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Evaluation of Antidiabetic Activity of *Dichanthium annulatum*, *Saccharum benghalense* and *Hippaestrum vittatum* Ethanolic Extracts Against Streptozotocin-Induced Diabetic Rats

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ABSTRACT
Objective: The current work aimed to evaluate the antidiabetic activity of ethanolic leaf extracts of *Dichanthium annulatum*, *Saccharum benghalense*, and *Hippeastrum vittatum* against Wistar rats having diabetes induced by streptozotocin (STZ). **Materials and methods:** The plant materials were collected from Uttarakhand, India, authenticated, and extracted using a Soxhlet apparatus. Acute oral toxicity was assessed using OECD guidelines. An intraperitoneal application of STZ (65 mg/kg) caused diabetes, and animals were divided into nine groups, including normal control, inducer, standard glibenclamide (5 mg/kg), and groups treated with two doses (100 mg/kg and 200 mg/kg) of each extract. Over 15 days, body weight and blood glucose level were monitored, with lipid profiles and pancreatic histopathology assessed at the end of the study. **Results:** The finding showed that all extracts, particularly at 200 mg/kg, improved lipid markers and significantly reduced blood glucose levels, with *D. annulatum* exhibiting effects comparable to glibenclamide. Histopathology revealed reduced pancreatic damage and signs of β-cell regeneration in treated groups. **Conclusion:** These findings suggest that *D. annulatum*, *S. benghalense*, and *H. vittatum* possess promising antidiabetic activity.

INTRODUCTION:

The symptoms of diabetes mellitus involve the breakdown of carbohydrates, proteins, and lipids, leading to an increase in blood glucose levels due to insufficient or no insulin synthesis[1]. The International Diabetes Federation predicts that more than 783 million people will develop diabetes by 2045, notably 537 million adults and 124.87 million in India[2]. Diabetes is becoming more prevalent all over the world, with serious consequences for global well-being and economic expansion[3]. Diabetes, if not managed appropriately, may lead to a variety of consequences such as heart problems, damage to the nerves, renal damage, and damage to the eyes[4]. The use of natural therapies in disease therapy and management has been increasing rapidly in the past few decades, with many people all throughout the world depending on them as part of their main health-care system[5]. Herbal medicinal products came from a wide range of different sources, such as plants grown on land, terrestrial microbes, aquatic creatures, and terrestrial vertebrates as well as invertebrates, but herbal products play an important role as a source of synthetic as well as herbal drugs[6].

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Dichanthium annulatum is from the Poaceae family and is a perennial grass commonly recognized as marvel grass. Numerous diseases, including diabetes, hypertension, inflammation, astringent, anthelmintic, ulcerative colitis, diuretic, and antioxidant properties, have been treated with Poaceae plants in traditional medicine[7, 8].

Saccharum benghalense, from the Poaceae family, is commonly known as *Tripidium bengalense* and is generally referred to as munj grass. It grows in desert areas and along riverbanks. It had previously been used for the following conditions: vertigo, fever, inflammation, bleeding wounds, burning sensations, etc[8].

Hippaestrum vittatum is a perennial annual herb from the family Amaryllidaceae[9]. The plant focuses primarily on its alkaloidal concentration. The valuable alkaloids in this plant have demonstrated noteworthy sedative, anxiolytic, anticonvulsant, antidepressant, and cytotoxic characteristics. Still, considering their good standing, plants from the Amaryllidoideae subfamily of the Amaryllidaceae have received little attention for their anti-diabetic characteristics, notably their mucilages[10].

Thus, this is the objective of this investigation, which had been planned to explore the in vivo anti-diabetic activity of extracts derived from ethanol of *D. annulatum, S. benghalense* and *H. vittatum* leaves with glibenclamide in STZ-induced diabetic rats.

