EVALUATION OF ANTIDIABETIC ACTIVITY OF WHOLE PLANT OF LEPTADENIA PYROTECHNICA (FORSSK.) DECNE AGAINST STREPTOZOTOCIN INDUCED DIABETES IN RATS.

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Summary

Present study is undertaken to evaluate the antidiabetic potential of Methanolic extract of Leptadenia pyrotechnica (MELP) against streptozotocin induced diabetic rat. The extract exhibited the dose dependent lowering of blood glucose level in diabetic rats. Elevated serum cholesterol and triglyceride level were significantly reduced to normal limits after 3 weeks of treatment with the extract. Depleted glycogen and total protein level in the rats showed marked improvement and were almost equivalent to normoglycemic rats. A marked improvement was also observed in the weight of diabetic rat treated with the extract as compared to diabetic control. These results strongly indicate the antidiabetic potential of Leptadenia pyrotechnica.

Key words: Leptadenia pyrotechnica, Streptozotocin, Diabetes

Introduction

Leptadenia pyrotechnica (Forssk.) Decne (Synonym-L. Spartinum Wight) locally known as Khimp or Khip (Rajasthan), Khimparlo, Thahawar, Ranser (Gujarat), Broom bush (English) is an erect, ascending, shrub up to 1.5m-3m high with green stem and pale green alternating bushy branches with watery sap. Leaf is rarely found and are deciduous when present are 2.5-6.5x 0.2-0.3 cm, sessile, narrowly linear to linear lanceolate, caduceus. Flowers are in cluster lateral umbellate cymes, greenish yellow. Corolla –lobes valvate, outer corona is of 5 scales, staminal corona of raised undulate fleshy ring. Each flower is bisexual pentamorous actinomorphic, sepals joined as base only, corolla sympetalous. Follicles 7.0-14.0x0.5-0.8 cm, terete, lanceolate, tapering to selender beak, glabrous. Seeds are 5-7 mm long, ovate lanceolate, glabrous, comose (hairy) with tufted hairs 2.5-3.5 cm long. Flowering and fruiting occurs from August to January.
It is common throughout the state of Rajasthan and found in dry habitats particularly in desert zones. In India it is commonly found in Banswara, Palod, Dungarpur and Kota [1].

Whole plant seeds and flowers are used for different purpose. Its fiber is used as antihistaminic and expectorant [2]. Fresh juice of the plant is used for abortion[3]. Plant sap is applied to eczema and other skin disease and is also given in diabetes [4]. Whole plant is used in treating wound in Yemen folks and proved to have antibacterial activity against *Staphylococcus aureus & Bacillus subtilis*[5,6]. The latex or the leaf paste is applied over the thorn injury for thorn removal [7]. Whole plant infusion is mixed with buttermilk and given for uterine prolapse and stomach disorders in sariska region of Rajasthan [8]. It is used to cure constipation and is considered good for health in Bikaner region of Rajasthan [9]. In the sudanodeccanian region of central Sahara it is traditionally used in fever, cough, kidney disorders, stones, urinary disease [10].

On phytochemical screening of aerial part of *Leptadenia pyrotechnica* it is found to have terpenes, saponins, flavonoids and tannins, alkaloids, β sitosterol, and polyhydroxy pregnane glycosides[11-12]. A new pentacyclic triterpenoid named leptadenol was isolated from the n-hexane extract of *Leptadenia pyrotechnica*[13]. Twenty-four alkaloids and six simple amines were detected from the aerial parts for the first time in *Leptadenia pyrotechnica*. On GC-MS analysis of isolated alkaloids it was found that they belonged to pyridine, pyrrole, pyrazine, and indole types[14]. It is also proved that Simultaneous administration of *L. pyrotechnica* extract along with cholesterol significantly (p<0.001) prevented the rise in serum total cholesterol, LDL-cholesterol, VLDL-cholesterol, triglycerides and atherogenic index Hepatic and aortic total cholesterol, triglycerides, and lipid peroxidation (TBARs) were also lowered significantly in the extract treated rabbits. The Plant extracts also significantly prevented the atheromatic changes and plaque formation in the aorta and favoured increased fecal cholesterol output. Thus, the results indicate hypolipidemic and antiatherosclerotic effect of methanolic extract of *L. pyrotechnica* [15]. Six flavonoids namely kaempferol-3-O-α-l-rhamnopyranosyl (1’’→6’’) - O-β-d-glucopyranoside (E-I.1), kaempferol-3-O-β-d-rhamnopyranosyl (1’’→6’’) - O-β-d-glucopyranoside (E-I.2), texasin-7-O-β-d glucopyranoside E-II.2, kaempferol-3-O-β-d-glucopyranoside (E-III.1), kaempferol (E-IV.1) and kaempferide-3-O-α-l-rhamnopyranosyl (1’’→6’’) - O-β-d-glucopyranoside (E-I.1a) were isolated. Using Sephadex LH-20 low pressure liquid chromatography (LPLC), preparative paper chromatography (PC), and high performance liquid chromatography (HPLC)[16].

