Antidiabetic Activity of *Borreria Hispida* Linn on Streptozotocin Induced Diabetic in Albino Rats

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ABSTRACT

The present study was aimed to identify the anti-diabetic effect of *Borreria hispida* (BH) on streptozotocin-induced diabetic rats. Acute toxicity of BH was studied according to the guidelines of OECD- 423. The preliminary screening method was done by using an oral glucose tolerance test (OGTT). Diabetes was induced in rats by intraperitoneal injection of streptozotocin (60 mg/kg). After induction of diabetes, rats were treated with methanolic extracts of *Borreria hispida* Linn (MEBH) (200 and 400 mg/kg p.o., respectively) and Glibenclamide 600 µg/kg for 28 days. Blood was collected by puncturing retro-orbital plexus and serum was analyzed for biochemical parameters MEBH caused significant reduction in elevated blood glucose levels in diabetic rats. Histopathological study revealed that regeneration of pancreatic β cells in the MEBH-treated animals. Based on the results, MEBH showed significant anti-diabetic effect against streptozotocin-induced diabetes rats.

Key Words: *Borreria hispida Linn, Streptozotocin, Anti-diabetic and Glucose.*

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INTRODUCTION

For thousands of years, mankind has been utilizing plant source to alleviate or cure illnesses. Plants are a source of new chemical compounds which are of potential application in medicine and other uses [1]. Throughout human history, herbs have been utilized in food, cosmetics, and fragrances [2]. In 1985, 119 plant secondary metabolites were identified which were utilized as drugs. In addition, plants are the source of conventional medicines which are applied for the treatment of different diseases [3].

Diabetes mellitus (DM) is an important health issue being the third greatest reason of death worldwide, and if not treated, it is responsible for several complications influencing different organs in the body [4]. Patients with chronic hyperglycemia experience injury and failure of different organs [5]. These end up with more problems including retinopathy, cardiovascular disorders, polyuria and polyphagia [6].

Normally, insulin is secreted by the β-cells located at the islets of Langerhans in response to high levels of blood sugar. It improves the ability of muscles, red blood cells, and fat cells to absorb sugar out of the blood and use it in other metabolic processes, which restores the sugar levels to the normal level [7].

According to the literature survey, a wide range of traditional uses has been reported for the plant *Borreria hispida* Linn, but it has not been scientifically proven. The World Health Organization (WHO) and Philippine Alternative Medicine have proposed further assessment of the traditional plant treatment for diabetes. The present study was carried out to assess the anti-diabetic activity of methanolic extract of *B. hispida* (MEBH).

MATERIALS AND METHODS

Plant Material

The Plants were collected from Anaikuttam, a place near Sivakasi, Virudhunagar District, Tamil Nadu and it was identified and authenticated by taxonomist Dr. V. Ganesan, professor and head of Department of Botany, Ayyanadar Janakiammal College of Arts and Science,

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Sivakasi, Virudhunagar District, Tamil Nadu. The plant specimen was identified as *Borreria hispida* Linn.

Preparation of Maceration

The whole plant of *B. hispida* was air dried and powdered. Then, about 150 g of the powdered material was added to 1000 ml solvent (methanol and aqueous) in a stopped container for a defined period with frequent agitation until the soluble matter was dissolved. The extracts were concentrated by distillation and the solvents were recovered [8].

Phytochemical analysis

The methanolic extract of the whole plant was subjected to chemical tests for characterization of its phyto-constituents [9, 10].

Selection of Animal

Albino rats weighing 150-200 g were utilized for the study. They were kept at room temperature (25 ± 1 ℃) under 12 hr light/12hr dark cycle in the animal house. Rats were fed with a commercial pellet diet and water *ad libitum* freely during the study [11]. All animal procedures were performed after approval from the IAEC and accordance with the recommendations and proper care (Ref. No. SBCP/2013-14/CPCSEA/IAEC-I/3(i)).

Acute toxicity study

Acute oral toxicity test was carried based on OECD-423 guidelines. All the animals were randomly distributed into one control group and four treated groups, including three animals per group. 5, 50, 300 and 2000 mg/kg body weight of the extract was orally administered for the groups 1, 2, 3, and 4, respectively. The animals were inspected continuously for the first 72 hours and 7 days for any signs of behavioral, toxicity, mortality and body weight alterations [12].

Experimental Design

Albino rats were fasted overnight (16 hours) before the experiment. Glucose was loaded orally (2g/kg) 30 minutes after the standard, saline or the plant extract administration and blood samples were taken before and 0, 30, 60, 90 and 120 min after the glucose administration for the measurement of glucose with a glucometer [13].

