Herbal Anti-Hyperglycemic Compound Improves Glycemic Control and Insulin Sensitivity in Diabetic Rats

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Abstract

Objective: To study the effect of HPLC purified herbal anti-hyperglycemic active compound (FIIc) isolated from the fruit pulp of Eugenia jambolana in diabetic rats.

Methods: 24 male wistar rats were taken and diabetes was induced in group B, C and D rats (n = 6 each) by injecting Streptozotocin at a dose of 45 mg/kg of body weight 15 minutes after Nicotinamide at a dose 230 mg/kg body weight intraperitoneally after overnight fasting. Active compound (FIIc) was given to group C and Pioglitazone to group D at dose of 20 mg/kg of body weight orally for 4 weeks respectively. Glycemic and lipid profile, protein tyrosine kinase activity and serum DPP-4 levels were measured and compared between all the 4 study groups.

Results: Significant Improvement in body weight, glycemic profile, dyslipidemia and tyrosine kinase activity (4.90 ± 1.28 U/mg protein) were observed in FIIc treated rats at week 4 of the study compared to diabetic control rats. Serum DPP-4 levels (26.41 ± 0.43 pg/ml) were also found to be decreased in FIIc treated rats at week 4 of the study compared to diabetic control rats. This is possibly due to increased serum insulin levels and increased insulin sensitivity after treatment with active compound FIIc.

Conclusion: FIIc significantly reduced hyperglycemia and dyslipidemia by inhibiting DPP-4 levels and improves insulin sensitivity by increasing protein tyrosine kinase activity and serum insulin levels.

Keywords: Eugenia jambolana; FIIc; Diabetes; Tyrosine kinase; Dipeptidyl Peptidase 4; Pioglitazone; Wistar rats; HPLC

Introduction

Type 2 Diabetes mellitus is a disorder of carbohydrate metabolism which is characterized by a combination of peripheral insulin resistance and impaired insulin secretory capacity of pancreatic beta cell. In due course of time when it is unable to maintain sufficient hyper-insulinemic response, overt diabetes ensues. Modern synthetic medicines have several side effects compared to herbal drugs, due to which the popularity of traditional and complimentary medicines has increased[1].

The anti-hyperglycemic activity of Eugenia jambolana (Botanical name- Syzgium cumini) from its seeds, fruit pulp, bark and roots has been well established[2-5]. It has also known to have several anti diabetic compounds such as ferulic acid, cuminoside, α-hydroxy succinamic acid, stigma sterol, β-sitosterol, lupeol, ellagic acid, gallic acid, quercetin and kempferol[6]. Attenuation of renal dysfunction by anti-hyperglycemic compound isolated from fruit pulp of Eugenia jambolana in Streptozotocin-induced diabetic rats has also been reported[7]. The fruit extract (250 mg/kg) lowered glucose in overnight fasted streptozotocin induced diabetic rats; the treatment increased glycogen content in liver, induced degranulation in β-cells. The fruit may stimulate insulin secretion. The water extract of the fruit pulp also showed anti-hyperglycemic activity in normal and alloxan-induced diabetic rats[8].

Sharma et al has already isolated the active antihyperglycemic compound known as alpha hydroxy succinamic acid (FIIc) (US Patent number 6,426,826 dated 6th August 2002; Indian Product Patent number. 2,30,753 February 2009) from the fruit pulp of Eugenia jambolana[8].

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Incretin hormones such as GLP 1 and GIP have shown to control post prandial glucagon release, delay gastric emptying and increase satiety[9,10]. It has also been reported to stimulate insulin secretion[11,12], stimulate beta cells proliferation and differentiation[13,14]. However, GLP 1 has a very short half-life. It is rapidly degraded inside our body by the enzyme Dipeptidyl Peptidase 4 (DPP-4). Therapeutic agents that can block the DPP-4 enzyme (DPP-4 inhibitors) can increase the endogenous GLP-1 level and thus enhances the incretin action. Dipeptidyl Peptidase inhibitors, which act via enhancing the incretins represents another new therapeutic approach for the treatment of type 2 diabetes.

Glucose uptake in peripheral tissues is also stimulated by Insulin receptor tyrosine kinase and under pathologic conditions may result in a range of clinical manifestations including diabetes[15,16]. In a recent study it was shown that when non-obese diabetic mouse treated with tyrosine kinase inhibitors showed improved glycemic control and increased insulin sensitivity[17,18]. Hence, the development of tyrosine kinase inhibitors offers increasing opportunities for the treatment of various diseases including diabetes.

