ANTIDIABETIC AND ANTIHYPERLIPIDEMIC EFFECT OF 
BORASSUS FLABELLIFER IN STREPTOZOTOCIN (STZ) INDUCED 
DIABETIC RATS.

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ABSTRACT

Borassus flabellifer is used extensively in the indigenous system of medicine as an anti-diabetic agent. The current investigation focus on 
the antihyperglycemic and antihyperlipidemic property of acetone insoluble ethanolic fraction of Borassus flabellifer extract on 
streptozotocin induced diabetic rats. The diabetes induced animals 
were fed with inflorescence extracts of Borassus flabellifer at the 
increasing doses of 150mg, 300mg and 600mg of body weight. The 
extracts at dose of 600mg/kg body weight administrated animals 
revealed a significant (P<0.01) decrease blood glucose level and higher 
reduction in hyperlipidemia when compared to the diabetic control rats 
(P<0.01). The histological studies of the endocrine region of pancreas 
of diabetic animals revealed that shrinkage of β cells of islets of 
langerhans. The extracts treated animals revealed restoration of β-cells. 
The restoration of β cells was evident at higher dose level i.e.600mg/kg 
body weight extracts fed groups.

KEYWORDS: Borassus flabellifer, streptozotocin (STZ), hyperglycemia, hyperlipidemia.

INTRODUCTION

Diabetes is one of the most challenging diseases facing health care professionals today. Its 
increasing prevalence puts a large burden on society and the public health sector. [1] Type 1 
diabetes is characterized by an absolute deficiency of insulin secretion, associated with auto-
immune destruction of pancreatic β-cells, and this disease is more likely to occur in relatives of an affected person. [2] Type 2 diabetes, which accounts for more than 90% of cases, is caused by a combination of resistance to insulin action and impaired insulin secretion. [3] Plants have been an exemplary source of drugs, and many of the currently available drugs have been derived directly or indirectly from them. It is reported that about 800 plants may possess anti-diabetic potential. [4]

*Borassus flabellifer* Linn is widely distributed plant in coastal and forest area in India and are very likely to be cultivated for both medicinal as well as commercial reason. There are innumerable medicinal uses for all parts of the *B. flabellifer*. Briefly, the young plant is said to relieve biliousness, dysentery, and gonorrhea. Young roots are diuretic and antihelmintic, and a decoction is given in certain respiratory diseases. [5] The cabbage, leaf petioles, and dried male flower spikes all have diuretic activity. The pulp of the mature fruit relieves dermatitis. [6] Decoction of the root is used in gastritis, hiccups and it is taken by labour class people for the treatment of diabetes. [7]

However, no systematic study was carried out on the inflorescence extracts of *B. flabellifer* Linn, for anti diabetic activity. Hence present study was planned to investigate the antidiabetic and antihyperlipidemic activity of acetone insoluble fraction of ethanolic inflorescence extract of *B. flabellifer* in streptozotocin induced diabetic rats.

**MATERIAL & METHOD**

**Plant Material**

The plant of *Borassus flabellifer* has been collected from Salem district, Tamil Nadu, with the help of field botanist. The plant of *Borassus flabellifer* have been authenticated by Dr. G.V.S. Murthy, Scientist, ‘F’& Head of Office, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu, India. (Ref. BSI/SRC/23/2011-12/Tech 1083). The whole plant was dried initially under shade. It was preserved in a tightly closed container and powdered as per requirements.

**Preparations of Extracts**

The dried inflorescence part of plant was subjected to size reduction to a coarse powder by using dry grinder and passed through sieve. About 150g of this powder was packed into soxhlet apparatus and extracted successively with petroleum ether, chloroform, and ethanol (yield 1.62%, 4.48%, 7.59%, respectively). Again ethanolic extract was fractionated by
acetone and % yield of acetone soluble and insoluble fraction was obtained as (36.83%, 63.00% of ethanolic extract) respectively.

**Experimental Animals**

Male wistar rats (150-180 g) were used to assess acute toxicity, antidiabetic and antihyperlipidemic activity. All animals were housed in standard laboratory conditions temperature ($22^\circ$C± 2) and humidity (45±5%) with [12h day: 12h night cycle]. The standard laboratory diet was provided to the animals and they were allowed to drink water ad libitum. Studies were carried out after the approval of Institutional Animal Ethical Committee in accordance with institutional ethical guidelines for the care of laboratory animals of Goenka College of Pharmacy, Lacchmangarh, Sikar, India (approval no.1224/ac/08/CPCSEA).

