ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF TRICHOPUS ZEYLANICUS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT
The aim of present study was externally induced oxidative stress and evaluates Antioxidant Activity and antidiabetic activity of ethanolic extract of Trichopus zeylanicus. Family Malvaceae leaves in streptozotocin induced diabetic rats. The ethanolic extract of Trichopus zeylanicus was studied for antidiabetic activity in streptozotocin induced diabetic rats by oral administration of extract 400mg/kg body weight for 15 days. The effect was compared with standard drug of Glibenclamide at oral dose of 0.5mg/kg. Determination of blood glucose level by GOD-POD kit method. The result shows the ethanolic extract of Trichopus zeylanicus leaves significantly lowered the blood glucose of hyperglycemic rats. From the toxicity study it was observed that ethanolic extract of Trichopus zeylanicus was nontoxic up to 5g/kg body weight and phytochemical study showed the presence of phytosterols, flavonoids and glycosides. It is concluded that Trichopus zeylanicus leaf extract has significant antidiabetic activity, which lowered the fasting blood glucose level in Streptozotocin induced diabetic rats.

KEYWORDS: Anti diabetic activity, Trichopus zeylanicus, Streptozotocin, GOD-POD.

INTRODUCTION
Diabetes mellitus (DM) is a group of diseases characterized by high levels of blood glucose resulting from decreases in insulin production, insulin action, or both. According to WHO prediction, the prevalence of diabetes is likely to increase by 47%. Currently, there are over
150 million diabetic patients worldwide and this is likely to increase to more than 500 million of in the year of 2025. Statistical data analysis suggests that in the number of diabetic patient will rise from 20 million in 1995 to 57 million in the year 2025; Indians are highest number of diabetics in the world.\textsuperscript{[2]} Reasons for this rise include increase in sedentary lifestyle, consumption of junk diet, obesity, etc. Where diabetes mellitus rates could rise to twofold to threefold than the present rates.\textsuperscript{[3]} Evaluation of plant products to treat diabetes mellitus is of growing interest as they contain many bioactive substances like glycosides, flavonoids alkaloids have therapeutic potential. Based on Ethano botanical studies shows medicinal plants have efficacy and safety of treatment of in diabetes mellitus.

**MATERIALS AND METHODS**

**Plant material**

Fresh leaves were collected from in month of October from Gingee, Villupuram Dist, Tamilnadu, India and authentified by Prof. Jeyaraman, Botanist, plant Anatomy Research Centre, Tambram. And the specimen was submitted for further reference.

**Extraction**

The leaves were shade dried and powdered with help of dry mechanical grinder, and size separation and made coarse powder. The leaves powdered were extracted with different organic solvents using continuous hot percolation method by gradient elution technique. The extracts were concentrated to dryness and the phytochemical screening was done to find out active constituents.

**Animals**

Swiss albino mice of female sex weighing 20-25gms were employed for toxicity study. Wistar albino rats of male sex weighing 200-250 gms were employed for antidiabetic study. Animals were housed in standard environment condition are maintain and fed with standard rodent diet of ab libitum with water. Ethical clearance for the animal study was obtained from Institutional Animal Ethical Committee (09MP03AUG2009) of CPCSEA (887/ac/CPCSEA).

**Toxicity Study**

An acute oral toxicity study was performed as per OECD guidelines 423. In acute toxic class method Swiss albino mice of female sex weighing 20-25gms were used for the study. Acute toxic class method is a stepwise procedure with use of three animals of a single sex per group, depending on mortality or morbidity status of the animals. Average 2-4 steps may be
judgment on the acute toxicity of the substance. Three rats were used for each step. The animal were observed individually any states of toxicity, morbidity or mortality during the first 24hrs, with special given observation during the first 4 hours and daily thereafter for a total of 14 days.\[9\]

**Induction of diabetes**

All the rats were fasted overnight before the administration of Streptozotocin. By induction of diabetes in rats by intra peritoneal injection of Streptozotocin dissolved in buffer solution containing 0.1M sodium citrate buffer pH4.5 at the dose of 50mg/kg body weight. After the injection animals had free access to food and water. The development of diabetes was confirmed by glucometer after 48hrs of Streptozotocin injection. The fasting blood glucose level more than 200mg/dl were considered as diabetic rats and used for the experimentation. Diabetic animals were grouped after five days induction of diabetes Effect of Ethanolic Extract of Trichopus zeylanicus in Streptozotocin induced diabetes in rats.

