Antidiabetic activity of ethanolic leaf extract of *Bridelia scandens* in streptozotocin induced diabetic rats

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**ABSTRACT**

The aim of present study to evaluate antidiabetic activity of ethanolic extract of *Bridelia scandens* (Roxb) Wild. (Family: Euphorbiaceae) leaf in streptozotocin induced diabetic rats. The alcoholic extract of *Bridelia scandens* (Roxb) Wild was studied for antidiabetic activity in streptozotocin induced diabetic rats by oral administration of extract 200&400mg/kg body weight for the period of 28 days. The preliminary phytochemical study showed the presence of flavonoids, carbohydrates and glycosides, phenolic compounds and tannins. From the toxicity study it was observed that ethanolic extract of *Bridelia scandens* (Roxb) Wild was nontoxic up to the dose of 2000mg/kg body weight. The determination of blood glucose level by GOD-POD kit method. The effect was compared with oral dose of 0.6mg/kg Glibenclamide. The result shows the alcoholic extract of *Bridelia scandens* (Roxb) Wild level significantly lowered the blood glucose of hyperglycemic rats in the dose dependent manner and it was also comparable to that of the standard drug glibenclamide.

**KEYWORDS:** Anti-diabetic, *Bridelia scandens* (Roxb) Wild, Glibenclamide, Streptozotocin.

**INTRODUCTION:**

Diabetes Mellitus (DM) is a chronic disorder of carbohydrate, fat and protein metabolism characterized by increased blood glucose level (hyperglycemic) resulting from the defects in insulin action, or both. It is one of the alarming worldwide health problems at present leading to micro vascular(retinopathy, neuropathy and nephropathy) and macro vascular(heart attack, stroke and pheripheral vascular disease) complication(Umar et al., 2010). It is excepted that about 366million peoples are like to be diabetic by the year2030(wild et., 2004). Hyperglycemia is known to produce reactive oxygen species(ROS) which plays a central role in complication of diabetes[1].

Hyperglycemia is known to produce reactive oxygen species(ROS) which play a central role in complications of diabetes. Antioxidants are substance or nutrients in our food which act as free radical scavengers by preventing and repairing damage caused by ROS and reactive nitrogen species(RNS), and therefore can enhanced the immune defense and lower the risk of diabetic complication[2]. Through insul and other synthetic ant diabetic drugs are available none of them gives long duration glycemic control without causing any adverse side effects(Singh et., 2007). Thus there is a growing interest in using herbal remedies for the treatment of DM(Sunmil et., 2012). World Health Organization(WHO) has given sufficient stress in utilizing traditional plants for diabetic since they are non- toxic, efficient, with less or no side effect and very cost- effective.

*Bridelia Scandens* belongs to the family Euphorebiacae. It is distrib- uted in the warm regions of India and Southeast Asia. This plant used as antimicrobial activity[3]. The bark decoction has been used in the traditional medicine for the treatment of asthma, intestinal worms and cough and leaves are used against colics. Tannins were isolated from the bark. The fatty acids C_{22}H_{46}O, named bridelyl alcohol besides fatty acids and a phlobatannin were isolated from the leaves of *Bridelia Scandens*[4]. Taraxenone was isolated from root hexane extract[5].

**Collection and authentication of plant material**
The leaves of *Bridelia scandens*(Roxb) Willd. were collected from the Panpozhi,Thirunelveli Distric of Tamilnadu,India. in the month of November-2014. Taxonomic identification was made from Botanical Survey Medical Plants Unit Siddha,Government of India.
Preparation of the extract

The leaves of *Bridelia scandens* (Roxb) Willd was collected and air dried under shade and then coarsely powdered with the help of mechanical grinder. The powder was passed through sieve no.40 and dried under shade and then coarsely powdered with the help of mechanical grinder. The powder was passed through sieve no.40 and stored in an airtight container for the extraction.

The collected, cleaned and powdered leaves of *The leaves of Bridelia scandens* (Roxb) Willd was used for the extraction purpose 500 gms of powdered material was evenly packed in the Soxhlet apparatus. It was then extracted with various solvents from non-polar to polar such as petroleum ether, chloroform, acetone and ethanol. The solvents used were purified before use. The extraction method used was continuous hot percolation and carried out with various solvents, for 72 hours.

Preliminary Phytochemical Screening

The phytochemical examination of the selected extracts showed the presence of various constituents. From that ethanolic and extracts showed maximum phytoconstituent especially flavonoids, tannins and phenolic compounds. The phytochemical screening of the alcoholic and aqueous leaves extracts *Bridelia scandens* (Roxb) Willd. Showed the presence of flavonoids, carbohydrates and glycosides, phenolic compounds and tannins.[6]

Experimental Animals

Studies were carried out using male Wistar albino rats (150 – 200g) and Swiss albino mice(20 – 25kg). They were procured from Sri Venkateswara Enterprises, Bangalore, India. The animals were grouped and housed in polyacrylic cages (38 × 23 × 10cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycle (12/12h). The animals were fed with standard pellet supplied by Hindustan Lever Ltd. Bangalore, India and fresh water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All procedures described were reviewed and approved by the University Animal Ethical Committee. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC No.-60/2013/IAEC/VMCP.) after scrutinization. The animals received the drug treatment by oral gavage tube.