**Chemicals**

The estimation of biochemical parameters was carried out using commercially available kits (Primal Healthcare Limited, Lab Diagnostic Division, and Mumbai, India). STZ and other chemicals were procured from Himedia Laboratories, Mumbai, India.

**Acute Toxicity Study**

Acute oral toxicity study was performed as per Organization for Economic Cooperation and Development guidelines 423 (acute toxic classic method). After the oral administration of inflorescence of *Borassus flabellifer* (2,000 mg/kg), animals were observed individually at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4h, and daily thereafter, they were observed for a total of 14 days for toxicity determination.

**Induction of Experimental Diabetes in Rats**

STZ was dissolved in freshly prepared 0.1 M cold citrate buffer (pH 4.5) and administered by intraperitoneal route (60mg/kg) to the overnight fasted rats. After 6h of STZ injection, rats were received 5% dextrose solution for the next 24h to prevent STZ induced fatal hypoglycemia as a result of massive pancreatic insulin release after its administration. Diabetes was confirmed 72h after induction by measurement of tail vein blood glucose levels using glucose meter (Glucocard™ 01-mini, Arkray Factory, Inc., Japan) by glucose oxidase-peroxidase method using strips. Diabetic rats were kept 14 days under standard laboratory condition for the stabilization of blood glucose levels. After 14 days induction of diabetes,
blood glucose was again determined and animals with a blood glucose level greater than 250 mg/dL were selected for the study.

**Phytochemical Screening**

The preliminary phytochemical screening of the crude extract of *Borassus flabellifer* was carried out in order to ascertain the presence of its constituents utilizing standard conventional protocols.\(^{[12]}\)

**Experimental Design**

The Streptozocin-induced diabetic wistar rats were randomly assigned into six groups (I-VI) of six rats (n=6) each as follows, namely.

Group I- Received normal saline 10 ml/kg of body weight, per orally.

Group II- Diabetic control.

Group III- Received glibenclamide 10 mg/kg of body weight, per orally.

Group IV- Received *B. flabellifer* acetone insoluble fraction of ethnolic extract 150 mg/kg of body weight, per orally.

Group V- Received *B. flabellifer* acetone insoluble fraction of ethnolic extract 300 mg/kg of body weight, per orally.

Group VI- Received *B. flabellifer* acetone insoluble fraction of ethnolic extract 600 mg/kg of body weight, per orally.

**Determination of Blood Glucose Levels**

Blood samples were collected by cutting the tail-tip of the rats, for blood glucose determination at intervals of 2, 4, 6, 8 hr (Acute study) and 7, 14, 21, 28 days (Sub Acute study) by the glucose-oxidase principle \(^{[13]}\) using the one touch basic instrument \(^{[14]}\) and results were reported as mg/dl. \(^{[15]}\)

**Biochemical Analysis**

The biochemical estimation of serum TC \(^{[16]}\), TG, \(^{[17]}\) LDL, HDL, and VLDL \(^{[17]}\) were carried out.
Histopathology
The pancreatic tissues were dissected out and washed on ice cold saline immediately. A portion of pancreatic tissue was fixed in 10% neutral formalin fixative solution for histological studies. After fixation tissues were embedded in paraffin, solid sections were cut at 5μm and the sections were stained with haematoxylin and eosin.\(^{[18]}\)

Statistical Analysis
Blood glucose levels were expressed in mg/dl as mean ± SEM. The data were statistically analyzed using ANOVA with multiple comparisons versus control group. The values of \(p<0.01\) were considered as significant.\(^{[19]}\) The criterion for statistical significance was considered as \(P\) value <0.001. The difference between test and controls were evaluated by student’s t-test.

RESULTS
Phytochemical Analysis
Freshly prepared extracts were subjected to preliminary phytochemical screening test for various constituents. This revealed the presence of tannins, carbohydrate, terpenes, saponins, flavonoids and alkaloids.