OGTT (Oral glucose tolerance test)

Group I - Normal control (saline)
Group II - Glucose (2g/kg) p.o.
Group III - Glibenclamide (600µg/kg) p.o + Glucose (2g/kg) p.o.
Group IV - MEBH (200mg/kg) p.o + Glucose (2g/kg) p.o.
Group V - MEBH (400mg/kg) p.o. + Glucose (2g/kg) p.o

The animals of group II to V were allowed to drink a 5% glucose solution overnight to overcome STZ-induced diabetics. On 3rd day of STZ injection, their blood glucose was determined. On 4th day, the respective dosing was started considering it as 1st day of treatment and was continued till 28 days. Body weight and blood glucose level were observed on 0, 7, 14, 21, and 28th day of post-treatment. On the 28th day, the rats were sacrificed under mild ether anesthesia. The pancreas was transferred to 10% formalin solution and directly processed by the paraffin method. The sections were stained by haematoxylin and eosin for histopathological analysis.

Statistical analysis

Values were presented as mean± SEM. The mean difference in paw volume and biochemical parameters were analyzed utilizing one-way ANOVA followed by Dunnett's test. The values were considered significant at $P<0.001$, $P<0.01$, and $P<0.05$. The analysis was carried out by Graph Pad prism statistical software (Version 5.03).

RESULT AND DISCUSSION

The plant *B. hispida* was subjected to different solvent extractions and the extracts were dried, and finally, percentage yield also was calculated. The chemical tests were done on different extracts of *B. hispida*. The phyto-constituents were present in different extracts like glycosides, steroids, flavonoids, alkaloids, and saponins. The methanolic extract revealed the presence of maximum phyto-constituents and was used for further study. The results are shown in Table 1.

The MEBH was tested for acute toxicity study in order to fix the dose for the study. During 14 days of observation of animals no toxicity, behavioral changes and mortality were reported in a dose of 2000 mg/kg. Hence, the effective dose was identified as 1/5th and 1/10th of the maximum tolerated dose, and so 200 and 400 mg/kg b.w. were selected as the doses for testing the activity.

The administration of OGTT shows a significant rise in the blood sugar level in all the glucose-treated groups. Administration of standard drug Glibenclamide 600 µg/kg p.o. shows a significant decrease in the blood sugar level when compared to the glucose-treated group. Administration of MEBH 400 mg/kg p.o. shows a significant reduction of the blood sugar level when compared to MBHE 200 mg/kg p.o. The results are presented in Table 2.

The administration of STZ 60 mg/kg i.p. showed a significant rise in the blood sugar level, which attain a hyperglycemic condition within 48 hrs. After a hyperglycemic condition, animals were divided into four groups except for normal control. Administration of standard drug Glibenclamide 600 µg/kg p.o. showed a gradual decrease in the blood sugar level until the end of 14th hrs. MEBH 200 mg/kg and 400 mg/kg p.o. decreased blood sugar when compared to the diabetic control group. The results are shown in Table 3.

200 mg/kg and 400 mg/kg of MEBH increased the level of HDL and decreased the level of LDL, TG, and TC

when compared to the diabetic control group. The results are shown in Table 4.

Histopathological evaluation revealed that administration of streptozotocin induced diabetes with the alteration in the pancreas. Administrations of 200mg/kg and 400mg/kg p.o. MEBH showed normal acini and ducts; stroma revealed scattered lymphocytes compared to the normal and Glibenclamide-treated pancreas.

Phytochemical Substance	Methanol extract
Alkaloids	+
Steroid	+
Triterpenoids	-
Fixed Oils	+
Tannins	+
Proteins& Amino acids	+
Saponins	+
Glycosides	+
Flavonoid	+
Carbohydrate	+

Table 1: Phytochemical screening of whole plant extracts of Borreria hispida Linn.

Table 2: Effect of MEBH on oral glucose tolerance test (OGTT) in normal and streptozotocin-induced diabetic rats.