The term diabetes mellitus is described as a metabolic disorder of multiple etiology which is characterized by chronic hyperglycemia (high blood sugar) with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both.[17]. Diabetes mellitus is also known to cause hyperlipidemia due to insulin deficiency which has been known to stimulate lipolysis in the adipose tissue and gives rise to hyperlipidemia and fatty liver. Thus, in diabetes hypercholesterolemia and hypertriglyceridemia often occurs [18]. Currently there are 190 million people with diabetes in the world and their number may double in next three decades [19]. Out of total diabetics about 85-90% suffer from type 2 diabetes mellitus, which is now recognized as vascular disease [20]. Though *Leptadenia pyrotechnica* has number of uses, and is traditionally used in the treatment of diabetes however there is no scientific data available to claim the antidiabetic activity of *Leptadenia pyrotechnica* hence the need to embark on this study become necessary.
Material and Methods

Plant material

The shade dried, aerial parts of the plant *Leptadenia Pyrotechnica* (forsk) decne, Asclepiadaceae, was collected from the Rajasthan, in the month of October 2010 and authenticated by Dr. D.C. Saini, senior scientist, palaeobotany, Birbal Sahni institute of palaeobotany, Lucknow India. A voucher specimen no.11721 was deposited in the department of Pharmaceutical technology MIET Meerut. The plant material was further size reduced and stored until further use in an air tight container.

Animals

Adult albino wistar rats of either sex weighing 150-200 gm were acclimatized for a period of 10 days at room temperature 50% relative humidity, at 12 hr light and day cycle and were maintained on a standard pellet diet (Hindustan Liver, Mumbai, India) and water *ad libitum*. The animal described fasted were deprived of food for 18 hr but had free access to water. The rats were maintained as per guideline of CPCSEA, India, and the study was approved by college ethical committee.

Preparation of the extract

The plant washed with water and dried in shade and successive solvent extraction was done using soxhlet with Pet ether (60-80°C), chloroform (yield 1.6%), methanol (9.40%) and ethanol (yield 8.7%). All the extracts were dried below 45°C in rotary evaporator and stored in airtight containers in refrigerator below 10°C. The various extracts obtained were tested phytochemically for the presence of various active constituents. Methanol gave the highest extractive value and was found positive for the presence of alkaloids, carbohydrate, flavonoids, proteins, amino acids, phenols, tannins, glycosides and steroids and was chosen for the present study.

Toxicity studies

The animals were divided into six groups separately and were treated orally with methanolic extracts of *Leptadaenia pyrotechnica* at 100, 200 and 400 mg/kg, body weight doses. The animals were continuously observed for 1 hr., then frequently for 14 days. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion [21].

Induction of Diabetes

Group of rats were fasted for 18 hr and were made hyperglycemic by intraperitoneal injection of streptozotocin dissolved in citrate buffer at a dose of 50 mg/Kg. After 48 hrs, their blood glucose level was estimated and the rats having plasma glucose level above 250mg% were selected and divided into 6 groups of 6 animals each [22].

- Group 1- Normal control received equal volume of normal saline and 2% gum acacia.
- Group 2- Diabetic control received normal saline (10 ml/kg) with 2% gum acacia
- Group 3, 4, 5- Received Methanolic extract of L. Pyrotechnica at a concentration of 100, 200, 300 mg/kg) with 2% gum acacia respectively.
- Group 6- Received Glibenclamide (10 mg/Kg) with 2% gum acacia.

The blood drop was collected from the tail for the estimation of glucose, just before drug estimation on first day, and 1hr after the drug estimation on 7, 14, 21 day.(Table 1)[23].

Biochemical parameters
Triglycerides, cholesterol, total proteins and Liver glycogen, were estimated from the serum by using commercially available diagnostic Kits.(Randox,UK) [24].

Statistical Analysis
Results are expressed as the mean ± S.E. and statistical significance between treated and diabetic control group was analyzed by one-way ANOVA followed by Student’s t-test and P<0.005 was considered significant.

