IMPROVED GLUCOREGULATION, INSULIN RESISTANCE AND LEPTIN LEVELS BY A POLYHERBAL DRUG IN HIGH FAT DIET AND LOW DOSE STREPTOZOTOCIN TYPE 2 DIABETES MODEL

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Key words: high fat diet, streptozotocin, polyherbal extract, insulin resistance, leptin

SUMMARY

The aim of the present study was to evaluate the anti-diabetic and lipid lowering potential of a polyherbal formulation of six different Indian medicinal plants. Animals were divided into groups as follows: (a) normal control; (b) high fat diet control; (c) high fat diet + streptozotocin (STZ) control; (d) high fat diet + STZ + polyherbal formulation; and (e) high fat diet + STZ + rosiglitazone. The rats were fed a high fat diet for a period of 2 weeks and then injected streptozotocin (65 mg/kg body weight ip). Intragastric administration of polyherbal formulation (250 and 500 mg/kg body mass) for the next 30 days significantly improved glucose tolerance, and decreased glycemic indices, glycated hemoglobin and lipid levels. Supplementation with polyherbal extract also displayed a renoprotective and hepatoprotective potential by reducing urea, creatinine, aspartate aminotransferase and alanine aminotransferase levels. The hypoglycemic principles of the polyherbal extract increased peripheral glucose utilization by improving succinate dehydrogenase and glycogen synthase levels and reducing glucose-6-phosphatase and glycogen phosphorylase levels. Furthermore, polyherbal extract also restored altered plasma insulin and leptin levels.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) prevalence has been increasing rapidly over the last decade and is now considered a worldwide epidemic (1).

T2DM is a heterogeneous disorder characterized by a progressive decline in insulin action (insulin resistance), followed by the inability of β-cells to compensate for insulin resistance (β-cell dysfunction) (2). If not controlled, these may result in other micro- and macrovascular complications such as cardiovascular disease, renal failure, neuropathy and poor wound healing. Although several drugs are available for the treatment of diabetes, adverse effects...
and drug resistance are of great concern. As an alternative, a greater number of people are seeking natural products or dietary interventions to prevent or treat diabetes. The World Health Organization (WHO) has urged researchers to examine whether traditional medicines produce any beneficial clinical results (3).

Indian system of medicine is one of the oldest and there are more than 100 medicinal plants mentioned in this system including folklore medicines for the treatment of diabetic complications; the mentioned plants are active at their best either individually or in combination. The plant kingdom represents a largely unexplored reservoir of biologically active compounds not only as drugs, but also as unique templates that could serve as a starting point for synthetic analogs and an interesting tool that can be applied for better understanding of biological processes. The present study involves a polyherbal aqueous extract using leaves, bark and seeds of different plants comprising of *Cassia fistula*, *Ocimum sanctum*, *Annona squamosa*, *Terminalia arjuna*, *Azadirachta indica* and *Aegle marmelos*. The plants used in the present study have been reported to show blood glucose lowering effect in diabetic animals and some of them also have anti-lipidemic and cholesterol lowering effects. A major objective of selecting these plants is that they are commonly available with minimal or no cost of procurement and hence very cost effective.

The experimental model selected to test the efficacy of the herbal extract in the present study was high fat diet (HFD) fed rat model followed by a low dose streptozotocin (STZ) injection. It is suggested that HFD might be a better way to initiate insulin resistance (IR), which is one of the important features of T2DM. At the same time, STZ is widely used to reproducibly induce both insulin dependent and non-insulin dependent diabetes mellitus by inducing β-cell death through alkylation of DNA (4). Although high dose STZ severely impairs insulin secretion mimicking T1DM, low dose STZ has been known to induce a mild impairment of insulin secretion, which is similar to the feature of the later stage of T2DM (2,5). The general strategy is using HFD feeding for a period with the purpose to induce mild IR at first and then an injection of low dose STZ to cause partial dysfunction of β-cell for suppressing insulin secretion, which works as a compensation for IR with the result of persistent hyperglycemia. In 2000, Reed et al. (5) suggested the fat fed/STZ treated rats as a novel animal model for T2DM, suitable for testing of antidiabetic compounds. Therefore, the present study was designed to evaluate the effects of polyherbal formulation/supplementation on the metabolic risk factors in rats treated with HFD+STZ for insulin deficient T2DM.