Acute Toxicity Study (LD50)
The sign of toxicity were first noticed after 10-12 hours of extract administration. There was decreased locomotor activity and decreased in sensitivity to touch. Also there was decreased feed intake, and prostration after 18 hours of extract administration. The median lethal dose (LD50) in rats was calculated to be 2000 mg/kg body weight.
Table: 1 Effect of *Borassus Flabellifer* on Blood Glucose Level in Streptozotocin Induced Diabetic Male Wister Rats Treated by Various Doses of Ethanol Extracts (Acute Study).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Blood Glucose Level mg/dl</th>
<th>Mean % Reduction after 6hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0hr</td>
<td>2hrs</td>
</tr>
<tr>
<td>I</td>
<td>Normal control (vehicle)</td>
<td>68.16±3.44</td>
<td>68.66±3.62</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>379.83±8.04</td>
<td>384.66±8.61</td>
</tr>
<tr>
<td>III</td>
<td>Glibenclamide (10 mg/kg)</td>
<td>395.00±9.83</td>
<td>257.33±6.80</td>
</tr>
<tr>
<td>IV</td>
<td>Ethanolic extract (150 mg/kg)</td>
<td>393.83±9.10</td>
<td>363.83±9.63</td>
</tr>
<tr>
<td>V</td>
<td>Ethanolic extract (300 mg/kg)</td>
<td>391.66±11.70</td>
<td>353.00±8.63</td>
</tr>
<tr>
<td>VI</td>
<td>Ethanolic extract (600 mg/kg)</td>
<td>373.33±10.04</td>
<td>282.33±8.61</td>
</tr>
</tbody>
</table>

n=6, *p<0.05- significant, **p<0.01-more significant v/s diabetic control, SEM= standard error mean, n= number of animals.
Table: 2 Effect of *Borassus Flabellifer* on Blood Glucose Level in Streptozotocin Induced Diabetic Male Wister Rats Treated by Various Doses of Ethanolic Extracts (Sub Acute Study).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Blood Glucose Level mg/dl</th>
<th>Mean % reduction after 28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control (vehicle)</td>
<td>68.26±3.44</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>379.83±8.04</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Glibenclamide (10mg/kg)</td>
<td>395.00±0.63</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Ethanolic extract (150mg/kg)</td>
<td>393.83±8.10</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Ethanolic extract (300mg/kg)</td>
<td>391.66±11.70</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>Ethanolic extract (600mg/kg)</td>
<td>373.33±10.04</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>0 Day</th>
<th>7 Days</th>
<th>14 Days</th>
<th>21 Days</th>
<th>28 Days</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>68.26</td>
<td>68.96</td>
<td>70.16</td>
<td>71.83</td>
<td>72.13</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>379.83</td>
<td>384.66</td>
<td>389.66</td>
<td>393.16</td>
<td>402.83</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>395.00</td>
<td>266.16</td>
<td>194.66</td>
<td>183.83</td>
<td>145.00</td>
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</tr>
<tr>
<td>IV</td>
<td>393.83</td>
<td>316.66</td>
<td>308.66</td>
<td>293.66</td>
<td>240.50</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>391.66</td>
<td>294.00</td>
<td>292.66</td>
<td>282.33</td>
<td>223.50</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>373.33</td>
<td>276.50</td>
<td>208.83</td>
<td>198.16</td>
<td>165.66</td>
<td></td>
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</tbody>
</table>

n=6, *p<0.05- significant, **p<0.01- more significant v/s diabetic control, SEM= standard error mean, n= number of animals

Table: 3 Effect of *Borassus Flabellifer* on Various Biochemical Parameters of Serum Lipid Profile (Mg/Dl) in Streptozotocin Induced Diabetic Male Wister Rats After 28 Days Treatment by Various Doses of Ethanolic Extracts.

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Parameters</th>
<th>Normal control (vehicle)</th>
<th>Diabetic control</th>
<th>Glibenclamide (10mg/kg, b. w.)</th>
<th>Ethanol extract (150mg/kg, b.w.)</th>
<th>Ethanol extract (300mg/kg, b.w.)</th>
<th>Ethanol extract (600mg/kg, b.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TG (mg/dl)</td>
<td>81.10±0.41</td>
<td>152.17±1.47**</td>
<td>66.84±1.19**</td>
<td>92.09±1.16**</td>
<td>87.19±1.17**</td>
<td>69.13±1.13**</td>
</tr>
<tr>
<td>2</td>
<td>TC (mg/dl)</td>
<td>78.26±1.66</td>
<td>146.28±1.87**</td>
<td>69.10±1.21**</td>
<td>76.16±1.55**</td>
<td>72.28±1.31**</td>
<td>71.96±1.87**</td>
</tr>
<tr>
<td>3</td>
<td>HDL (mg/dl)</td>
<td>26.28±0.73</td>
<td>14.14±0.48*</td>
<td>23.07±0.78*</td>
<td>17.00±0.68*</td>
<td>18.62±0.47*</td>
<td>19.54±0.26**</td>
</tr>
<tr>
<td>4</td>
<td>LDL (mg/dl)</td>
<td>40.18±1.18</td>
<td>90.10±1.01**</td>
<td>62.15±0.63*</td>
<td>69.08±1.09**</td>
<td>72.24±0.60**</td>
<td>65.19±0.72**</td>
</tr>
<tr>
<td>5</td>
<td>VLDL (mg/dl)</td>
<td>16.07±0.24</td>
<td>30.09±0.42**</td>
<td>18.92±0.59**</td>
<td>17.26±0.38**</td>
<td>15.27±0.35**</td>
<td>16.25±0.57**</td>
</tr>
</tbody>
</table>
Table: 4 Effect Of *Borassus Flabellifer* On Various Biochemical Parameters Of Serum Lipid Profile (Mg/Dl) In Streptozotocin Induced Diabetic Male Wister Rats Mean % Reduction After 28 Days Treatment By Various Doses Of Ethanolic Extracts.