MATERIALS & METHODS:

Plant materials:
Freshly gathered air-dried leaves of *D. annulatum, S. benghalense* & *H. vittatum* were collected locally from Roorkee, Uttarakhand, India. The botanical identification of *D. annulatum, S. benghalense* and *H. vittatum* was carried out by former Chief Scientist Dr. Sunita Garg, Head RHMD & Chief Scientist Mr. R.S. Jayasomu, and Head RHMD in CSIR-NIScPR (Delhi). The leaves had been washed thoroughly with water to get rid of dust particles & then left to dry at the natural temperature in the shade. The leaves that were air-dried were ground using a blender to obtain the coarse powder & preserved until needed in an airtight container.

Preparation of extracts:
The air-dried and powdered leaves of *D. annulatum, S. benghalense and H. vittatum* were put in a thimble of a Soxhlet apparatus after being sequentially defatted with petroleum ether solvent. The entire extraction process was carried out for 8-10 hours using an ethanol solvent on a heating mantle at a 40 to 60°C temperature. Following the extraction process, the extract material from the sample was filtered & fully dried. The highly concentrated dry extracts of leaves were collected in an encapsulated airtight container[11].

Animal and diet:
The Wistar rats, which weighed between 150 to 200 g, were procured from the PBRI, Bhopal. The animals were kept in standard big, spacious, hygienic cages made from polypropylene & kept at 22 ± 2°C temp with a 12-hour cycle of light & dark. The method of experiment and protocol applied in this present study was reviewed by the Institutional Animal Ethical Committee, Reg. No: 1824/PO/RcBi/S/15/CPCSEA, and was in accordance with the guidelines of the CPCSEA. Water was administered regularly until the end of the research, and each test subject received a commercially accessible normal rat pellet diet (NPD) from Keval Sales Corporation in Vadodara.

Experimental design:
The verified hyperglycaemic rats were subsequently separated into 9 groups with 6 rats in each group for 15 days of study[4,12]. The following grouping was done:
Group 1: Normal Group
Group 2: Inducer Group
Group 3: Diabetic rats (STZ-injected) treated with 5 mg/kg body wt. glibenclamide;
Group 4: The test group received 100 mg/kg body weight DA extract;
Group 5: The test group received 200 mg/kg body weight DA extract;
Group 6: The test group received 100 mg/kg body weight HV extract;
Group 7: The test group received 200 mg/kg body weight HV extract;
Group 8: The test group received 100 mg/kg body weight SB extract;
Group 9: The test group received 200 mg/kg body weight SB extract;

The freshly prepared solution of extracts was administered orally every day for 15 days. Body weight and blood glucose level determine the onset of the study and the end of the study.

Acute oral toxicity study:
The LD50 for the extract was estimated by adopting the OECD-423 guidelines[13]. In this toxicology investigation, Wistar rats weighing 150–200 g (n =3) were randomly selected. Prior to the experiment, the rats had been starving for four hours and kept at standard temperature and humidity levels (22°C and 55°C, respectively). The rats were permitted to have unfettered access to water. The *D. annulatum, S. benghalense and H. vittatum* leaf extracts were dissolved in DW & taken orally through gavage along with the initially administered doses. Over a duration of fourteen days, the general behavior of

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the test animals was closely monitored for any possible sign of toxicities and latency of mortality[14].

Induction of type-2 diabetes:
The animals used for research employed within this investigation were healthy Wistar rats weighing between 150-200 g. Wistar rats were fasted for 12 h before being induced with diabetes. Rats were given an intraperitoneal (IP) injection of 65 mg/kg body wt. of streptozotocin mixed with 0.1 M citrate buffer at pH 4.5 for inducing diabetes. Diabetic rats with fasting blood glucose levels more than 250 mg/dl were utilized in this research after one week[15].

Biochemical Parameters:
Blood was drawn from the rat tail's veins at regular intervals until the study had ended. A glucometer was used to measure the blood glucose level when the subject was fasting. Glucose was observed at the day of onset of the study and the end of the study and the weight of each rat was recorded using a digital electric balance. Erba Diagnostics standard kits were used to estimate total cholesterol, HDL, LDL, and triglycerides.