Plants contain several phytochemicals, which may target several signaling pathways at the same time and may bring about benefits through a synergistic or additive action. The attributed antihyperglycemic effects of these plants are due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. Hence the treatment with herbal drugs has an effect on protecting β-cells and smoothing out fluctuation in glucose levels. In general, there is very little biological knowledge on the specific modes of action in the treatment of diabetes, but most of the plants have been found to contain phytocompounds like glycosides, alkaloids, terpenoids, flavonoids, etc., that are frequently implicated as having antidiabetic effects. In the traditional system of Indian medicine, plant formulation and combined extracts of plants are used as the drug of choice rather than individual plant extract. Thus, the present study was aimed to evaluate the efficacy of the polyherbal formulation to combat diabetes associated with obesity.

**MATERIALS AND METHODS**

**Experimental model and treatment regimen**

Adult female Charles Foster rats weighing 230-250 g were fed pellet diet (Pranav Agro, Baroda) and given water ad libitum. The experiment was carried out according to the guidelines for experiments on animals, India, and approved by the Ethics Committee of the Department of Zoology, The M. S. University of Baroda, Vadodara (approval no. 827/ac/04/CPCSEA).
The animals were divided into six groups of six rats each:

a) normal control (NC): received 1% carboxymethylcellulose (CMC)
b) high fat diet (HFD) control: received high fat diet for 2 weeks + 1% CMC
c) streptozotocin/high fat diet (STZHFD) control: fed high fat diet for 2 weeks and injected with STZ (65 mg/kg) + 1% CMC
d) STZHFD + polyherbal (PH): fed high fat diet for 2 weeks and injected with STZ (65 mg/kg) + 250 mg/kg body mass in 1% CMC for the next 30 days
e) STZHFD + polyherbal (PH): fed high fat diet for 2 weeks and injected with STZ (65 mg/kg) + 500 mg/kg body mass in 1% CMC for the next 30 days
f) STZHFD + rosiglitazone: fed high fat diet for 2 weeks and injected with STZ (65 mg/kg) + rosiglitazone (50 mg/kg oral in 1% CMC) for the next 30 days

Preparation of the extract

All plants were procured from local market (M/s G.Y Hakim and Sons, Vadodara) and authenticated (Table 1). All plants were shade-dried, powered and equal proportions (100 g each) were mixed thoroughly. The mixture (600 g) was further boiled in distilled water (3 L at 100 °C for 60 minutes) and filtered. The filtrate was evaporated to dryness, lyophilized and stored at 4 °C. The extractive value in terms of yield was 38% (w/w). TLC fingerprinting of polyherbal formulation was carried out to avoid batch-to-batch variations. It was suspended in 1.0% sodium carboxymethyl cellulose (as vehicle) and used for subsequent experiments.

Animals

At the end of the treatment schedule, animals were fasted for 16 h and oral glucose tolerance test (OGTT) was performed. Insulin response test was performed in another subset of animals. Animals were sacrificed by cervical dislocation and liver and muscle were quickly excised, blotted free of blood and washed in phosphate buffer solution (pH 7.4) and stored in -80 °C freezer until further analysis. Blood samples were centrifuged immediately at 2500 rpm for 10 min and plasma was separated.

Chemicals

Streptozotocin, pyruvate kinase were from Sigma, St. Louis, USA. Glucose-1-phosphate, sodium lauryl sulfate, 1-amino 2-naphthol 4-sulfonic acid (ANSA) were purchased from SRL, Mumbai. 2-Thiobarbituric acid (TBA), trichloro acetic acid (TCA), triphenyl tetrazolium chloride (TTC) and all other chemicals were of analytical grade.

Biochemical estimations

Blood glucose, HDL-C, LDL-C, triacylglycerol, phospholipids, creatinine and urea were estimated by enzymatic kits (Merck Diagnostics, Mumbai). HbA1c was estimated by HPLC (Varian) method. Plasma Insulin and plasma leptin were quantified using ELISA kits (Mercodia, Uppsala, Sweden).