The present study was therefore planned to evaluate the effect of purified active principle (FIIc) on Streptozotocin-Nicotinamide induced diabetic rats and to ascertain its mechanism of action by evaluating its effect on DPP-4 levels and tyrosine kinase activity.

Material and Methods

Isolation and purification of active anti-hyperglycemic compound (FIIc)

Preparation of crude aqueous extract: Fresh fruit pulp of Eugenia jambolana was grinded in a mixer grinder with distilled water (500 mL). After filtration through muslin cloth, the filtrate was centrifuged at 10,000 rpm at 4°C and then lyophilized to store it for a longer duration. The yield of lyophilized water extract was about 10 g from 650 g of fruit pulp, which was obtained from 1 kg fruits of E. jambolana.

Ion exchange chromatography was carried out for the isolation and purification of active compound (FIIc) using diethyl amino ethyl cellulose- 52 (DEAE-52) as the stationary phase. Fractions were then eluted with 0.1 M phosphate buffer (pH 6.0). A total of four fractions (FI to FIV) were obtained out of which FII has the significant high anti-hyperglycemic activity among the four fractions. FII was again subjected to Ion exchange chromatography and active compound (FIIc) was obtained. The US and Indian patents have already been granted[8].

Chemical characterization of active compound (FIIc): Homogeneity of FIIc was confirmed by HPL-C which gave a single peak after employing it on chromolith column (chromolith performance HPL-C column RP-18E 100 – 4.6 mm). FIIc was eluted with mobile phase (Water : Methanol : Acetonitrile :: 70 : 15 : 15) and monitored by PDA detector at wavelength 220 nm (Instrument Shimadzu HPL-C model SPD-M20A). A single peak was observed in chromatogram, suggesting that FIIc was almost homogenous as demonstrated in previous studies done by Sharma et al[8].

Experimental animals: Male Wistar albino rats (weighing 220 - 250 grams) were procured from Central Animal House of University College of Medical Sciences (UCMS), University of Delhi, India. The animals were housed in standard conditions of temperature (22 ± 2°C) and at 12 hour light-dark cycle. The rats were fed with commercial diet (Hindustan liver Ltd., Mumbai) and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC), UCMS, Delhi, India (UCMS/IAEC/26 granted on 30th December 2009).

Induction of diabetes: To induce diabetes, a freshly prepared solution of streptozotocin (45 mg/kg of body weight in 0.1 M citrate buffer, pH 4.5) was injected intraperitoneally to overnight fasted rats. Nicotinamide at a dose of 230 mg/kg body weight was given 15 minutes prior to STZ injection for the development of stable type 2 diabetes mellitus[9]. After 48 hours of STZ administration, fasting blood glucose (FBG) levels were measured.

Study design: Male Wistar rats were taken for the present study and divided into following groups (6 rats each group).

Group A: Healthy control (normal saline)
Group B: Diabetic control (normal saline)
Group C: Diabetic treated with FIIc (20 mg/kg of body weight)
Group D: Diabetic treated with Pioglitazone (20 mg/kg of body weight)

Animals in group A and B were given standard chow diet. Purified active compound (FIIc) was given orally to group C at a dose of 20 mg/kg of body weight day for 4 weeks, which is the effective dose of FIIc standardized in previous studies done by Sharma et al[9]. Pioglitazone was given as a standard drug orally at a dose of 20 mg/kg of body weight /day for 4 weeks to group D. An equal volume of vehicle was given to healthy control group A and diabetic untreated group B.

Glycemic parameters and hormonal assays: Blood was drawn from retro orbital plexus by using micro-capillary technique from all overnight fasted animals on day 1 and afterwards at week 4 of the study. Whole blood was drawn for the estimation of Glycosylated hemoglobin and plasma was separated from blood for the estimation of Glucose. Serum was separated for the estimation of lipids, Insulin and Dipeptidyl Peptidase-4. These samples were carefully processed and stored in -80°C deep freezer. These parameters were measured using commercially available kits: plasma glucose (Fortress diagnostics, United Kingdom), HbA1c (Fort-
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tress diagnostics, United Kingdom), total serum cholesterol (Fortress diagnostics, United Kingdom), serum triglycerides (Fortress diagnostics, United Kingdom), HDL-C (Fortress diagnostics, United Kingdom), Insulin (Mercodia rat ELISA kit, Sweden), tyrosine kinase (Bio medical Assay rat ELISA kit, China) and DPP-4 (Bioassay technology laboratory rat ELISA kit, China) respectively. Each time quality control sera (Bio-Rad, USA) were run along with the samples. Results of the unknown sample and quality control sera were reproducible.