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Parameters</th>
<th>Glibenclamide (10mg/kg. b. w.)</th>
<th>Ethanolic extract (150mg/kg. b.w.)</th>
<th>Ethanolic extract (300mg/kg. b.w.)</th>
<th>Ethanolic extract (600mg/kg. b.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TG (mg/dl)</td>
<td>16.15</td>
<td>9.86</td>
<td>12.51</td>
<td>14.61</td>
</tr>
<tr>
<td>2</td>
<td>TC (mg/dl)</td>
<td>16.23</td>
<td>8.52</td>
<td>8.99</td>
<td>11.43</td>
</tr>
<tr>
<td>3</td>
<td>HDL (mg/dl)</td>
<td>23.02</td>
<td>8.8</td>
<td>13.89</td>
<td>19.39</td>
</tr>
<tr>
<td>4</td>
<td>LDL (mg/dl)</td>
<td>24.19</td>
<td>15.28</td>
<td>20.00</td>
<td>22.28</td>
</tr>
<tr>
<td>5</td>
<td>VLDL (mg/dl)</td>
<td>18.66</td>
<td>8.37</td>
<td>11.12</td>
<td>16.3</td>
</tr>
</tbody>
</table>

TG = Triglycerides, TC = Total cholesterol, HDL = High density level cholesterol,
LDL= Low density level cholesterol, VLDL = Very low density level cholesterol.

Values are given as mean ± S.E.M. for groups of six rats of each.

Fig-1. The Pancreatic islets of langerhans of normal rat showing alpha cells and beta cells.
Fig-2: STZ induced diabetic damaged pancreatic islets showing reduced size and increased damaged beta cells.

Fig-3: Borassus flabellifer extract (150mg/kg) treated pancreatic islet show partial revealed better restoration, when compared to the STZ induced diabetic control rats

Fig-2: Borassus flabellifer extract (300mg/kg) treated pancreatic islet show partial revealed better restoration, when to the STZ induced diabetic and also (150mg/kg) treated rats
Fig 5: Borassus flabellifer extract (600mg/kg) treated pancreatic islets shows partial proliferation of beta cells. The animals revealed better restoration / proliferation from the STZ induced damage when compared to control as well as 300 mg/kg treated animal.

RESULTS
Tables-1, showed results of the effects of Borassus flabellifer extracts, glibenclamide and control groups in streptozocin-induced diabetic male wistar rats. Acute studies were carried out on STZ-induced diabetes rats. The ethanolic extract Borassus flabellifer (150, 300 and 600 mg/kg, body wt.) has shown a significant (P<0.01) reduction in blood glucose levels of about 37.36%, 39.95% and 47.85%, respectively, after 6 h of treatment. At the same time, glibenclamide caused a significant (P<0.01) reduction of blood glucose levels of 60.12%.

In Table-2, data showed on repeated administration (sub acute treatment) of vehicle, glibenclamide, ethanolic extract of Borassus flabellifer for 28 days, a significant (P < 0.01) decrease in glucose level of the diabetic rat were seen at a dose of 150, 300, and 600 mg/kg, body weight,( 26.39%, 27.91%, 46.92%) in dose-dependent manner as compared with diabetic-treated group. On the other hand, glibenclamide showed a significant (P < 0.01) decrease in blood glucose at a dose of 10 mg/kg, p.o., (63.29% decrease) as compared with diabetic-treated group. Maximum activity of Borassus flabellifer was seen with a significant decrease (P < 0.01) in blood glucose levels at the dose of 600 mg/kg.