Enzymatic measurements

(a) Glucose-6-phosphatase (E.C. 3.1.3.9) was estimated by the method of Harper 1963 (6). The inorganic phosphate released was measured by

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Family</th>
<th>Common name</th>
<th>Parts used</th>
<th>Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cassia fistula L.</td>
<td>Leguminosae</td>
<td>Golden shower tree</td>
<td>Pod</td>
<td>100 g</td>
</tr>
<tr>
<td>Ocimum sanctum L</td>
<td>Lamiaceae</td>
<td>Holy basil</td>
<td>Leaves</td>
<td>100 g</td>
</tr>
<tr>
<td>Annona squamosa L</td>
<td>Annonaceae</td>
<td>Sugar apple</td>
<td>Seeds</td>
<td>100 g</td>
</tr>
<tr>
<td>Terminalia arjuna Roxb.</td>
<td>Combretaceae</td>
<td>Arjuna</td>
<td>Bark</td>
<td>100 g</td>
</tr>
<tr>
<td>Azadirachta indica A</td>
<td>Meliaceae</td>
<td>Neem</td>
<td>Leaves</td>
<td>100 g</td>
</tr>
<tr>
<td>Aegle marmelos (L) Correa ex Roxb.</td>
<td>Rutaceae</td>
<td>Holy fruit tree</td>
<td>Leaves</td>
<td>100 g</td>
</tr>
</tbody>
</table>
the method of Fiske-Subbarow (1925) (7). The enzyme activity is expressed as µg phosphorus released/mg protein/10 min.

(b) Succinate dehydrogenase (E.C. 1.3.9.1) was estimated by the method of Kun and Abood 1949 (8). The enzyme activity is expressed as µg formazon formed/mg protein/20 min.

(c) Glycogen was estimated by the method of Seifter et al., 1950 (9). It is expressed as mg glycogen/100 mg tissue.

d) Glycogen phosphorylase (E.C. 2.4.1.1) was estimated by a modified method of Cori et al., 1940 (10) and adapted by Cahill et al., 1957 (11). The inorganic phosphate released was measured by the method of Fiske-Subbarow (1925) (7). The enzyme activity is expressed as µg phosphorus released/mg protein/10 min.

c) Glycogen synthase (E.C. 2.4.1.11) was assayed by the method of Leloir and Goldenberg (1962) (12)]. The enzyme activity is expressed as nmol UDPG incorporated in glycogen/min/mg protein.

**Statistical analysis**

Statistical evaluation of data was done by one-way ANOVA followed by post-hoc Bonferroni test and results are expressed as mean ± SE using Graph Pad Prism version 3.0 for Windows (Graph Pad Software, San Diego, California, USA).

**RESULTS**

**Metabolic status (Table 3)**

Significant mean body mass increase was seen in HFD+STZ treated animals \( P<0.0001 \). Treatment with low dose of extract proved to be more potent in reducing body mass as compared to high dose of herbal extract. There was no significant change in food consumption throughout the treatment period.

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**Table 2. Composition of regular and high fat diet**

<table>
<thead>
<tr>
<th></th>
<th>Normal diet (%)</th>
<th>High fat diet (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat flour</td>
<td>45</td>
<td>44</td>
</tr>
<tr>
<td>Casein</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>20.0</td>
<td>-</td>
</tr>
<tr>
<td>Lard</td>
<td>-</td>
<td>20.0</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>DL methionine</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**Table 3. Effect of low and high dose polyherbal extract on body mass and food intake in high fat diet and streptozotocin treated animals**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HFD</th>
<th>HFD + STZ</th>
<th>HFD + STZ + PH250</th>
<th>HFD + STZ + PH 500</th>
<th>HFD + STZ + ROZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (g)</td>
<td>224.7 ± 4.29</td>
<td>312.7 ± 8.42*</td>
<td>284.4 ± 6.84*</td>
<td>247.8 ± 8.4a 3</td>
<td>263.3 ± 7.3 • 3</td>
<td>232.6 ± 8.4 53</td>
</tr>
<tr>
<td>Food intake /rat/day (g)</td>
<td>12.53 ± 0.82</td>
<td>18.42 ± 1.55</td>
<td>16.42 ± 1.62</td>
<td>14.51 ± 1.88</td>
<td>14.71 ± 1.39</td>
<td>13.72 ± 1.48</td>
</tr>
</tbody>
</table>