**EXPERIMENTAL DESIGN**

In the experiment rats were divided into the following four groups with six animals each

- **Group I**: Normal control of Wister rats received 1% w/v gum acacia 1ml/kg for 15 days orally.
- **Group II**: Diabetic control of Wister rats received 1% w/v gum acacia 1ml/kg for 15 days orally.
- **Group III**: Diabetic rats received ethanolic leaves extract of Trichopus zeylanicus 400mg/kg body weight once a day orally for 15 days.
- **Group IV**: Diabetic rats treated with standard drug of Glibenclamide 0.5mg/kg orally once a day for 15 days.

Rats were fasted overnight and the blood was withdrawn from the orbital sinus of the eye on the 5th day, 15th day and 20th day post induction of diabetes to determine blood glucose by GOD-POD kit method. Diabetes rats change body weight also observed throughout treatment period in experimental animals.

**ESTIMATION OF BIOCHEMICAL PARAMETERS IN BLOOD SERUM**

After the completion of experiment, the blood were collected through the retro orbital puncture with of eye of animal under sterile condition with mild anesthesia in Eppendorff’s tube (1 mL)containing 50 μL of anticoagulant (10 %trisodium citrate) and the serum was
separated by centrifuging at 6,000 rpm for 15 min. and estimate The blood biochemical parameters like SOD, Catalyase, GSH,GPX And LPO were analyzed.

STATISTICAL ANALYSIS
All values were expressed as Mean ± S.D. The differences between control and treatment groups were tested for significance using ANOVA followed by Dunnet’s t test. P<0.05 were considered significant.

RESULTS
The preliminary phytochemical studies indicate the presence of bio active substance carbohydrates, phytosterols, Flavonoids and glycosides, confirmed by phytochemical tests in ethanolic extract Trichopus zeylanicus leaf. In acute toxicity study the ethanolic extract of Trichopus zeylanicus did not produce lethality up to the dose level of 2000mg/kg.

Table- 1: Effect of Trichopus zeylanicus leaf extract on body weight in Streptozotocin induced diabetic rates
Values are expressed as Mean ± S.E. n=6.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight in gms(Mean±SEM)</th>
<th>Post induction days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5th day</td>
<td>15th day</td>
</tr>
<tr>
<td>Control</td>
<td>167.2±3.25</td>
<td>173±3.54</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>163.8±3.34</td>
<td>136.8±2.10*</td>
</tr>
<tr>
<td>Diabetic rats+ Control</td>
<td>162.3±1.98</td>
<td>172.3±1.76*</td>
</tr>
<tr>
<td>Diabetic rats+ glibenclamide</td>
<td>165.1±2.77</td>
<td>170.8±2.62*</td>
</tr>
</tbody>
</table>

P*<0.05 Experimental groups were compared with diabetic control.
P*<0.05 Diabetic groups were compared with control group.

In the antidiabetic activity, the effects of etanolic extract Trichopus zeylanicus on body weight is measured on 5th, 15th and 20th day of post induction diabetes were compared with normal and diabetic control groups. As per above shown in Table No-1. Streptozotocin induced diabetic rats showed a significant decrease (P<0.05) in body weight compared to normal rats. In oral administration of leaf extract of Trichopus zeylanicus at the dose of 400mg/kg showed a significant) increase (P<0.05) in body weight on 15th and 20th day of post induction when compared to untreated diabetic rats.
Table- 2: Effect of Trichopus zeylanicus leaf extract on blood sugar level in streptozotocin induced diabetic rats.

