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Original Article

ANTIDIABETIC ACTIVITY OF CLERODENDRUM PHLomidis L. AGAINST STREPTOZOTOCIN (STZ) INDUCED DIABETICS IN RATS

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Abstract

The investigation was carried out to study the effects of Clerodendrum phlomidis leaves extract on blood glucose and lipid profile level. The antidiabetic efficacy of the ethanolic extract of the leaves was evaluated in normal and Streptozotocin (STZ) induced diabetic rats. The extract exhibited significant hypoglycemic and hypolipidemic activity in animal model when compared with control group. The activity was also comparable to that of the effect produced by standard antidiabetic agent Glibenclamide. The hypoglycemic produced by the extract may be due to increased uptake og glucose at tissue level or increase in pancreatic betal cell function or due to inhibition of intestinal absorption of glucose. The study indicated that the ethanolic extract is a potential antidiabetic agent and lends scientific support for its else’s in folk medicine.

Keywords:- Glucose, Clerodendrum phlomidis, Streptozotocin, Lipid profile.

1. Introduction

Diabetes is a metabolic disorder of carbohydrate, fat and protein, affecting a large number of population in the world (Pareek et al., 2009). Diabetes mellitus is not a single disorder but it is a group of metabolic disorder characterised by chronic hyperglycemia, resulting from defects in insulin secretion, insulin action, or both. Increased thirst, increased urinary output, ketonemia and ketonuria are the common symptoms of diabetes mellitus, which occur due to the abnormalities in carbohydrate, fat, and protein metabolism. When ketones body is present in the blood or urine, it is called ketoacidosis, hence proper treatment should be taken immediately, else it can leads to other diabetic complications (Craig et al., 2009). Diabetes mellitus has caused significant morbidity and mortality due to microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications (Thevenod, 2008). Diabetes is mainly attributed to the rapid rise in unhealthy life style, urbanization and aging.

Herbal medications have been used for the treatment of variety of ailments, a huge number of population in the world is entirely dependent on traditional medicines (Feshani et al., 2011). A number of medicinal plants and their formulations are used for treating diabetes in Ayurvedic medicine system as well as in ethnomedicinal practices (Pareek et al., 2009). In India, indigenous remedies have been used in the treatment of diabetes mellitus since the time of Charaka and Shusrutha. From the ethnobotanical information, about 800 plants which may possess anti-diabetic potential have been found (Warjeet Singh, 2011; Patel et al., 2011). In the present study to investigate the antidiabetic activity of Clerodendrum phlomidis leaves on Streptozotocin (STZ) induced diabetic rats.

2. Materials and Methods

2.1 Animals

Male albino rats of Wistar strain approximately weighing 160-180g were used in this study. They were healthy animals purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2º C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided ad libitum. They were acclimatized to the environment for one week prior to experimental use. The animal feed composition is crude protein (22.3%), crude oil (4.01%), crude fibre (4.02%), Ash (8.02%) and sand silical...
Table 1: Effect of Clerodendrum phlomidis on glucose and lipid profile in experimental rats

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Glucose (mg/dl)</td>
<td>84.74 ±5.93</td>
<td>347.45 ±28.32*</td>
<td>86.61 ±6.76</td>
<td>103.38 ±7.23</td>
</tr>
<tr>
<td>2.</td>
<td>Hb</td>
<td>17.90 ±1.25</td>
<td>8.24 ±0.57*</td>
<td>15.62 ±1.09</td>
<td>14.20 ±0.99</td>
</tr>
<tr>
<td>3.</td>
<td>Triglyceride (mg/dl)</td>
<td>77.77 ±5.44</td>
<td>252.77 ±17.69*</td>
<td>133.33 ±9.33</td>
<td>138.88 ±9.72</td>
</tr>
<tr>
<td>4.</td>
<td>Cholesterol (mg/dl)</td>
<td>36.36 ±2.54</td>
<td>136.36 ±9.54*</td>
<td>57.57 ±4.02</td>
<td>63.63 ±4.45</td>
</tr>
<tr>
<td>5.</td>
<td>HDL – Cholesterol (mg/dl)</td>
<td>39.58 ±2.76</td>
<td>18.75 ±1.31*</td>
<td>29.16 ±2.04</td>
<td>29.16 ±2.04</td>
</tr>
<tr>
<td>6.</td>
<td>VLDL – Cholesterol (mg/dl)</td>
<td>15.55 ±1.08</td>
<td>50.55 ±3.33*</td>
<td>26.66 ±1.86</td>
<td>27.77 ±1.94</td>
</tr>
<tr>
<td>7.</td>
<td>LDL – Cholesterol (mg/dl)</td>
<td>12.33 ±0.86</td>
<td>104.56 ±7.31*</td>
<td>55.07 ±3.85</td>
<td>62.24 ±4.35</td>
</tr>
</tbody>
</table>

Values are expresses as Mean ± SD for six rats
Significantly different from group I, III and IV (p<0.05)

(1.02%).