Control vs. rest of the groups: \( P<0.01=\*, \ P<0.001=\#\, \ P<0.0001=\@\)

HFD vs. rest of the groups: \( P<0.01=1, \ P<0.001=2, \ P<0.0001=3\)

HFDSTZ vs. rest of the groups: \( P<0.01=\alpha, \ P<0.001=\beta, \ P<0.0001=\delta\)
Plasma glucose, insulin and HbA1c levels

(Table 4)

HFD+STZ group recorded significantly elevated blood plasma glucose levels \((P<0.0001)\). The administration of polyherbal formulation to STZ+HFD treated animals showed significant reduction in blood glucose levels. Low doses of the extract (PH 250 mg/kg body mass) proved to be more potent than high dose of the extract (PH 500 mg/kg body mass).

HFD+STZ group recorded significant increase in glycated hemoglobin \((P<0.001)\). High dose of the extract was not able to induce significant changes in glycated hemoglobin and was comparable to rosiglitazone treated animals. Insulin titters in HFD+STZ treated animals were significantly lowered \((P<0.01)\) and markedly elevated by low dose PH formulation.

Glucose tolerance and insulin response tests

(Figs. 1 and 2)

Analysis of glucose tolerance pattern during 120 minute test period in control and experimental animals revealed that HFD+STZ treated rats developed...
glucose intolerance. The rate of glucose disposal was more or less stable during the 90-120 minute period in HFD+STZ treated animals. Polyherbal extract (PH 250 mg/kg body mass) supplemented rats showed lower glucose elevation and faster glucose disposal rates, thereby displaying significant ($P<0.0001$) improvement in glucose tolerance pattern. During insulin response test, HFD+STZ treated rats showed constant decrease in plasma glucose levels throughout the 120 minute test period, while the rats supplemented with low and high dose of the polyherbal extract displayed improved recovery rates attributed to improved insulin sensitivity.

**Plasma lipid profile and leptin levels (Table 5)**

HFD+STZ animals recorded significant increase in plasma total cholesterol ($P<0.001$), plasma triglycerides ($P<0.0001$), plasma LDL-C ($P<0.0001$), plasma VLDL-C ($P<0.0001$) and plasma free fatty acids ($P<0.0001$), while HDL-C levels showed significant decrement in HFD+STZ treated animals ($P<0.01$). Low dose of extract (PH 250 mg/kg body mass) did not have significant effect in decreasing the lipid profile as compared to the high dose formulation, which was potent in decreasing plasma lipid levels and increasing HDL-C levels. There was no change observed in leptin titers in HFD+STZ animals and HFD+STZ treated with low and high dose of polyherbal extract.

**Liver lipid profile (Table 6)**

HFD+STZ animals recorded significant increase in total cholesterol ($P<0.001$), triacylglycerol ($P<0.0001$) and free fatty acids ($P<0.0001$), while both low and high doses of polyherbal formulation had non-significant ($P>0.05$) influence in lowering lipid profile levels in the liver.