Results
In the present study antidiabetic effect of methanolic extract of Leptadenia pyrotechnica (MELP) was evaluated for 21 days after administration of daily dose of 100, 200, & 300 mg/Kg of MELP. and it was found that there was a dose dependent reduction of plasma glucose from 7th day onwards which was found to be 27.25%, 39.63% &56.35% respectively on 21st day. Normal control and diabetic control did not show any significant change in their plasma glucose concentration where as Glibenclamide (10mg/kg) treated rats also showed a significant decrease in plasma glucose concentration.(Table 1)

The variation in body weight of different group of rats is depicted in (table 2). Before starting the experiment all the groups have no significant difference in their body weight .The Streptozotocin induced diabetic rat exhibited loss of bodyweight. A significant decrease was observed in the body weight of diabetic control group compared to normal control from 7th day onwards. A significant increase was observed in glibenclamide treated group. No significant increase in weight was observed in rats treated with 100mg/kg MELP.A significant increase in bodyweight was observed in rats treated with 200 & 300 mg/kg of MELP.

Serum triglycerides and serum cholesterol were found elevated in diabetic control. A significant dose dependent decrease in serum triglycerides and serum cholesterol was observed in animals treated with 300mg/kg of MELP and similar results were observed with the glibenclamide treated group (Table 3).

The diabetic rat exhibited the decreased level of liver glycogen and total protein. Diabetic rats treated with different concentration of MELP exhibited significant restoration of these parameters and same results were obtained with glibenclamide treated group.(Table 4).
Table 1- Effect of Methanolic extract of whole plant of Leptadenia pyrotechnica in streptozotocin induced hyperglycemia

<table>
<thead>
<tr>
<th>Treatment and dose</th>
<th>Plasma glucose concentration (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day (mg/ml)</td>
</tr>
<tr>
<td>Normal Control</td>
<td>104.67± 3.77</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>278.26±0.58</td>
</tr>
<tr>
<td>MELP(100 mg/Kg)</td>
<td>276.85±0.50</td>
</tr>
<tr>
<td>MELP (200mg/Kg)</td>
<td>274.64±0.33</td>
</tr>
<tr>
<td>MELP (300mg/Kg)</td>
<td>274.45±1.03</td>
</tr>
<tr>
<td>Glibenclamide (10mg/kg)</td>
<td>275.23±0.31</td>
</tr>
</tbody>
</table>

Each value represents mean± S.E.M (n=6)
Number in the parenthesis denotes percentage of reduction (plasma glucose)
<sup>a</sup>p<0.01, <sup>b</sup>p<0.001 vs control saline.

Table 2- Body weight variation of different groups of rats during treatment

<table>
<thead>
<tr>
<th>Group of Animals</th>
<th>Body Weight of Different Group (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0&lt;sup&gt;th&lt;/sup&gt; Day</td>
</tr>
<tr>
<td>Normal control</td>
<td>247.5±2.54</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>245.24±2.45</td>
</tr>
<tr>
<td>Diabetic+MELP(100mg/kg)</td>
<td>248.34±1.34</td>
</tr>
<tr>
<td>Diabetic+MELP(200mg/kg)</td>
<td>244.56±3.45</td>
</tr>
<tr>
<td>Diabetic+MELP(300mg/kg)</td>
<td>243.46±1.22</td>
</tr>
<tr>
<td>Diabetic+Glibenclamide (10mg/kg)</td>
<td>242.43±2.42</td>
</tr>
</tbody>
</table>
Values are expressed as mean ± S.E.M (n=6)  *P < 0.05 vs. Diabetic control.

Table 3- Serum Triglycerides and Serum Cholesterol in various groups of Streptozotocin induced diabetic rats after 21 days.

<table>
<thead>
<tr>
<th>Group of Animals</th>
<th>Serum triglycerides (mg/dl)</th>
<th>Serum cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>134±1.32</td>
<td>54.66±2.13</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>194±2.24</td>
<td>90.36±1.36</td>
</tr>
<tr>
<td>Diabetic+MELP(100mg/kg)</td>
<td>167±1.45*</td>
<td>74.21±2.49*</td>
</tr>
<tr>
<td>Diabetic+MELP(200mg/kg)</td>
<td>150±2.38*</td>
<td>63.89±1.86*</td>
</tr>
<tr>
<td>Diabetic+MELP(300mg/kg)</td>
<td>132±3.24*</td>
<td>57.35±2.49*</td>
</tr>
<tr>
<td>Diabetic+Glibenclamide (10mg//kg)</td>
<td>131±1.36*</td>
<td>55.92±1.66*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M (n=6)  *P < 0.05 vs. Diabetic control

Table 4- Liver Glycogen and total protein concentration of different groups of animals after 21 days.