Values are expressed as Mean ± S.E. n=6.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose level in mg/dl (Mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Post induction days</td>
</tr>
<tr>
<td></td>
<td>5th day</td>
</tr>
<tr>
<td>Control</td>
<td>62.2±1.22</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>260.2±1.34</td>
</tr>
<tr>
<td>Diabetic rats+ EETZ</td>
<td>273.10±7.04</td>
</tr>
<tr>
<td>Diabetic rats+ glibenclamide</td>
<td>263.20±3.59</td>
</tr>
</tbody>
</table>

P*<0.05 Experimental groups were compared with diabetic control.
P*<0.05 Diabetic groups were compared with control.

The effect Trichopus zeylanicus leaf extract on fasting blood glucose level is measured on 5th, 15th and 20th day of post induction of diabetics and compared with normal and diabetic control groups. The values are shown in table No-2. Streptozotocin induced rats showed a significant increase (P<0.05) in fasting blood glucose level compared to normal rats. Oral administration of ethanolic extract of Trichopus zeylanicus at the dose of 400mg/kg body weight showed a significant decrease (P<0.05) in blood glucose level in 10 and 15 days of treatment. The fasting blood glucose level on 15th day of post induction (10 days of treatment) was 135.4±3.99 mg/dl compared to fasting blood glucose of diabetic control animal 260.2±1.34mg/dl. The group treated with standard drug of Glibenclamide 0.5 mg/kg showed fasting blood glucose level of 126.06±8.07 mg/dl. On 20th day of post induction (15days of treatment ), the leaves extract treated group showed a fasting blood glucose level of 70.61 ± 2.24 mg/dl, compared to untreated diabetic animal which showed a fasting blood glucose level of 269.8 ± 1.88 mg/dl. The group treated with standard drug of Glibenclamide 0.5 mg/kg orally showed fasting blood glucose level of 64.06± 1.28 mg/dl.
Table- 2: Effect Of Trichopus zeylanicus Leaf Extract Biochemical Parameters In Streptozotocin Induced Diabetic Rats.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>BIOCHEMICAL PARAMETERS IN BLOOD SERAM (MEAN ±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SOD</td>
</tr>
<tr>
<td>Control</td>
<td>20.15 ± 0.85</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>0.66 ± 0.09*</td>
</tr>
<tr>
<td>Diabetic rats+ EEXX</td>
<td>13.1 ± 0.83*</td>
</tr>
<tr>
<td>Diabetic rats+ glibenclamide</td>
<td>16.36 ± 0.59*</td>
</tr>
</tbody>
</table>

Superoxide dismutase (SOD) and Catalase are an enzyme is act as anti oxidant, to prevent free radical damage to the body. SOD assayed by the method of kakkar (1984). Catalase assayed by sinha (1972) Reduced glutathione (GSH) is important in the normal function of the immune cells. Is assayed by reddy Low levels have been associated with impaired immune function. Glutathione peroxide (GPX) Glutathione peroxide was estimate by the method of Rotruck, et.al. (1973). Ethanolic extract of Trichopus zeylanicus Linn significantly decreases the lipid peroxidation level compared with un treated group (P< 0.05).

DISCUSSION

In the present study the hypoglycemic activity of ethanolic extract of Trichopus zeylanicus leaves was evaluated in Streptozotocin induced diabetic rats. The continuous administration of Trichopus zeylanicus extract for a period of 15 days produced a significant decrease in blood glucose level in diabetic rats which is comparable to that of standard drug Glibenclamide which is used in treatment of type II diabetes mellitus. The standard drug of Glibenclamide stimulates the beta cells and increase secretion of insulin .From the study plant extract decreases the blood glucose level and increases the SOD, catalyses, GSH and GPX level and also decreases the lipid peroxidation level.

CONCLUSION

The ethanolic extract of Trichopus zeylanicus leaf exhibited significant hypoglycemic activity in streptozotocin induced diabetic rats. From the phytochemical test it was found that the major chemical constituents of the leaf extract were flavonoids and glycosides. On the basis of above study realy on it is possible that the presence of flavonoids may be responsible for the observed antidiabetic and antioxidant activity.
REFERENCES