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**Table 5. Effect of low and high dose polyherbal extract on plasma leptin, cholesterol, triglyceride and lipoprotein levels and atherogenic index in high fat diet and streptozotocin treated animals**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HFD</th>
<th>HFD+STZ PH250</th>
<th>HFD + STZ PH 500</th>
<th>HFD + STZ ROZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma cholesterol (mmol/L)</td>
<td>2.60 ± 0.21</td>
<td>7.03 ± 0.31*</td>
<td>4.87 ± 0.40*</td>
<td>4.37 ± 0.41*</td>
<td>4.31 ± 0.483</td>
</tr>
<tr>
<td>Plasma triglycerides (mmol/L)</td>
<td>2.88 ± 0.29</td>
<td>9.32 ± 0.40*</td>
<td>7.38 ± 0.31*</td>
<td>4.85 ± 0.38*</td>
<td>4.36 ± 0.473</td>
</tr>
<tr>
<td>Plasma HDL-C (mmol/L)</td>
<td>1.43 ± 0.09</td>
<td>0.68 ± 0.09*</td>
<td>1.04 ± 0.05*</td>
<td>0.93 ± 0.05*</td>
<td>0.99 ± 0.09*</td>
</tr>
<tr>
<td>Plasma LDL-C (mmol/L)</td>
<td>0.69 ± 0.06</td>
<td>4.48 ± 0.13*</td>
<td>2.35 ± 0.14*</td>
<td>2.47 ± 0.11*</td>
<td>2.44 ± 0.09*</td>
</tr>
<tr>
<td>Plasma VLDL-C (mmol/L)</td>
<td>0.59 ± 0.05</td>
<td>1.85 ± 0.07*</td>
<td>1.47 ± 0.06*</td>
<td>0.97 ± 0.07*</td>
<td>0.87 ± 0.009</td>
</tr>
<tr>
<td>Plasma FFA (mmol/L)</td>
<td>1.42 ± 0.09</td>
<td>4.04 ± 0.11*</td>
<td>3.70 ± 0.14*</td>
<td>2.10 ± 0.26*</td>
<td>2.43 ± 0.24*</td>
</tr>
<tr>
<td>Atherogenic index</td>
<td>0.10</td>
<td>0.56</td>
<td>0.26</td>
<td>0.24</td>
<td>0.18</td>
</tr>
<tr>
<td>Plasma leptin (ng/L)</td>
<td>1.12 ± 0.003</td>
<td>0.13 ± 0.01</td>
<td>0.04 ± 0.003</td>
<td>0.06 ± 0.006</td>
<td>0.08 ± 0.04</td>
</tr>
</tbody>
</table>

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**Table 6. Effect of low and high dose polyherbal extract on liver cholesterol, triglyceride and free fatty acid levels in high fat diet and streptozotocin treated animals**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HFD</th>
<th>HFD + STZ PH250</th>
<th>HFD + STZ PH 500</th>
<th>HFD + STZ ROZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver cholesterol (mg/g)</td>
<td>25.32 ± 2.45</td>
<td>68.42 ± 4.61*</td>
<td>45.66 ± 3.75*</td>
<td>33.67 ± 3.05*</td>
<td>37.91 ± 2.86*</td>
</tr>
<tr>
<td>Liver triglycerides (mg/g)</td>
<td>35.67 ± 5.72</td>
<td>128.96 ± 8.35*</td>
<td>102.5 ± 8.51*</td>
<td>86.74 ± 7.42*</td>
<td>93.65 ± 6.32*</td>
</tr>
<tr>
<td>Liver free fatty acids (mg/g)</td>
<td>23.67 ± 2.53</td>
<td>93.64 ± 4.65*</td>
<td>64.73 ± 5.33*</td>
<td>45.76 ± 3.76*</td>
<td>48.98 ± 5.38*</td>
</tr>
</tbody>
</table>
**Hepatic glycogen and glucogenic enzymes (Table 7)**

Liver glycogen levels decreased significantly \( (P<0.0001) \) in the STZ+HFD treated group, while in low and high dose aqueous extract treated groups there was a non-significant increase in liver glycogen level \( (P>0.05) \) with respect to STZ+HFD treatment. Glycogen phosphorylase and glucose-6-phosphatase activity increased significantly \( (P<0.0001 \text{ and } P<0.0001, \text{ respectively}) \) in the STZ+HFD treated group. Moreover, animals treated with polyherbal aqueous extract showed significant decrease in the enzymatic activity compared to normal control, while the same significantly decreased \( (P<0.0001 \text{ and } P<0.001, \text{ respectively}) \) from the levels recorded in the STZ+HFD treated rats. Low dose of the extract showed significant increase in the activity of both glycogen synthase and SDH \( (P<0.0001 \text{ both}) \).

**Muscle glycogen and glucogenic enzymes (Table 7)**

Muscle glycogen levels decreased significantly \( (P<0.0001) \) in the STZ+HFD treated group compared to normal control animals. Glycogen phosphorylase activity increased significantly \( (P<0.0001) \) in the STZ+HFD treated group. Glycogen synthase and SDH activity decreased significantly \( (P<0.0001) \) in STZ+HFD animals, while the animals with low dose extract showed significant increase \( (P<0.01) \) in the levels of SDH.