2.2 Chemicals:
Streptozotocin (STZ), Ethylene Diamine Tetra Acetic Acid (EDTA)), Glibenclamide (Prudence Pharma Chem, India), Chlороform were purchased for Sigma chemical company, Mumbai All other chemicals and reagents used in this study was of analytical grade with high purity and were obtained from Glaxo laboratories and Sisco Research laboratories, Mumbai, India.

2.3 Plant materials and preparation of extract
The fully mature Clerodendrum phlomidis leaves were collected in April 2013 from Poyyundar kottai, Thanjavur District, Tamil Nadu, India from a single herb. The leaves were identified and authenticated by Botanist, Dr. S John Britto, Department of Botany, St. Josephs College, Tiruchirappalli, Tamil Nadu, India. A Voucher specimen (VL 0.05) has been deposited at the Rapinat Herbarium, St. Josephs College, Tiruchirappalli, Tamil Nadu, India. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with 70% ethanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used.

2.4 Streptozotocin (STZ) Induced Diabetic rats
Diabetes was induced in all groups except vehicle control following overnight fasting(deprived of food for16h allowed free access to water) by a single intraperitoneal injection of 65mg/kg of streptozotocin (STZ) dissolved in a freshly prepared 0.1M citrate buffer (pH4.5) (Liu et al., 2008).The animals of vehicle control (Group I) were injected with buffer alone. Diabetes was confirmed 72 h after induction by measurement of tail vein blood glucose levels by glucose oxidase-peroxidase method using strips. Plant extracts at a dose of 500mg/kg was orally given once a day for 15 days after hyperglycemia was confirmed by the elevated glucose levels in blood determined at 72 h, Glibenclamide is used as a standard at dose of 0.25mg/kg (Arulmozhi et al., 2010).

2.5 Experimental Design
The animals were divided into four groups of six animals each as follows:
- Group I- Vehicle control were injected with buffer alone (Non-diabetic)
- Group II- Diabetic control
- Group III- Clerodendrum phlomidis leaves extract 500mg/kg was orally given once a day for 21 days.
- Group IV- Diabetic standard as 0.25mg/kg of Glibenclamide, p.o was orally given once a day for 21 days.

2.6 Collection of blood and preparation of plasma sample
At the end of the experimental period, the animals were anaesthetized using chloroform vapour prior to dissection. The blood was collected with EDTA as anticoagulant. The blood was allowed to clot by standing at room temperature for 30 minutes. The blood was centrifuged at 3000rpm for 10minutes, and then the plasma (supernatant) was isolated and stored at refrigerated until required for analysis.

2.7 Biochemical estimations
Glucose was estimated by GOD/POD method (Trinder, 1969). Cholesterol was estimated by Allain et al (1974). Triglyceride was determined by the method of Werner et al (1981). HDL cholesterol was estimated by the method of Allain et al (1974). LDL VLDL cholesterol was calculated as per Friedewald’s (1972) equation. Haemoglobin was estimated by Cyanmethaemoglobin method (Dacie and Lewis, 1968) (Beacon Diagnostic Kit).

2.8 Statistical Analysis
Values were expressed as mean ± SD for six rats in each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey’s test for multiple comparisons (Harvey and Paige, 1998). The results were statistically analyzed by Graphpad Instat Software (Graphpad Software, San Diego, CA, USA) version 3 was used and p<0.05 was considered to be significant.

3. Results
The ethanolic extract of of Clerodendrum phlomidis was administered orally in an aqueous solution at a dose of 500mg/kg body wt, to diabetic rats to assess the synergetic impact of the plant extract. The plant extracts were fed with normal and diabetes induced rats. The blood glucose levels was significantly (P<0.05) reduced when compared to the specific diabetic control animals.

The lipid profile such as TC, TG, LDL and VLDL levels were significantly increased in diabetic control animals (DC) whereas HDL levels were decreased when compared to the control rats. The plant extract was administered orally at a dose of 500mg/kg body wt., to diabetic rats significant (P<0.05) depletion in the total cholesterol, TG, LDL, and VLDL levels and increment of
Figure 1: shows the % of Antidiabetic activity of Clerodendrum phlomidis in experimental rats

Table 2 - % of Antidiabetic activity of Clerodendrum phlomidis in experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>% of Antidiabetic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>--</td>
</tr>
<tr>
<td>Group II</td>
<td>--</td>
</tr>
<tr>
<td>Group III</td>
<td>75.07</td>
</tr>
<tr>
<td>Group IV</td>
<td>70.24</td>
</tr>
</tbody>
</table>

HDL levels were recorded in the diabetic animals (Table 1).

