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ORIGINAL ARTICLE

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

PRADEEP GOYAL¹, M. PHARM¹, NILESH JAIN¹, G. K. SINGH², B. P. NAGORI²

For author affiliations, see end of text.

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ABSTRACT

Evaluation of hypoglycemic activity of inflorescence of *Borassus flabellifer* extract in male wistar rats has been investigated. Antidiabetic potentials were studies by the orally administration of plant extract at the doses of 150, 300 and 600 mg/kg in streptozocin-induced diabetic male wistar rats. Treatment of streptozocin-induced diabetic male wistar rats with the extracts caused a significant (p<0.01) reduction in the blood glucose levels when compared with control. The dose of 600 mg/kg showed a significant decrease in blood sugar (p<0.05) after 2, 4, 6 and 8 hours of extracts administration when compared to control normal saline. The highest activity resided at the dose of 600 mg/kg with mean percentage glycemic change of 39.50% after 8 hours of extract administration while the other two doses 150 and 300 mg/kg had glycemic change of 30.34% and 31.42% respectively after 8 hours of extract administration. The phytochemical screening revealed the presences of tannins, carbohydrate, terpenes, saponins, flavonoids and alkaloids. This result suggests that the inflorescence of *Borassus flabellifer* extracts possess antidiabetic effect on streptozocin-induced diabetic male wistar rats.

Keywords: Hypoglycemic activity, Streptozocin, Borassus flabellifer, Diabetes mellitus

Diabetes mellitus is an endocrine metabolic disorder characterized by hyperglycemia, altered lipids, carbohydrates, proteins metabolism and it increases risk of cardiovascular diseases complications [1]. 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 way [2]. 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 complications [3]. 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 by handful 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 medicine [4]. 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 complications [5]. 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 diabetes [6]. 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 diabetes.

MATERIALS 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 whole plant was subjected to size reduction to a coarse powder using dry grinder and passing through sieve. About 150 g of this powder was packed into soxhlet apparatus and extracted successively with petroleum ether, chloroform, and water (yield 1.81%, 1.94%, 1.70%, respectively). The solvent was recovered by distillation in vacuum and extracts were stored in desiccators to use for subsequent experiments.

Experimental animals

Male wistar rats (150-180 g) were used to assess acute toxicity and anti-diabetic activity. All animals were housed in the standard laboratory conditions temperature ($22^{\circ C} \pm 2$) and humidity $45 \pm 5\%$ 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* (2000 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 4 h, 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 (60 mg/kg) to the overnight fasted rats [10]. After 6 h of STZ injection, rats were received 5% dextrose solution for the next 24 h to prevent STZinduced fatal hypoglycemia as a result of massive pancreatic insulin release after its administration-Diabetes was confirmed 72 h after induction by measurement of tail vein blood glucose levels using glucose meter (GlucocardTM 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 five groups (1-5) of five rats (n=5) each as Follows:

Group 1- Received normal saline 10 ml/kg of body weight, per orally

Group2- Received glibenclamide 10 mg/kg of body weight, per orally

Group3- Received *B. flabellifer* extract 150 mg/kg of body weight, per orally

Group4- Received *B. flabellifer* extract 300 mg/kg of body weight, per orally

Group5- 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 h by the glucose-oxidase principle [13] using the one touch basic instrument [14] and results were reported as mg/dL [15].

Table 1. Effect of *Borassus flabellifer* on reduction of blood glucose levels (mg/dL) in streptozocin-induced diabetic male wister rats treated by various doses of ethanolic extract (acute study)

Treatment Groups		Time after treatment									
	0 h	2 h	4 h	6 h	8 h	24 h					
Normal control (vehicle)	68.16±3.44	68.66±3.62	69.16±3.74	71.83±4.44	72.13±3.44	72.83±3.44					
Diabetic control (vehicle)	379.83±8.04	384.66±8.61**	388.33±9.84**	392.16±9.83**	396.83±9.48**	398.00±9.06**					
Glibenclamide (10 mg/kg/b.w.)	395.00±9.83	257.33±6.80**	220.16±7.47**	157.50±6.76**	229.00±11.23**	322.66±10.47**					
Ethanolic extract (150 mg/kg/b.w.)	401.50±8.33	375.66±10.49 ^{ns}	319.50±8.47**	231.50±8.56**	279.66±9.95**	368.33±11.08**					
Ethanolic extract (300 mg/kg/b.w.)	392.00±8.41	356.00±8.15**	274.16±7.47**	215.16±9.74**	268.83±8.32**	358.50±10.38**					
Ethanolic extract (600 mg/kg/b.w.)	392.83±8.07	292.33±7.28**	218.66±6.88**	188.50±6.14**	253.00±7.46**	348.0±9.31**					

⁻Blood glucose levels in control and experimental groups of rats on initial (0 h) and final (24 h) of the treatment periods.