Table-3 & 4, data showed the lipid profile such as TC, TG, LDL and VLDL levels were significantly increased in diabetic control animals (DC) where as HDL levels were decreased when compared to the diabetic control rats. The extract was administered orally at increasing dose levels of 150mg, 300mg, and 600mg/kg body wt., to diabetic rats. The diabetic animals at
150mg/kg doses recorded a non significant change in the TC, TG, HDL, LDL and VLDL levels. On the other hand when dosage levels were increased to 300mg and 600mg/kg body wt., a significant (P<0.01) depletion in the total cholesterol level was recorded in the diabetic animals. The depletion in the TC, TG, LDL, and VLDL was dose dependent and the highest reduction in the cholesterol recorded was 14.61%, TG-11.43%, LDL- 22.28% and VLDL-16.31% in 600mg/kg body wt., when compare to the diabetic control animals. The depleted high density lipoprotein (HDL) in the diabetic rats, increased significantly (P<0.01) after the administration of the plant extract. The highest increment was recorded at 600mg/kg body wt., dosage level (19.39% %).

Histological sections of endocrine regions of pancreas of STZ induced diabetic rats revealed a significant reduction in the size of the islets when compared to that of normal groups. Further the study revealed the presence of damaged β-cell population. These damage of the β-cells due to STZ induction. The reduction in β-cell number can be as low as 50% during diabetes (Hayashida et al 1983). On the other hand, studies on the supplementation of extracts the diabetic rats revealed restoration of size of the islets along with β-cells repair. This recovery of the β-cells was recorded as dose dependant that is form 300mg to 600mg/kg body wt of the extract given animals. The plant extract fed animals revealed better restored β-cells of pancreas from the STZ induced damage. The restoration of β-cells was evident at higher dose level of 600mg/kg body wt extract fed groups.

**DISCUSSION**

Diabetes is a chronic metabolic disorder affecting a major proportion of the population worldwide. A sustained reduction in hyperglycemia will decrease the risk of developing micro vascular diseases and reduce their complications. The conventional therapies for diabetes have many shortcomings like side effects and high rate of secondary failure. On the other hand herbal extracts are expected to have similar efficacy without side effects as that of conventional drugs. The present investigation reports that anti-diabetic and anti-hyperlipidemic effect of *Borassus flabellifer* in streptozotocin (STZ) induced diabetic rats. STZ injection resulted in diabetes mellitus, which is probably due to the destruction of β cells of islets of Langerhans as proposed by many authors. It is generally accepted that severe diabetes (SD) is of IDDM type and mild diabetes (MD) is of NIDDM type. This effect is being depicted by the high level of
blood glucose in animals. The main aim of this study was to assess the multiple roles of flavonoids rich extract from the *Borassus flabellifer* inflorescence as anti-diabetic agent for correction of IDDM where β cells degeneration is dramatic and MD or NIDDM where β cell degeneration is partial. The flavonoid rich ethanol extract resulted in the significant reduction of peak level of sugar within 6h time and this fact further strengthens the anti-diabetogenic potentiality. Further the plant extract also significantly decreased the blood glucose level in glucose loaded rats (GTT) and this fact could be attributed to the potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from existing β cells or its release from bound insulin. In this context a number of other plants have been observed to have similar pattern of hypoglycemic effects.

**CONCLUSION**

In the present study antidiabetic and antihyperlipidemic effect of *Borassus flabellifer* was evaluated in streptozocin-induced diabetic rat. Single-dose study with 150, 300, and 600 mg/kg showed significant (P < 0.01) decrease blood glucose level maximum at 6 h. Continuous treatment with the ethanol extract of leaves of *Borassus flabellifer* (150, 300, and 600 mg/kg) for a period of 28 days showed a significant decrease (P < 0.01) in the blood glucose level in diabetic rat. Maximum reduction of blood glucose level occurred at the dose of 600 mg/kg; p.o. LD50 determination (>2000 mg/kg) indicated safety profile of the drug. The levels of serum lipids are usually elevated in diabetes. Such elevation might lead to a higher risk for cardiovascular diseases in some cases. Lowering of serum lipids concentration through dietary or drug therapy is associated with a decrease in the risk of cardiovascular diseases.\(^{[23]}\) A regular administration of 600 mg/kg bodyweight of *Borassus flabellifer* not only lowered TC level, but also enhanced the cardioprotective lipid HDL level.

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