Anti-Diabetic and Antihyperlipidemic Activities of Alternanthera Sessilis in Experimentally Induced Type 2 Diabetes

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Abstract: The antidiabetic and antihyperlipidemic effects of ethanolic extract of whole plant of Alternanthera sessilis (EEAS) was investigated in Streptozotocin (STZ) induced type 2 diabetic rats. Diabetes was confirmed after 3 days of single intraperitoneal injection of STZ (35mg/kg) in albino Wistar rats. EEAS (100 and 200mg/kg) and glibenclamide (10mg/kg) orally administered daily for 15 days respectively, blood was withdrawn for glucose determination on 1, 7, 15 days respectively. On 15th day, overnight fasted rats were sacrificed and blood was collected for determination of high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), total cholesterol (TC), triglycerides (TG). EEAS at doses of 100mg/kg and 200mg/kg showed significant reduction in blood glucose and lipid profiles when compared to diabetic control group. We concluded that EEAS possess antidiabetic and antihyperlipidemic activities.

Keywords: Alternanthera sessilis, Streptozotocin, Antidiabetic, Antihyperlipidemic

I. Introduction

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia and affecting nearly 10% of the population all over the world [Burke J.P, et al. 2003]. In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus [Sumana G, et al. 2001]. There is increasing demand by patients to use natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents [Holmann et al. 1991]. The world health organization has also recommended the effectiveness of plants in condition where we lack safe mode drugs.

Alternanthera sessilis Linn. is an aquatic plant belonging to family of Amaranthaceae is a perennial herb. It is locally called as Ponnagantikoora. The leaves are used in eye diseases, cuts, wounds and antidote to snake bite; skin diseases. It is also reported about the wound healing property of Alternanthera sessilis. The plants aerial parts also have shown a hepatoprotective activity. Till date many of the plants were investigated for antidiabetic activity and this research continues on several other medicinal plants to reduce use of synthetic antidiabetics. The present study focused to evaluate ethanolic extract of Alternanthera sessilis, at various doses in streptozotocin induced diabetic rats as most of the species of amaranthaceae show antidiabetic activity. Most of the phytoconstituents dissolve in the ethanolic extract producing little side effects on animals (Praveen nayak et. al., 2010).

II. Materials & Methods

PLANT MATERIAL:
Alternanthera sessilis was collected from Natural habitat near Padmaakshamma temple, Hanamkonda in the month of August 2010, and authenticated by Professor E.N.Murthy, Department of Botany, University College of Pharmaceutical sciences, Warangal.

PREPARATION OF EXTRACT OF ALTERNANTHERA SESSILIS:
Fresh plant after collection was washed under tap water and was dried under shade for about one week. The dried whole plant were grounded and made into powder with the help of a laboratory mixer. Then the powder obtained was weighed and subjected to extraction. The dried whole plant is macerated in 90% ethanol and evaporated to dryness and concentrated under reduced pressure below 50°C, until a soft mass obtained and then preserved in desiccator

EXPERIMENTAL ANIMALS:
Wistar Rats weighing about 200 to 250g were purchased from Mahaveer enterprises, Hyderabad, India. The animals were housed in standard polypropylene cages, and maintained under standard laboratory conditions (12:12 hr light and dark cycle; at an ambient temperature of 25±5°C). They were fed standard laboratory diet and were given sterilized water ad libitum. The experimental protocol has been approved by Institutional animal ethics committee (CPCSEA/1407/ac/07).
INDUCTION OF TYPE 2 DIABETES IN RATS:

After four weeks feeding of high energy diet, the rats were fasted overnight. The rats were made diabetic by single i.p. injection of Streptozotocin at a dose of 35mg/kg body weight freshly dissolved in ice cold citrate buffer (pH 4.4). It was followed by administration of 5% Glucose solution for the next 24hrs. The high energy diet was fed for rats for the remaining experiment period. (Srinivasan K et al., 2008, Hui Jie Wang et al., 2007). High energy diet constitutes include 140 g total milk protein and 722 g carbohydrate/kg of diet(Fang Xie, Han Fu 2011). Acute oral toxicity tests were performed as per OECD guidelines and the therapeutic dose was selected based on the maximum cutoff value i.e., 2000mg/kg body weight (Sunil S. Jalalpure et al., 2008). Thirty rats were randomly divided into five groups among which one group serves as normal control i.e., without inducing diabetes and the remaining four groups were induced with diabetes.