4. Discussion

Insulin is the dominant hormone which influencing the regulation of glucose metabolism. One of the major effects of insulin is to enhance overall glucose disposal by stimulation of glucose uptake into the target tissues (Tiwari and Madhusudana, 2002). In diabetes the increased blood sugar levels might be due to either insulin resistance of the body cells or decreased secretion of insulin from beta cells manifest in the decreased serum insulin levels (Mohammad Ali, et al., 2004). Streptozotocin (STZ) (2-deoxy-d-(3-methyl-3-nitrosuuredio)-D-gluco-pyranose) is commonly used for experimental induction of type-I diabetes mellitus, which causes selective pancreatic islet β-cell cytotoxicity mediated through the release of nitric oxide (NO). This results in rapid reduction in pancreatic islet pyridine nucleotide concentration and subsequent β-cell necrosis. The action of STZ on mitochondria generates SOD anions, which leads to diabetic complications (Papaccio et al., 2000). Based on the above perspectives, in the present study, the antidiabetic activity has been assessed in rats made diabetic by STZ. Sulfonylureas such as glibenclamide are often used as a standard antidiabetic drug in STZ-induced diabetes to compare the efficacy of variety of antihyperglycemic compounds (Anderson et al., 1974).

The STZ selectively destroys the pancreatic cells and induce hyperglycemia (Gilman et al., 1990, Kurup and Bhide, 2000, Jarvenin, 1995). The reduction in the serum insulin levels in the STZ treated rats might be attributed to the reduced secretion of the hormone which might be due to the damage of the beta cells of endocrine pancreas were recorded earlier studies (Yoon and Ray, 1985). Nitric oxide has been demonstrated to participate in the beta cell damage during STZ induced diabetes (Duran Reges et al., 2004). Administration of Clerodendrum phlomidis to diabetic rats restored the levels of glucose. Present finding is in agreement with Subramaniam et al. (2012) studies.

The insulin deficiency depletes the activity level of lipoprotein lipase, thus leading to deranged lipoprotein metabolism during diabetes (Ranganathan et al., 2000). Diabetes affects both glucose and lipid metabolism (Sperling et al., 2000). In the post prandial state elevated serum insulin increases lipoprotein lipase activity in adipose tissue and promotes fuel storage as triglycerides in normal metabolism (Bhagavan, 2002).

The elevated hypertriglyceridemia was increased in the synthesis of triglyceride rich lipoprotein particles (very low density lipoprotein, VLDL) in liver diminished catabolism in diabetic rats reported by Ginsberg (1991). The lipoprotein levels in the STZ induced diabetic rats of the present study reveal a significant alter in lipoprotein metabolism. The serum total cholesterol content increased significantly in diabetic animals. Since insulin has a potent inhibitory effect on lipolysis in adipocytes, insulin deficiency is associated with excess lipolysis and increased influx of free fatty acids to the liver (Coppack, 1994, Ohno, 2000 ). The increased levels of low-density lipoprotein (LDL) and very low density lipoprotein (VLDL) in the diabetic animals might be due to over production of LDL and VLDL by the liver due to the stimulation of hepatic triglyceride synthesis as a result of free fatty acid influx (Coppack, 1994). The high density lipoprotein (HDL) was significantly reduced in the diabetic rats which indicate a positive risk factor for atherosclerosis (Bopanna, 1997). Supplementation of Clerodendrum phlomidis leaf extract to diabetic rats restored the lipid profile. Our results concord with the earlier work done by Kesari et al. (2006), where it has been reported that lipid profile level in the
plasma is restored with the treatment of *Aegle marmelos* seed extract in diabetic rats. After the administration of the extract of *Clerodendrum phlomidis* to the STZ induced diabetic rats revealed augmented serum insulin levels. The increment of serum insulin levels might be due to increased secretion of the hormone, which might reflect the probable ‘repair’ of the damaged beta cells of the endocrine of the pancreas due to STZ.

The levels of serum TC, TG, LDL, and VLDL were found to be significantly reduced in the plant extracts treated diabetic animals. The blood glucose level of *Clerodendrum phlomidis* extract fed animal was significantly (*P*<0.05) reduced. This might be due to the reduced hepatic triglyceride synthesis and or reduced lipolysis that might be due to the increase in serum insulin levels in the plant extract treated rats. The HDL increased significantly in the plant extract treated rats indicating a reversed atherogenic risk.

Glycosylated haemoglobin (HbA1) was found to increase in patients with diabetes mellitus to approximately 16% (Koenig et al., 1976) and the amount of increase is directly proportional to the blood glucose level (Jackson et al., 1979). In uncontrolled or poorly controlled diabetes there is an increased glycosylation of a number of proteins including haemoglobin and α-crystalline of lens (Alberti and Press, 1982). During diabetes the excess glucose present in blood reacts with haemoglobin. Therefore, the total haemoglobin level is decreased in STZ diabetic rats.

The present study demonstrated that the *Clerodendrum phlomidis* extract possess hypoglycemic effect revealed by decreased serum lipid levels, restored glucose and haemoglobin and therefore attributes to therapeutic value of *Clerodendrum phlomidis* leaves extract to combat the diabetic condition in rats. The potential antidiabetic activity of *Clerodendrum phlomidis* extract due to the phytochemicals.

5. **Acknowledgement**

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6. **References**


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