Acute oral toxicity studies:

An acute oral toxicity study was performed as per OECD guidelines 423. By acute toxic class method Swiss albino mice of female sex weighing 20-25gms were used for the study. The alcoholic leaf extracts of *Bridelia scandens* (Roxb) Willd did not showed any lethal effect on the animals up to the doses of 2000 mg/kg and the animals were observed for further 14 days for any sign of delayed toxicity. The LD$_{50}$ value considered as 2000mg/kg. So, the ED$_{50}$ dose 200mg/kg[7].

Induction of diabetes:

Streptozotocin (STZ) induced hyperglycaemia has been described as a useful experimental model to study the activity of hypoglycaemia agents. After overnight fasting (deprived of food for 16 hours, had been allowed free access to water), diabetes was induced in rats by intraperitoneal injection of STZ dissolved in 0.1 M sodium citrate buffer pH 4.5 at a dose of 50mg/kg body weight. After the injection they have free access to food and water. The animals were allowed to drink 5% glucose solution overnight to overcome the hypoglycaemic shock. The development of diabetes was confirmed after 48 h of the streptozotocin injection. The animals having fasting blood glucose levels more than 200mg/dl were considered as diabetic rats and used for experimentation[6].

Experimental protocol:

In the diabetic rat, 5 days after the induction of diabetes, animals were divided into four groups each having 6 rats. Non diabetic animal are grouped for control. Total of 5 groups of animal of six each were used for study.

**Group I:** Received 2% w/v gum acacia 1ml/kg orally served as control group (non diabetic control).

**Group II:** Served as STZ induced diabetic control received 2% w/v gum acacia 1ml/kg orally for 28 days.

**Group III:** Streptozotocin induced diabetic animal received the standard drug Glibenclamide 0.6 mg/kg body weight once daily orally for 28 days.

**Group IV:** Streptozotocin induced diabetic animals received ethanol ether extract of *Bridelia scandens* (Roxb) Willd leaves 200 mg/kg body weight once daily orally for 28 days.

**Group V:** Streptozotocin induced diabetic animals received ethanol ether extract of *Bridelia scandens* (Roxb) Willd leaves 400 mg/kg body weight once daily orally for 28 days.

All the group of animal received the treatment for 21 days. Rats were fasted overnight and the blood was withdrawn from the retro-orbital plexus on the 7th day of induction of diabetes and 14th, 21st day and
28th day post induction to determine the blood glucose level by glucose oxidase – peroxidase (GOD/POD) method. The change in body weight was observed throughout the treatment period in experimental animals.

**Estimation of Biochemical parameters:**
On 28th day, blood was withdrawn from the retro-orbital plexus kept wide for ½ hrs for clotting serum was separated by centrifuge the sample at 6000rpm for 20min. The serum was analysed for cholesterol, LDL, HDL, and triglycerides.

**Statistical analysis:**
All the grouped data were expressed as mean ± SEM. Difference between the control and treatment groups were tested for significance using ANOVA followed by Dunnet’s test. P<0.05 were considered significant.

**RESULTS:**

**Phytochemical screening:**
The phytochemical screening of the alcoholic and leaves extracts *Bridelia scandens* (Roxb) Willd. showed the presence of flavonoids, carbohydrates and glycosides, phenolic compounds and tannins.

**Acute oral toxicity studies:**
The alcoholic leaf extracts of *Bridelia scandens* (Roxb) Willd did not showed any lethal effect on the animals up to the doses of 2000 mg/kg and the animals were observed for further 14 days for any sign of delayed toxicity. The LD50 value considered as 2000mg/kg. So, the ED50 dose 200mg/kg.

**Anti Diabetic Activity:**
The effect of ethanolic extract of *Bridelia Scandens* (Roxb) Wild (EEBS) on fasting blood glucose level was measured on 7th, 14th, 21st and 28th day of post induction and compared with normal and diabetic control groups. The values are shown in the Table No: 1 STZ treated diabetic rats showed significant increase in the levels of blood glucose when compared to normal rats. After treatment with EEBS at 200mg/kg and 400mg/kg the blood glucose was significantly (P = 0.01) reduce compared to the diabetic rats.

**Body weight test:**
The body weight of experimental rats were measured drugs the period of study of antihyperglycemic activity. In diabetes mellitus the body weight loss was common symptoms. Diabetic control group animals loss their body weight continuously but the treated group after some time they start to regain their body weight. The alcoholic extracts shows the significant regain of body weight during study period Table No: 2.