**Table 7. Effect of low and high dose polyherbal extract on liver and muscle glycogen and glucogenic enzymes in liver and muscle in high fat diet and streptozotocin treated animals**

<table>
<thead>
<tr>
<th>Control</th>
<th>HFD</th>
<th>HFD + STZ + PH250</th>
<th>HFD + STZ + PH 500</th>
<th>HFD + STZ + ROZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver glycogen (mg/100 mg)</td>
<td>43.67 ± 2.49</td>
<td>33.87 ± 2.71</td>
<td>14.78 ± 1.96*</td>
<td>22.87 ± 2.71*</td>
</tr>
<tr>
<td>Muscle glycogen (mg/100 mg)</td>
<td>5.63 ± 0.42</td>
<td>4.16 ± 0.45</td>
<td>3.22 ± 0.38♦</td>
<td>2.62 ± 0.38♦</td>
</tr>
<tr>
<td>Liver glucose-6-phosphatase (µg phosphorus released/mg protein/15 min)</td>
<td>0.115 ± 0.003</td>
<td>0.101 ± 0.002</td>
<td>0.216 ± 0.005*</td>
<td>0.163 ± 0.005*</td>
</tr>
<tr>
<td>Liver glycogen phosphorylase (µg phosphorus released/mg protein/10 min)</td>
<td>0.046 ± 0.001</td>
<td>0.058 ± 0.002</td>
<td>0.087 ± 0.002*</td>
<td>0.068 ± 0.002*</td>
</tr>
<tr>
<td>Muscle glycogen phosphorylase (µg phosphorus released/mg protein/10 min)</td>
<td>0.051 ± 0.002</td>
<td>0.061 ± 0.002</td>
<td>0.091 ± 0.002*</td>
<td>0.073 ± 0.002*</td>
</tr>
<tr>
<td>Liver glycogen synthase (nmol UDPG incorporated in glycogen/min/mg protein)</td>
<td>0.546 ± 0.01</td>
<td>0.472 ± 0.01*</td>
<td>0.188 ± 0.01*</td>
<td>0.361 ± 0.02*</td>
</tr>
<tr>
<td>Muscle glycogen synthase (nmol UDPG incorporated in glycogen/min/mg protein)</td>
<td>0.248 ± 0.01</td>
<td>0.218 ± 0.01</td>
<td>0.093 ± 0.01*</td>
<td>0.132 ± 0.01*</td>
</tr>
<tr>
<td>Liver succinate dehydrogenase (µg formazon formed/mg protein/20 min)</td>
<td>0.916 ± 0.016</td>
<td>0.778 ± 0.012*</td>
<td>0.394 ± 0.014*</td>
<td>0.557 ± 0.014*</td>
</tr>
<tr>
<td>Muscle succinate dehydrogenase (µg formazon formed/mg protein/20 min)</td>
<td>0.125 ± 0.008</td>
<td>0.114 ± 0.005</td>
<td>0.077 ± 0.004*</td>
<td>0.091 ± 0.005*</td>
</tr>
</tbody>
</table>

Control vs. rest of the groups: \( P<0.01=●, P<0.001=■, P<0.0001=♦ \)
HFD vs. rest of the groups: \( P<0.01=1, P<0.001=2, P<0.0001=3 \)
HFDSTZ vs. rest of the groups: \( P<0.01=α, P<0.001=β, P<0.0001=δ \)
Plasma urea and creatinine levels (Table 8):

Significant increment in the levels of urea was observed in HFD+STZ treated animals ($P<0.01$). Plasma creatinine levels increased significantly ($P<0.001$) in HFD+STZ rats compared to normal control animals, while treatment with high dose extract was slightly effective in lowering the same.

Liver function tests (Table 8)

Significant ($P<0.01$) increment in the levels of AST and ALT enzymatic activity was seen in HFD+STZ treated rats, while non-significant ($P>0.05$) decrease was observed with both doses of the polyherbal extract.

