Hypoglycemic Activity of Inflorescence of *Borassus flabellifer* Extracts on Blood Glucose Levels of Streptozocin-Induced Diabetic Rats

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**ABSTRACT**

**Background:** The prevalence of diabetes mellitus continues to rise worldwide and treatment with oral hypoglycemic drugs leads to numerous side effects and huge monetary expenditure. Therefore active research on identification of new anti diabetic drugs with minimal side effects from medicinal plants is a challenge according to WHO recommendations. In this aspect, the present study was undertaken to evaluate the antidiabetic potential of *Borassus flabellifer* inflorescence in streptozotocin (STZ) induced diabetic rats. **Methods:** Diabetes was induced in male wister rats by intraperitoneal administration of STZ (60 mg/kg.b.w). Fasting blood glucose (FBG) levels were measured by glucose-oxidase & peroxidase method. The statistical analysis of results was carried out using one-way analysis (ANOVA) followed by Student t-test. **Results and Discussion:** Antidiabetic potentials of inflorescence of *Borassus flabellifer* extract has been investigated at the doses of 150, 300 and 600 mg/kg body weight orally administered against streptozocin induced diabetes male wistar rats. Treatment of streptozocin diabetic male wistar rats with the extracts caused a significant (P<0.01) reduction in the blood glucose levels. The highest activity resides at the dose of 600 mg/kg body weight with mean percentage glycemic change of 52.52% after 6 hours of extract administration while the other two doses 150 and 300 mg/kg have glycemic change of 42.34% and 45.11% respectively after 6 hours of extract administration. This result suggests that the inflorescence of *Borassus flabellifer* extracts possess antidiabetic effect on streptozocin induced diabetic male wistar rats. **Conclusions:** The plant extract is capable of managing hyperglycemia and complications of diabetes in STZ induced diabetic rats. Hence this plant may be considered as one of the potential sources for the isolation of new oral anti-hyperglycemic agent(s).

**KEYWORDS:** Hypoglycemic activity, Streptozocin, *Borassus flabellifer*, Diabetes mellitus

**INTRODUCTION**

Diabetes mellitus is an endocrine metabolic disorder characterized by hyperglycemia, altered lipids, carbohydrates, proteins metabolism and it increases risk of cardiovascular diseases complications1. The two forms of diabetes, type 1 and 2, differ in their basic mechanisms of development and in physiologic characteristics such as associations with obesity, age, and insulin. But, both types of the diabetes share the common characteristics of hyperglycemia, micro vascular and macro vascular complications. Moreover, the alterations of lipoproteins metabolism are involved to the pathogenesis of the cardiovascular disease in both forms of diabetes in a similar way2. Also, diabetes is usually accompanied by increased generation of free radicals or impaired antioxidant defenses. Oxidative stress is also responsible for the development and progression of diabetes and its complications3. Diabetes has a considerable impact on the health, life style, life expectancy of patients and its related complications are major healthcare problems. Currently, diabetes is controlled of available drugs such as oral hypoglycemic agents and insulin, but they have their own limitations. Traditionally, many herbal medicines and medicinal plants have been used for the treatment of diabetes as an alternative medicine4. Presence of various phytoconstituents in medicinal plants is thought to act on a different series of targets by multiple modes and mechanisms. Hence, plants have the potential to impart therapeutic effect in complicated disorders like diabetes and its complications5. Screening of medicinal plants is one of the alternative and valid approaches in the drug development process because they contain diverse phytoconstituents which may give new drug leads and may be effective and safe in diabetes6. In India, traditionally numbers of plants are used to manage the diabetic conditions...
and their active principles were isolated but few plants have been scientifically studied 7.

*Borassus flabellifer* (Areaceae) a south Indian plant known as Tad tree. Leaves, inflorescence, bark and fruits of this plant are traditionally employed in several regions for medicinal purposes 8. The present study was designed to test the hypoglycemic effect of inflorescence of *Borassus flabellifer* extract on streptozocin- induced diabetic rats.