Insulin Receptor (IR)-binding assay: After 4 weeks of treatment rats were sacrificed and their organs including liver and adipose tissues were dissected for the estimation of protein tyrosine kinase activity. These tissues were collected and solubilized by lysis buffer. IR-binding assay was performed using Rayto 2100c microplate ELISA reader (Rayto, China). The amount of phosphorylated IR was quantified by sandwich enzyme-linked immunosorbent assay (ELISA). The absorbance was measured at 450 nm through ELISA plate reader.

Statistical analysis: Two way ANOVA was applied for the comparison of parameters between the groups followed by Tukey’s test. Pearson’s coefficient of correlation was calculated for all the 3 groups together and separately for all the above mentioned parameters. Difference was assumed to be significant at the level of $p < 0.05$.

Results

Effect of FIIc on glycemic index

In the present study a significant improvement was observed in body weight, glycemic and lipid profile in FIIc treated rats at week 4 of the study compared to diabetic controls. Serum insulin and HDL-C were found to be significantly increased in FIIc treated rats at week 4 of the study compared to diabetic controls (Table 1).

Table 1: Showing body weight, glycemic, lipid and hormonal profile at week 0 and at week 4 after treatment with FIIc and Pioglitazone.

<table>
<thead>
<tr>
<th>Glycemic and lipid parameters</th>
<th>Time points</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Weight</strong></td>
<td>Week 0</td>
<td>222.5 ± 5.89</td>
<td>221.67 ± 9.83</td>
<td>229.17 ± 9.70</td>
<td>245 ± 27.20</td>
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<tr>
<td></td>
<td>Week 4</td>
<td>254.33 ± 31.97</td>
<td>218.5 ± 11.14*</td>
<td>236.67 ± 7.06*</td>
<td>251.67 ± 28.32</td>
</tr>
<tr>
<td><strong>FBG (mmol/l)</strong></td>
<td>Week 0</td>
<td>4.92 ± 0.037</td>
<td>10.41 ± 0.032</td>
<td>10.54 ± 0.048</td>
<td>10.1 ± 0.075</td>
</tr>
<tr>
<td></td>
<td>Week 4</td>
<td>4.68 ± 0.059</td>
<td>12.53 ± 0.068</td>
<td>8.47 ± 0.063</td>
<td>8.16 ± 0.058</td>
</tr>
<tr>
<td><strong>HbA1c %</strong></td>
<td>Week 0</td>
<td>5.1 ± 0.12</td>
<td>5.3 ± 0.12*</td>
<td>5.31 ± 0.22 b</td>
<td>5.36 ± 0.29**</td>
</tr>
<tr>
<td></td>
<td>Week 4</td>
<td>5.13 ± 0.10</td>
<td>8.58 ± 0.91*</td>
<td>5.38 ± 0.17**</td>
<td>5.33 ± 0.26**</td>
</tr>
<tr>
<td><strong>Serum TG (mg/dl)</strong></td>
<td>Week 0</td>
<td>64.3 ± 4.45</td>
<td>66.5 ± 7.58</td>
<td>64.17 ± 5.6</td>
<td>65.17 ± 7.52</td>
</tr>
<tr>
<td></td>
<td>Week 4</td>
<td>67.00 ± 6.29</td>
<td>114.00 ± 6.29</td>
<td>92.00 ± 4.8**</td>
<td>87.00 ± 4.5**</td>
</tr>
<tr>
<td><strong>Total CHL (mg/dl)</strong></td>
<td>Week 0</td>
<td>67.16 ± 4.91</td>
<td>64.17 ± 9.92</td>
<td>69.33 ± 5.31</td>
<td>66.67 ± 2.50</td>
</tr>
<tr>
<td></td>
<td>Week 4</td>
<td>67.5 ± 4.32</td>
<td>98 ± 7.26*</td>
<td>76 ± 6.54**</td>
<td>73 ± 7.3*</td>
</tr>
<tr>
<td><strong>HDL-C (mg/dl)</strong></td>
<td>Week 0</td>
<td>38.17 ± 1.60</td>
<td>37.33 ± 1.50</td>
<td>37.67 ± 1.50</td>
<td>37.67 ± 1.63</td>
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<tr>
<td></td>
<td>Week 4</td>
<td>38 ± 1.09</td>
<td>25.17 ± 1.16*</td>
<td>34 ± 1.09**</td>
<td>34.5 ± 1.04**</td>
</tr>
<tr>
<td><strong>LDL-C (mg/dl)</strong></td>
<td>Week 0</td>
<td>16.13 ± 5.09</td>
<td>13.53 ± 9.59</td>
<td>18.83 ± 6.62</td>
<td>15.97 ± 3.11</td>
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<td></td>
<td>Week 4</td>
<td>16.1 ± 3.19</td>
<td>50.03 ± 7.92*</td>
<td>23.60 ± 7.08**</td>
<td>21.10 ± 7.57**</td>
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<td><strong>DPP-4 (pg/ml)</strong></td>
<td>Week 0</td>
<td>32.7 ± 0.62</td>
<td>32.43 ± 0.56</td>
<td>32.43 ± 0.71</td>
<td>32.56 ± 0.50</td>
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<td>Week 4</td>
<td>32.40 ± 0.67</td>
<td>34.53 ± 0.48</td>
<td>26.41 ± 0.43**</td>
<td>37.61 ± 4.49</td>
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<tr>
<td><strong>Serum Insulin (pmol)</strong></td>
<td>Week 0</td>
<td>16.18 ± 0.59</td>
<td>9.1 ± 0.58*</td>
<td>9.00 ± 0.34**</td>
<td>9.08 ± 0.24**</td>
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<td>Week 4</td>
<td>16.3 ± 0.29</td>
<td>7.36 ± 0.19*</td>
<td>12.00 ± 0.44**</td>
<td>12.45 ± 0.35**</td>
</tr>
<tr>
<td><strong>Liver Tyrosine kinase (U/mg protein)</strong></td>
<td>Week 4</td>
<td>12.11 ± 0.85</td>
<td>2.18 ± 0.86*</td>
<td>4.90 ± 1.28**</td>
<td>5.31 ± 0.51**</td>
</tr>
<tr>
<td><strong>Adipose Tyrosine kinase (U/mg protein)</strong></td>
<td>Week 4</td>
<td>10.26 ± 0.28</td>
<td>1.63 ± 0.24*</td>
<td>3.21 ± 0.34**</td>
<td>4.38 ± 0.51**</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. (n = 6). (p < 0.001)