DISCUSSION

Diabetes mellitus is a syndrome resulting from a variable interaction and environmental factors and is characterized by depleted insulin secretion, hyperglycemia and altered metabolism of lipids, carbohydrates and proteins, in addition to damaged $\beta$-cells of the pancreas and an increased risk of complications of vascular disease (2).

In the present study, the high fat diet fed animals with exposure to low dose of STZ were used to test the dose dependent efficacy of polyherbal extract as potential concoction for improvement of hyperglycemia and insulin resistance and as a potent anti-lipidemic drug wherein the low dose of the polyherbal extract shows more favorable effects. The credited anti-hyperglycemic action of most of the herbs is due to their capacity to refurbish islet function by either increasing the insulin output or by inhibiting the intestinal absorption of glucose or by facilitation of metabolites generated due to insulin action. Hence treatment with herbal drugs shows an effect in protecting beta cells and improving fluctuations in the glucose levels. Our data showed that the polyherbal extract exerted a significant hypoglycemic effect marked by significantly lowered plasma glucose levels and improved plasma insulin levels. The blend of different herbs in the current polyherbal extract, i.e. *Cassia fistula* (13-15), *Ocimum sanctum* (16-20), *Annona squamosa* (21-24), *Terminalia arjuna* (25-27), *Azadirachta indica* (28-31) and *Aegle marmelos* (32-38), has been demonstrated to possess significant anti-diabetic/hypoglycemic effects. The polyherbal extract clearly showed the dose dependent efficacy with the decline in glycemic levels comparable to that of rosiglitazone treatment. HFDSTZ+PH 250 could be accredited to a greater extent for the improvement in insulin level, which was significantly brought down in HFDSTZ exposed rats. The lower insulin titer and marked hyperglycemia in diabetic control rats were also well reflected in elevated HbA1c, suggestive of protein glycosylation, which leads to many serious secondary complications encountered in diabetes. Treatment with PH 250 remarkably reversed the condition, which was very much similar to the results obtained by rosiglitazone treatment. The increase in the peripheral insulin titer and marked hypoglycemia were responsible for decrement in HbA1c in HFDSTZ+PH250 treated rats. Similar observations have been made even for other plant extracts (39,40).

**Table 8. Effect of low and high dose polyherbal extract on liver function tests and plasma urea and creatinine levels in high fat diet and streptozotocin treated animals**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HFD</th>
<th>HFD + STZ</th>
<th>HFD + STZ + PH250</th>
<th>HFD + STZ + PH 500</th>
<th>HFD + STZ + ROZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>108.6 ± 7.76</td>
<td>136.8 ± 21.7</td>
<td>187.8 ± 11.86●</td>
<td>166.5 ± 14.72</td>
<td>147.8 ± 13.77</td>
<td>138.7 ± 16.72</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>67.85 ± 7.88</td>
<td>88.7 ± 11.97</td>
<td>134.7 ± 12.76●</td>
<td>117.7 ± 13.62</td>
<td>118.6 ± 12.72</td>
<td>86.32 ± 13.62</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>30.71 ± 4.77</td>
<td>46.82 ± 5.39</td>
<td>67.9 ± 8.49●</td>
<td>36.7 ± 7.90</td>
<td>44.6 ± 7.27</td>
<td>43.82 ± 8.48</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.37 ± 0.03</td>
<td>0.44 ± 0.02</td>
<td>0.62 ± 0.05*</td>
<td>0.57 ± 0.02●</td>
<td>0.51 ± 0.03</td>
<td>0.44 ± 0.02 p</td>
</tr>
</tbody>
</table>

Control vs. rest of the groups: $P<0.01$=●, $P<0.001$=■, $P<0.0001$=♦
HFD vs. rest of the groups: $P<0.01$=1, $P<0.001$=2, $P<0.0001$=3
HFDSTZ vs. rest of the groups: $P<0.01$=α, $P<0.001$=β, $P<0.0001$=δ
Apart from the regulation of carbohydrate metabolism, insulin also plays an important role in the metabolism of lipids. Insulin is a potent inhibitor of lipolysis, since it inhibits the activity of the hormone sensitive lipases in adipose tissue and suppresses the release of free fatty acids. In diabetes, the enhanced activity of this enzyme increases lipolysis and releases more free fatty acids to the circulation. Increased fatty acid concentration also increases β-oxidation of fatty acids, producing more acetyl CoA and cholesterol in diabetes. In normal condition, insulin increases the receptor-mediated removal of LDL-cholesterol and the decreased activity of insulin in diabetes causes hypercholesterolemia. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic rats.