**MATERIAL AND METHODS**

**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 *B. flabellifer* 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 1.81%, 1.94%, 1.70%, respectively). The solvent was recovered by distillation in vacuum and extracts were stored in desiccators and used for subsequent experiments.

**Experimental animals**
Male wistar rats (150-180 g) were used to assess acute toxicity and antidiabetic activity. All animals were housed in standard laboratory conditions temperature (22°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) 9. 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 9.

**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 10. 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 11. 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 (1-5) of six rats (n=6) each as Follows, namely

**Group 1** - Received normal saline 10 ml/kg of body weight, per orally
**Group 2** - Diabetic control
**Group 3** - Received glibenclamide 10 mg/kg of body weight, per orally
**Group 4** - Received *B. flabellifer* extract 150 mg/kg of body weight, per orally
**Group 5** - Received *B. flabellifer* extract 300 mg/kg of body weight, per orally
**Group 6** - Received *B. flabellifer* 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 and 8 hours by the glucose-oxidase principle 13 using the one touch basic instrument 14 and results were reported as mg/dl 15.

**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. 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 2,000 mg/kg body weight.

**Anti diabetic study:**
Tables 1,2 show results of the effects of *Borassus flabellifer* extracts, glibenclamide and control groups in streptozocin-induced diabetic male wistar rats. Blood samples were collected before and at 0, 2, 4, 6, 8 and 24 hrs after glucose administration. Oral glucose tolerance test (OGTT) of rats was found to be glucose intolerance. Acute studies were carried out on STZ-induced diabetes rats. The ethanolic extract *Borassus flabellifer* (150, 300 and 600 mg/kg, b.w.) has shown a significant (*P*<0.01) reduction in blood glucose levels of about 42.34%, 45.11% and 52.52%, 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%.

**DISCUSSION**
Medicinal plants are widely used by the populations of underdeveloped countries as alternative therapy. In India, hundreds of plants are used traditionally for the management and/or control of diabetes mellitus. Unfortunately only a few of such Indian medicinal plants have received scientific scrutiny. The present work was therefore designed to study the anti-diabetic property of *inflorescence of Borassus flabellifer* extract in Streptozocin-diabetic rats. Streptozocin-induced hyperglycemia has been described as a useful experimental model to study the activity of hypoglycemic agents. Streptozocin selectively destroyed the pancreatic insulin secreting β-cells, leaving less active cell resulting in a diabetic state. Many secondary metabolites participate in a variety of anti-diabetic functions in vivo. The glycemic change in blood glucose levels of diabetic rats at different time intervals after oral administration *Borassus flabellifer* extract.
extract of at the doses of 150, 300, and 600mg/kg as showed in Table 1.

In relation to the diabetes rats that received 150, 300, and 600mg/kg bodyweight of *Borassus flabellifer* extract there was a significant (p<0.01) reduction in the blood glucose levels when compared to the control group after different time hours of extract administration as regard to the dose of 600 mg/kg body weight and the reference drug . In relation to the dose of 150 and 300 mg/kg body weight of the *Borassus flabellifer* there was a less significant change in the blood glucose levels after different time hour of extract administration. In relation to the reference drug glibenclamide 10 mg/kg of body weight given orally. The dose of 600 mg/kg body weight was found to be more effective in the glycemic change after 6 hours of extract administration than the other two doses of the extract 150 and 300 mg/kg body weight. The extract might possess glibenclamide like effect on peripheral tissues either by promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis. The phytochemical studies of *Borassus flabellifer* there was a less significant presence of tannins, carbohydrate, terpenes, saponins, flavonoids and alkaloids.

Effect of the flavonoids, quercetin and ferulic acid on pancreatic β-cells leading to their proliferation and secretion of more insulin have been proposed. The presence of these constituents leads to anti-diabetic activity caused by streptozocin in diabetic rats. The flavonoids present in *Borassus flabellifer* may also be acting similarly thereby decreasing the high blood glucose levels of streptozocin-diabetic rats.

In conclusion, the experiment evidence obtained in the present laboratory animal study indicate that inflorescence of *Borassus flabellifer* extract possess anti-diabetic properties which suggest the presence of biologically active components which may be worth further investigation and elucidation.

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