**Table 1: Effect of alcoholic extracts on blood glucose levels(mg/dL) in STZ induced diabetic rats.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 day</th>
<th>7th day</th>
<th>14th day</th>
<th>21st day</th>
<th>28th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>80.30±3.1</td>
<td>85.40±2.80</td>
<td>83.48±5.32</td>
<td>85.58±4.60</td>
<td>84.5±2.18</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>250.28±6.88</td>
<td>266.03±3.28</td>
<td>275.28±4.76</td>
<td>290.44±6.42</td>
<td>293.03±1.28</td>
</tr>
<tr>
<td>Glibenclamide (0.6mg/kg)</td>
<td>256.42±1.27</td>
<td>190.38±6.20**</td>
<td>163.37±5.11**</td>
<td>123.40±2.42**</td>
<td>110.03±3.72**</td>
</tr>
<tr>
<td>EEBS(200mg/kg)</td>
<td>270.48±5.60</td>
<td>210.82±5.12’</td>
<td>178.82±2.67’</td>
<td>144.48±4.27’</td>
<td>136.48±2.80’</td>
</tr>
<tr>
<td>EEBS(400mg/kg)</td>
<td>268.00±3.82</td>
<td>201.48±4.26’</td>
<td>169.38±4.38’</td>
<td>130.51±4.57’</td>
<td>120.38±1.78’</td>
</tr>
</tbody>
</table>

**Table no: 2 Effect of alcoholic extracts on body weight in STZ induced diabetic rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 day</th>
<th>7th day</th>
<th>14th day</th>
<th>21st day</th>
<th>28th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>156.32±4.12</td>
<td>162.5±4.18</td>
<td>165.66±4.84</td>
<td>171.66±4.08</td>
<td>175.00±3.65</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>152.5±6.12**</td>
<td>143.33±4.64*</td>
<td>133.16±8.49**</td>
<td>113.16±6.05**</td>
<td>86.66±6.05**</td>
</tr>
<tr>
<td>Glibenclamide (0.6mg/kg)</td>
<td>155.00±5.47*</td>
<td>140.16±6.64*</td>
<td>142.66±4.08*</td>
<td>143.16±3.76*</td>
<td>144.83±3.76*</td>
</tr>
<tr>
<td>EEBS(200mg/kg)</td>
<td>160.00±8.94*</td>
<td>141.66±4.08*</td>
<td>146.66±4.08*</td>
<td>148.33±6.0*</td>
<td>153.33±6.05*</td>
</tr>
<tr>
<td>EEBS(400mg/kg)</td>
<td>161.68±7.52*</td>
<td>150.16±10.68*</td>
<td>150.00±8.36*</td>
<td>154.16±7.36*</td>
<td>157.7±6.12*</td>
</tr>
</tbody>
</table>

All the values are expressed as mean ±SEM n=6 in each group. Value are significantly different from control. *P<0.05; **P<0.01; ***P<0.001. compared with diabetic control values.
DISCUSSION:
Any drug that is effective in diabetes will have the ability to control the rise in glucose level by different mechanisms and the ability of the extracts to prevent hyperglycaemia could be determined by hyperglycaemic animal model. In animals, diabetes can be induced by partial pancreatectomy or by the administration of diabetogenic drugs such as streptozotocin, alloxan, dinitrophenol and anti-insulin serum. Streptozotocin is a naturally occurring nitrosourea product of Streptomyces achromogenes, and it is widely used to induce diabetes in experimental animals. Usually, the intraperitoneal injection of a single dose (50 mg/kg body weight) of it exerts direct toxicity on β cells resulting in necrosis 48-72 h and causes a permanent hyperglycaemia. Streptozotocin was used in the present study for the induction of diabetes in rats[9].

In the present study the hypoglycaemic activity of ethanolic extract of *Bridelia Scandens* leaves was evaluated in Streptozotocin induced diabetic rats. The continuous treatment of leaves extract for a period of 28 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 Glibenclamide stimulates insulin secretion from beta cells of islets of langerhans. From the study, it is suggested that the possible mechanism by which the plant extract decreased the blood glucose level may be by potentiation of insulin effect either by increase in pancreatic secretion of insulin from beta cells of islets of langerhans or by increase in peripheral glucose uptake. However, we suggest that further work should be carried out at cellular and molecular levels to find out the absolute mechanism of action of the plant in experimental diabetic[10].

CONCLUSION:
The ethanolic extract of *Bridelia Scandens* leaves exhibited significant hypoglycaemic activity in streptozotocin induced diabetic rats. From the phytochemical analysis it was found that the major chemical constituents of the leaves extract were flavonoids and glycosides. On the basis of above evidence it is possible that the presence of flavonoids may be responsible for the observed antidiabetic activity.

REFERENCES:

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