<table>
<thead>
<tr>
<th>Group of Animals</th>
<th>Liver Glycogen (mg/of tissue)</th>
<th>Total protein (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>37.87 ±1.10</td>
<td>5.86 ± 0.19</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>21.31 ± 0.85</td>
<td>4.04 ± 0.42</td>
</tr>
<tr>
<td>Diabetic+MELP(100mg/kg)</td>
<td>30.32± 1.45*</td>
<td>5.11±0.34*</td>
</tr>
<tr>
<td>Diabetic+MELP(200mg/kg)</td>
<td>36.56± 1.38*</td>
<td>5.67±1.22*</td>
</tr>
<tr>
<td>Diabetic+MELP(300mg/kg)</td>
<td>41.48± 2.33*</td>
<td>6.65±0.35*</td>
</tr>
<tr>
<td>Diabetic+Glibenclamide (10mg//kg)</td>
<td>43.87±1.57*</td>
<td>6.45±0.66*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M (n=6)  *P < 0.05 vs. Diabetic control

Discussion

In the present study antidiabetic activity of Methanolic extract of Leptadenia pyrotechnica was evaluated. Streptozotocin induced high blood glucose level in untreated group of rats which may be due to selective destruction of beta cell of pancreas [25], this has caused severe insulin

deficiency leading to elevated glucose level disturbed biochemical parameters such as serum cholesterol, triglycerides, total proteins and liver glycogen. MELP exhibited dose dependent lowering of blood glucose level, maximum lowering of 56.35% took place at a concentration of 300mg/kg of extract and a lowering of 59.14% was observed with glibenclamide treated group. It is a well known fact that, most of the synthesized drugs are highly efficacious but induced several intolerable side effects The use of herbal medicines in diabetes treatment produces few side effects compared to synthesized drugs[26].The glucose lowering effect of MELP may be due to increased glucose uptake by adipocytes, or increased lipogenesis [27], increase glucose metabolism in cells[28], or stimulation of β cells leading to increased insulin release [29] may be some of the potential mechanism for glucose lowering effect which may be due to the presence of flavonoids, tannins, β sitosterol, polyhydroxy pregnane glycosides and pentacyclic triterpenoid present in Leptadenia pyrotechnica.

Significant reduction in the weight of diabetic rats is observed which may be due to increased amount of glucose and insufficient amount of insulin in the blood stream. This triggers the release of triglycerides from adipose tissue and catabolism of amino acids in the muscle tissue, leading to loss of fat and lean mass, which ultimately causes a significant reduction in body weight [30]. Serum triglyceride and serum cholesterol was found to be elevated in Diabetic group as compared to normal group. The possible mechanism behind it may be insulin resistance which play a major role in the development of increased level of cholesterol and triglycerides as there is an increased free fatty- acid release from insulin resistant fat cells [31,32,33,34].This increased flux of free fatty acids into the liver in the presence of glycogen promotes triglyceride production, which in turn stimulates the secretion of apolipoproteins B(ApoB) and VLDL cholesterol leading to increased VLDL cholesterol production and decreased HDL cholesterol production and hepatic fat accumulation [35,36]. There is significant reduction in triglycerides and cholesterol level in MELP treated group and glibenclamide treated group which may be due to the increased production of insulin as well as increased sensitivity of the cell towards insulin.

Diabetes acutely impairs the ability of liver to synthesize glycogen. It is reported that in the fed state the liver glycogen level were markedly decreased in short term diabetic animals and in fasting state the liver glycogen was depleted in acute diabetes. It is due to the fact that the level of glycogen synthase enzyme in its active form is lower in diabetic group than normal group [37]. So liver glycogen was least (21.31 mg/tissue) in diabetic rats whereas MELP treated rats and glibenclamide treated rats depicted significant level of liver glycogen which may be due to improved glycogenesis.

Due to destruction of β cells after administration of streptozotocin there is decrease in insulin level this hypoinsulinemia reduces protein synthesis in many tissue including skeletal muscles. [38,39,40]. The total protein was found significantly high in MELP treated group (6.65 gm/dl at as dose of 300mg/kg) as compared to diabetic group(4.04gm/dl) which may be due to increased secretion of insulin.

Conclusion

In the present study Methanolic extract of Leptadenia pyrotechnica in the varying concentration of 100, 200, &300 mg/kg was evaluated for its antidiabetic activity. The extract exhibited dose dependent lowering of serum glucose along with normalization of elevated serum cholesterol, triglycerides. It also exhibited maintenance of decreased liver glycogen, total protein and loss in
body weight. This study confirms the antidiabetic activity of *Leptadenia pyrotechnica* but further studies are required to search for the active principles behind its therapeutic effect.

References