G. No.	Drug& Treatment	Normal Glucose mg/dl	The blood Glucose level in (min)mg/dl				
			0	30	60	90	120
I	Normal Saline (Control)	83.67 ± 2.03	89.03 ± 1.29**	88.25 ± 1.49***	87.40 ± 1.17**	85.5 ± 1.12**	84.75 ± 1.11**
II	Diabetic Control Glucose 2gm/kg p.o.	81.33 ± 1.20	116.25 ± 2.62	128.75 ± 3.75	118.5 ± 1.96	117 ± 3.87	123.4 ± 3.02
III	Glibenclamide 600µg/kg p.o. + Glucose 2gm/kg p.o.	83.67 ± 0.88**	89.5 ± 2.22 **	112.5 ± 1.55**	103.5 ± 3.52**	82.25 ± 1.31**	86.5 ± 3.06**
IV	MEBH 200mg/kg p.o.+ Glucose 2gm/kg p.o.	87.67 ± 2.91	102.75 ± 1.10*	116 ± 1.68	101.75 ± 2.1*	97.5 ± 2.30*	95.6 ± 1.4**
V	MEBH 400mg/kg p.o.+ Glucose 2gm/kg p.o.	86.17 ± 1.01	94 ± 3.67**	122.5 ± 3.20	102 ± 2.05*	89 ± 1.43**	86.13 ± 1.82**

Values are expressed as the mean ± SEM. The experiment's statistical significance (p) was calculated by one-way ANOVA followed by Dunnett's multiple comparison test, *** P<0.001, ** p<0.01, *p<0.05, NS-non significant by comparing the treated group with diabetic control.

Table 3: Effect of whole plant extract of *Borreria hispida* Linn in streptozotocin-induced diabetic rats.

G. No.	Drug & Treatment	Normal sugar level (before) mg/dl	Blood sugar level (After 48 hrs) mg/dl	Blood glucose level after drug administration (hr) mg/dl				
				0	3	5	7	14
I	Normal Saline (Control)	82.75 ± 4.38	85.12 ± 23***	85.12 ± 23***	86.01 ± 1.01***	84.23 ± 1.21***	83.47 ± 0.98***	83.01 ± 0.87***
II	Diabetic control STZ 60 mg/kg i.p.	80.12 ± 0.98	332.26 ± 5.21	332.26 ± 5.21	340.12 ± 5.56	345.11 ± 4.98	343.27 ± 3.65	340.01 ± 3.02
III	Glibenclamide 600 µg/kg per day/per oral + STZ 60 mg/kg i.p.	83.64 ± 1.02	300.11 ± 6.12	300.11 ± 6.12*	241.03 ± 5.24**	180.23 ± 3.56***	123.03 ± 2.56***	90.14 ± 1.21***
IV	MEBH 200 mg/kg per day/per oral + STZ 60 mg/kg i.p.	82.36 ± 1.31	311.25 ± 6.23	311.25 ± 6.23	280.41 ± 5.01**	240.12 ± 2.21***	200.47 ± 1.98***	180.27 ± 1.11***
V	MEBH 400 mg/kg per day/per oral + STZ 60 mg/kg i.p.	84.11 ± 1.12	307.25 ± 11.01	307.25 ± 11.01*	245.21 ± 5.47**	201.54 ± 4.56***	148.67 ± 3.41***	100.21 ± 2.01***

Values are expressed as the mean ± SEM. The experiment's statistical significance (p) was calculated by one-way ANOVA followed by Dunnett's multiple comparison test, *** P<0.001, ** p<0.01, *p<0.05, NS-non significant by comparing the treated group with diabetic control.

Table 4: Blood parameters of whole plant extract of *Borreria hispida* Linn in streptozotocin-induced diabetic rats.

G. No.	Drug & Treatment	LDL (mg/dl)	HDL (mg/dl)	TG (mg/dl)	TC (mg/dl)
I	Normal Saline (Control)	23.01 ± 0.02***	31.02 ± 0.22**	75.01 ± 5.27**	95.02 ± 3.28**

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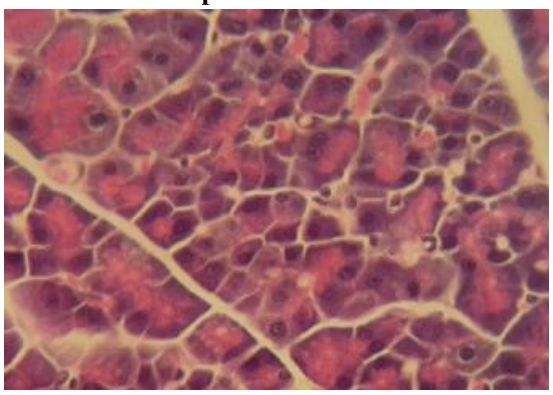
II	**Diabetic control STZ 60 mg/kg i.p.**	51.12 ± 0.01	19.25 ± 0.12	105.05 ± 6.46	132.14 ± 6.27
III	**Glibenclamide 600 µg/kg per day/per oral + STZ 60 mg/kg i.p.**	29.23 ± 0.01**	29.13 ± 0.02**	81.02 ± 2.01**	101.05 ± 2.13**
IV	**MEBH 200 mg/kg per day/per oral + STZ 60 mg/kg i.p.**	25.41 ± 0.01***	22.36 ± 0.11*	93.08 ± 3.27*	121.27 ± 4.31*
V	**MEBH 400mg/kg per day/per oral + STZ 60 mg/kg i.p.**	24.45 ± 0.02***	27.17 ± 0.13**	89.12 ± 2.28**	108.18 ± 2.81**