ASSESSMENT OF EFFECT OF EEAS ON BLOOD GLUCOSE LEVEL AND DIFFERENT BIOCHEMICAL PARAMETERS IN 14 DAYS (SUB CHRONIC) STUDY IN STZ-INDUCED DIABETIC RATS:

All the rats were overnight fasted.
Group 1 served as normal control received only vehicle (5% Gum Acacia, p.o.) containing six animals.
Remaining STZ-induced diabetic rats were divided into four groups of six animals.
Group 2 served as diabetic control received only vehicle (5% Gum Acacia,p.o).
Group 3 served as standard received Glibenclamide at a dose of 10mg/kg,p.o.
Group 4 and 5 received EEAS orally at the dose level of 100 and 200mg/kg,p.o.
Blood samples were drawn from Retro-orbital plexus of the rat under mild ether anesthesia. Serum was separated by centrifugation and serum glucose levels were estimated by Glucose oxidase and peroxidase method. Then the rats were treated with freshly prepared extracts once a day. From the starting day of extract supplementation, blood glucose levels were measured on Day 1, Day 7 and Day 15 (B.S. Ashok Kumar et al., 2010). All the rats were sacrificed on Day 15 and serum Total Cholesterol, serum Triglycerides, serum HDL - Cholesterol, serum LDL Cholesterol levels were estimated by standard kits.

III. Statistical Analysis

Data were presented as mean±SD and were analysed by student’s t test & one way ANOVA using GraphPad prism5.0 software. Values were considered significant when p values were <0.05 or less.

IV. Results

a. Antidiabetic activity of ethanolic extract of Alternanthera sessilis on STZ Induced type 2 diabetic rats subacute (15days) study:

The EEAS at doses of 100 and 200mg/kg was orally administered to diabetic rats once daily for 15 days, and the non-fasted blood glucose levels were determined at after 0th, 1st, 7th and 15th day. Table 1: showing the effect of ethanolic extract of A.sessilis on glucose level in STZ-induced diabetic rats after the daily treatment (100 and 200mg/kg) for 15 days. Showed significant (p < 0.005) percentage fall in blood glucose levels with the doses of 100, 200mg/kg. of EEAS and10mg/kg of Glibenclamide as compared to diabetic control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood glucose level in mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st day</td>
</tr>
<tr>
<td>I</td>
<td>Normal control</td>
<td>72.40±2.314</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>257.75±25.966</td>
</tr>
<tr>
<td>III</td>
<td>Glibenclamide(10mg/kg)</td>
<td>206.25±13.793***</td>
</tr>
<tr>
<td>IV</td>
<td>EEAS(100mg/kg)</td>
<td>235.25±30.346**</td>
</tr>
<tr>
<td>V</td>
<td>EEAS(200mg/kg)</td>
<td>240.5±15.154**</td>
</tr>
</tbody>
</table>

All the values are expressed in mean±SD. **p <0.05, p <0.001 compared with corresponding values of diabetic control animals

b. Antihyperlipidemic activity of EEAS (100 & 200mg/kg) in STZ induced type 2 diabetic rats:

Table 2: showing the effect of EEAS (100 and 200mg/kg) on Serum Lipid profile on STZ induced diabetic rats treated for 15days compared to diabetic control rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Triglycerides</th>
<th>Total Cholesterol</th>
<th>HDL Cholesterol</th>
<th>LDL Cholesterol</th>
<th>LDL/HDL Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>85.71±3.62</td>
<td>145.48±4.86</td>
<td>34.96±3.90</td>
<td>93.37±2.65</td>
<td>2.70±0.50</td>
</tr>
<tr>
<td>Diabetic-control</td>
<td>183.71±3.90</td>
<td>285.33±2.64</td>
<td>26.70±1.85</td>
<td>221.89±3.63</td>
<td>8.34±0.70</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>75.06±3.146**</td>
<td>133.28±5.55**</td>
<td>40.34±0.716**</td>
<td>77.93±4.09**</td>
<td>1.93±0.182**</td>
</tr>
<tr>
<td>EEAS 200mg/kg</td>
<td>92.03±3.68**</td>
<td>168.50±3.98**</td>
<td>34.5±4.18**</td>
<td>108.34±3.55**</td>
<td>3.40±0.64**</td>
</tr>
</tbody>
</table>

All values are expressed as Mean±SD, **p<0.05 when compared with the diabetic control.
V. Discussion

Among currently available drugs, synthetic drugs have potential adverse effects, which can be minimized to greater extent through natural compounds. Still there are many natural drugs which are yet to be investigated scientifically. In the present investigation, the whole plants were collected, dried and subjected to size reduction to get uniform coarse powder. Then the powder was subjected to maceration with 90% ethanol. The concentrated extracts were used for study. The study was undertaken to evaluate the hypoglycemic activity in Streptozotocin (STZ) induced diabetic rats. The present study deals with two dimensions for antidiabetic effects of ethanolic extract of whole plant of *Alternanthera sessilis* (EEAS). In one dimension, the blood glucose levels were measured. In the other dimension, the antihyperlipidemic potency of EEAS was studied as there is a close correlation between hyperglycemia and hyperlipidemia (Chhanda Mallick et al., 2006).

STZ significantly induced hyperglycemia and upon oral administration of EEAS for 14 days caused a significant (p<0.001) decrease in blood glucose levels which is similar to the significant hypoglycemic effect produced in the diabetic rats upon repeated oral administration of the water extract at a dose of 0.125 g/kg for 7 days (Penchom Peungvicha et al., 1998). Hypercholesterolemia and hypertriglyceridemia are primary factors involved in the development of atherosclerosis and coronary heart diseases which are secondary complications of diabetes. EEAS significantly (p<0.05) reduced serum triglycerides and total cholesterol in STZ induced diabetic rats which is similar to results obtained in a study where Oral administration of MEAS (200 and 400 mg/kg) for 15 days showed significant reduction in the elevated blood lipid levels. Thus it is reasonable to conclude that EEAS could modulate blood lipid abnormalities (Penchom Peungvicha et al., 1998, B.S.Ashok Kumar et al., 2010). Results showed that the antidiabetic activity of EEAS was not found dose dependent, as there was no significant differences between the 100 and 200 mg/kg extract on the treated groups. The obtained results in our study may suggest that the antihyperglycaemic activity of the EEAS, may be due to a stimulating insulin release from the remnant b cells in the islets of Langerhans. This is the same mechanism exerted by the sulfonyl drugs as glibenclamide which involves an improvement in insulin action at cellular level (Fatima Torrico et. al., 2007). There are many reports available to support the multiple mechanisms of antidiabetic plants to exert their blood glucose lowering effect, such as inhibition of carbohydrate metabolizing enzymes, enhancement of insulin sensitivity, regeneration of damaged pancreatic islet β-cells, and enhancement of insulin secretion and release (V. Sabitha, S. Ramachandran et al., 2011). The antidiabetic activity of EEAS may be due to its preventive effect on structural protein degradation.

VI. Conclusion

The ethanolic extract of whole plant of *Alternanthera sessilis* (EEAS) has antidiabetic activity as it significantly lowered blood glucose levels in diabetic rats in subacute (15days) study. The serum lipids were recovered to normal levels in diabetic rats treated with EEAS. The antidiabetic activity of EEAS may be due to its preventive effect on structural protein degradation. But mechanism of action of the extract has to be studied further at the molecular level.

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References


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