This was validated in the present study, wherein a significant increase (2- to 3-fold) was observed in various plasma lipid fractions in STZ induced diabetic rats. Similar observations in induced diabetic models are also reported elsewhere (25,41). Polyherbal extract treatment was found to be effective in lowering the diabetes specific plasma lipid parameters, with both PH doses demonstrating nearly the same significance. The potency of PH extract in normalizing various impaired lipid fractions was nearly similar to those obtained with rosiglitazone. The PH extract effectively lowered plasma and liver triglycerides, free fatty acids, cholesterol and LDL levels, while increasing HDL levels. There are similar reports documenting the lipid lowering actions of other medicinal plants (42,43). VLDL is usually converted to LDL via post heparin lipases. The results of the present study suggest PH 500 to be able to restore the catabolic metabolism of VLDL, which could be due to the increased lipolytic activity. The possible mechanism of action of PH extract in lowering lipid levels may be attributed to its ability to increase insulin level and consequent mechanism of action as brought about by increased insulin levels. The regression of diabetic state due to PH extract administration increases glucose utilization as a result of improved insulin titers and thereby depressing the mobilization of fat. The hypolipidemic efficiency of the PH extract can also be substantiated by the calculated cardiovascular risk and atherogenic index, and in spite of being a crude extract, its antilipidemic potency can be compared with the standard drug rosiglitazone. Increased adiposity is related to the secretion of leptin, an adipocytic hormone, whose serum level is known to be proportional to body fat mass (44). Leptin has a major role in the critical control of body mass by suppression of food intake through satiety center and promotion of energy expenditure (45-52). The HFD mice are also characterized by a tremendous increase in serum leptin level, which not only indicates increased adipose mass but also insulin resistance and β-cell dysfunction. The increased leptin level together with the invoked hypoinsulinemia and insulin resistance stand to prevent reduced glucose intake. Supplementation of PH extract significantly prevents augmented body mass gain and reduces serum lipid profile along with increasing the reduced leptin levels in HFD STZ animals, thus supressing food intake and promoting energy intake in these animals.

The hypoglycemic principles of the PH extract inhibit the activity of the glucose-6-phosphatase enzyme involved in dephosphorylation of glucose. Glucose phosphorylation to glucose-6-phosphate is a mandatory step in the glucose metabolism and glucose-6-phosphatase action helps in raising the blood glucose level. The in vivo inhibition of glucose-6-phosphatase activity is achieved with PH extract leading to lowering of the blood glucose level (53). The hypoglycemic activity of PH extract enhances the activity of SDH, which is a rate limiting enzyme in aerobic glycolysis. The increase in the activity of SDH speeds up the utilization of glucose, which in turn will lower the level of plasma glucose (54). The increased activity of glycogen phosphorylase and decreased action of glycogen synthase in HFD STZ treated animals lead to depletion of glycogen, which is restored to some extent in polyherbal treated animals due to reduced action of glycogen phosphorylase and increased activity of glycogen synthase.

The hepatoprotective and renoprotective action of PH extract is clearly evident by lowered plasma levels of AST, ALT, urea and creatinine produced in higher amounts in HFDSTZ rats. Apparently, PH extract has
a potent ability to afford protection against hepatic and renal damage generated in high amounts in HFDSTZ diabetic animals (41,55,56).

CONCLUSION

Overall, the results indicated that the active principles present in the polyherbal extract may possess diverse biological action and can be developed as a therapeutical based on its hypoglycemic and antilipidemic potential. The polyherbal extract has shown to afford significant protection against high fat fed and streptozotocin toxicity. Further studies are aimed at understanding the possible mechanism of action of the polyherbal drug.

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REFERENCES


