducing hypoglycemic state. The agent with these multiple advantageous properties viz., antidiabetic, antihyperlipidemic, antithrombogenic and antioxidant properties without causing hypoglycemia would be of greater therapeutic benefit in the management of DM complications in diabetes mellitus. Lancet 1, 785–789.

References