Statistical analysis

Blood glucose levels were expressed in mg/dL as mean \pm SEM. The data were statistically analyzed using ANOVA with multiple comparisons versus control group. The values of p<0.01 were considered significant [16]. 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 food intake, and prostration after 18 hours of extract administration. The median lethal dose (LD50) in rats was calculated to be 1064.9 mg/kg body weight.

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Anti diabetic study

Tables 1 and 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-induced diabetic rats. Streptozocin-

⁻Values are given as mean \pm S.E.M. for groups of five rats of each.

⁻Values are statistically significant at *p < 0.05., **p < 0.01, non significant = ns > 0.05.

⁻STZ-induced diabetic rats compared with control rats.

Table 2. Effect of Borassus flabellifer on mean percentage (%) reduction of blood glucose levels (mg/dl) in streptozocin-induced diabetic male wister rats treated by various doses of ethanolic extract (acute study)

Treatment Groups	Initial 0 Day	Percentage (%) reduction blood glucose levels				Mean % Reduction	24 h	Mean % reduction after24 h
		2 h	4 h	6 h	8 h			arter 24 ii
Normal control (vehicle)	68.16±3.44							
Diabetic control (vehicle)	379.83±8.04							
Glibenclamide (10mg/kg/b.w.)	395.00±9.83	34.85	44.26	60.12	42.02	45.31	18.31	39.91
Ethanolic extract (150mg/kg/b.w.)	401.50±8.33	6.43	20.42	42.34	30.34	24.88	8.23	21.55
Ethanolic extract (300mg/kg/b.w.)	392.00±8.41	9.18	30.06	45.11	31.42	28.94	8.54	24.88
Ethanolic extract (600mg/kg/b.w.)	392.83±8.07	25.58	44.33	52.52	35.59	39.5	11.41	33.88

⁻Comparison between controls vs. all treated groups.

induced hyperglycemia has been described as a useful experimental model to study the activity of hypoglycemic agents [17]. Streptozocin selectively destroyed the pancreatic insulin secreting β-cells, leaving less active cell resulting in a diabetic state [18]. Many secondary metabolites participate in a variety of anti-diabetic functions in vivo [19]. The glycemic change in blood glucose levels of diabetic rat at different time intervals after oral administration of Borassus flabellifer extract at the doses of 150, 300, and 600 mg/kg are shown in Table 1. In diabetes rats that received 150, 300, and 600 mg/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 at the dose of 600 mg/kg. In the doses of 150 and 300 mg/kg of the Borassus flabellifer, there was a less significant change in the blood glucose levels after different time hour of extract administration. The dose of 600 mg/kg was found to be more effective in lowering glucose 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 extract of revealed the presence of tannins, carbohydrate, terpenes, saponins, flavonoids and alkaloids [20]. Effect of the flavonoids quercetin and ferulic acid on pancreatic β-cells leading to their proliferation and secretion of more insulin have been proposed as the mechanism by which they reduced hyperglycemia caused by streptozocin in diabetic rats [21-22]. The flavonoids present in Borassus flabellifer may also be acting similarly to decrease the high blood glucose levels of streptozocin-induced 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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CURRENT AUTHOR ADDRESSES

- Pradeep Goyal, Goenka College of Pharmacy, Khuri Bari, Tehsil-Laxmangarh, Dist. Sikar (Rajasthan), India. E-mail: pradeep_goyal30@yahoo.com (Corresponding author)
- M. Pharm, Goenka College of Pharmacy, Khuri Bari, Tehsil-Laxmangarh, Dist. Sikar (Rajasthan), India.
- Nilesh Jain1, Goenka College of Pharmacy, Khuri Bari, Tehsil-Laxmangarh, Dist. Sikar (Rajasthan), India
- G. K. Singh, Lachoo Memorial College of Science and Technology (Pharmacy Wing), Sector - A, Shastri Nagar, Jodhpur (Rajasthan), India.
- B. P. Nagori, Lachoo Memorial College of Science and Technology (Pharmacy Wing), Sector - A, Shastri Nagar, Jodhpur (Rajasthan), India.