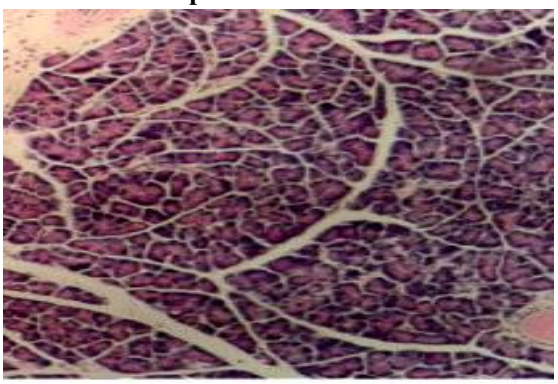
Values are expressed as the mean ± SEM. The experiment's statistical significance (p) was calculated by one-way ANOVA followed by Dunnett's multiple comparison test, *** P<0.001, ** p<0.01, *p<0.05, NS-non significant by comparing the treated group with diabetic control.

Group 1: Normal saline



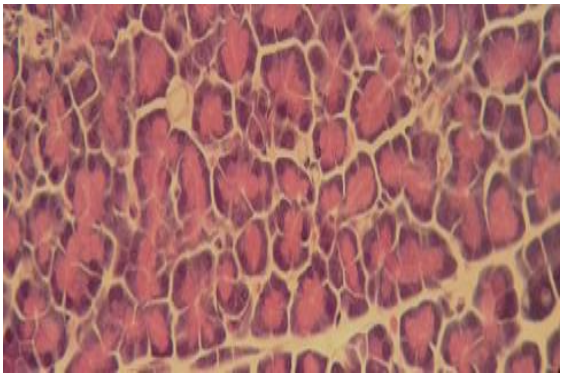
The pancreas of control rats showing normal acini cells and ducts, as well as normal islets.

Group II: Diabetic control



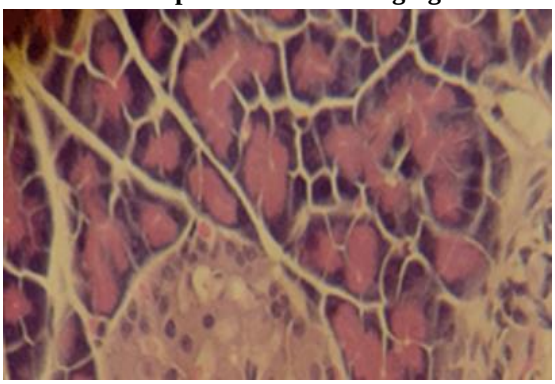
The pancreas of diabetic rats showing abnormal septa and cysticaly dilated ducts; stroma shows hemorrhagic areas

Group III: Glibenclamide 600 µg/kg



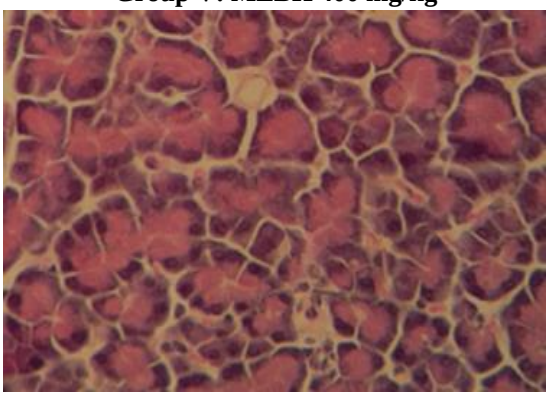
The pancreas of diabetic rats showing normal acini cells and normal langerhans.

Group IV: MEBH 200mg/kg



The pancreas of diabetic rats showing congestion and slight vaculation of langerhans cells

Group V: MEBH 400 mg/kg



The pancreas of diabetic rats showing normal acini cells and ducts with normal langerhans.

Figure 1: Histopathology section of the pancreas by STZ-induced diabetic rats.

CONCLUSION

Borreria hispida was found to have hypoglycemic activity. The present study provided the data for the anti-diabetic activity of methanolic extract of *B. hispida* that could be useful for future clinical studies of this plant. So, further beneficial studies are in progress at the Department of Pharmacology to confirm the virtue of its therapeutic effects.

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