Effect of FIIc on tyrosine kinase activity

After treatment with active compound (FIIc), we have observed 55.5% and 49.22% increase in tyrosine kinase activity in liver and adipose tissues respectively compared to the diabetic controls rats at week 4 of the study. Group D rats treated with Pioglitazone also demonstrated 58.94% and 62.7% increase in tyrosine kinase activity in liver and adipose tissues respectively compared to the diabetic controls (Table 1).
Increased serum insulin levels is also responsible for increased uptake of triglycerides from the blood into the muscles which are resistant to STZ-induced beta cell destruction[39,40]. Also GLP-1 treatment has been reported to prevent streptozotocin-induced beta cell apoptosis[39,40]. Studies have also reported that mice lacking both GLP-1 and GIP receptors clearly demonstrate that these incretins are acutely responsible for DPP 4 inhibition and glycemic controls[41]. A study by Zhang et al. reported that alogliptin restored the β-cell mass and islet morphology, and increased insulin secretion in their diabetic mouse model induced by STZ treatment[42]. Similar results were reported by Ahren et al.[43].

Increased serum insulin levels is also responsible for increased uptake of triglycerides from the blood into the muscles and adipose tissues, decreased rate of lipolysis in adipose tissues therefore lowers plasma fatty acid levels[44]. Hence we observed increased body weight and improved glycemic profile in FIIc treated rats compared to diabetic controls (Table 1). Rats treated with Pioglitazone also demonstrated similar results as it is known to have insulin sensitizing properties resulting in improved glycemic index and lipid profile.

Hence we conclude that purified active compound (FIIc) isolated from the fruit pulp of Eugenia jambolana has significant effect on glycemic control, serum lipid profile and insulin sensitivity. It also significantly decreased serum DPP-4 levels and improves the altered levels of protein tyrosine kinase which is involved in establishing a smooth insulin signaling mechanism.
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Figure 1: Showing regenerated Islets of pancreatic beta cell mass after treatment with active compound FIIc in group C rats at week 4 of the study.

Figure 2: Showing Streptozotocin-NAD induced degenerated Islets of pancreatic beta cell mass in group B rats at week 4 of the study.

Conclusion

Thus FIIc significantly reduces hyperglycemia, hyperlipidemia via inhibiting serum DPP-4 levels and improves serum insulin and tyrosine kinase activity, and it may prove potential herbal compound for the management of type 2 diabetes.

Acknowledgements: The authors acknowledge Indian Council of Medical Research, New Delhi for their financial support.

Conflict of Interests: The authors declare that they have no conflicting interests.

References