Histopathological Studies:
At the completion of the experiment, the experimental rats were euthanized for examination. The formalin-fixed sections of tissues from the pancreas were serially alcohol-dehydrated, cleansed using xylene, & fixed in paraffin blocks. After a routine process, the micro sections (4-5 microns thick) were separated and examined using hematoxylin and eosin (H and E) and tested for histopathological alteration[16].

Statistical Analysis:
The findings will be expressed as Mean ± SD (n=6). The results received were evaluated by statistical analysis using one-way analysis of variance (ANOVA), followed by the Bonferroni t-test. A significance level of $P < 0.05$ was utilized for the assessment of groups.

RESULTS:

Plants have been implemented for decades for curing a variety of disorders. India has a wide variety of medicinal plants that are considered to be biologically active. Because of the therapeutically active substances found in the plants, they are frequently utilized for treating a variety of diseases in diverse ethnic cultures. Diabetes is currently one of the most serious disorders of metabolism. Both domestic and foreign markets offer an extensive selection of drugs that manage or improve the symptoms of diabetes. However, the cost of these diabetic medications is too high, and they have a number of adverse consequences. Because they are less expensive and have fewer adverse effects, herbal medicines are crucial in this case for the management of diabetes. Therefore, determining the in-vivo antidiabetic effect against STZ-induced diabetic rats is the work's goal[15].

Acute toxicity study:
Test samples DA, HV, and SB have been examined for acute oral toxicity at various dosages of 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg. For sample DA, no reported death or significant behavioral variations were noted at any doses, with normal parameters across toxicological evaluations as well as a steady rise in body weight all throughout the fourteen-day period. Similarly, sample SB demonstrated no adverse effects, normal toxicological parameters, & an increase in body weight with no fatalities recorded at all dose levels. However, for sample HV, behavioral & physiological changes were noted, including watery stool & diarrhea at higher doses (≥50 mg/kg), pale eyes at 2000 mg/kg, and instances of aggression at 300 mg/kg, though no mortality occurred. Overall, the results indicate that DA and SB were well tolerated across doses, while HV presented mild to moderate signs of dose-dependent toxicity at higher levels.

Effects of extracts on body weight:
Our study's findings demonstrated a significant decrease in the body weight of the rats with STZ-induced diabetes. Over the period of the study, the diabetic rats' body weight decreased, a common indicator of diabetes carried due to the breakdown of proteins and lipids as a substitute for energy. Plant extracts (lower and higher doses of DA, HV, and SB) and glibenclamide treatment improved the body weight of all groups receiving treatment compared to the diabetes control group. There was significant difference ($P < 0.05$), as shown in Table 1 and Figure 1.

Table 1 Body weight in (g)

S. No.	Group	Body Weight (g)	
		Onset of study	End of study
1.	Normal	193.4±6.082	197.1±2.062
2.	Inducer	195.2±5.010	183.6±3.201
3.	Standard	188.5±7.211	188.3±10.40*
4.	DA 100mg/kg	190.3±6.325	192.6±8.524
5.	DA 200mg/kg	191.6±10.40	196.3±8.717*
6.	HV 100mg/kg	198.1±2.182	186.8±2.725
7.	HV 200mg/kg	192.2±9.632	194.6±6.333
8.	SB 100mg/kg	188.7±8.082	194.1±6.094
9.	SB 200mg/kg	195.4±5.072*	193.6±9.015**

Values will be shown as MEAN±SD for n=6, evaluated with a one-way ANOVA followed by a Bonferroni test, *P<0.050.

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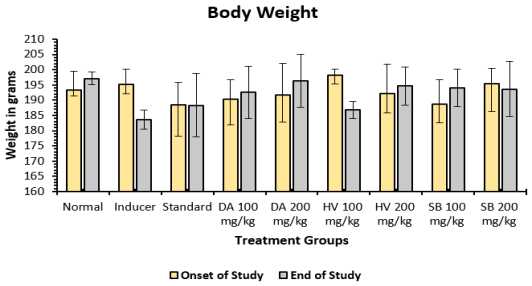

Fig. 1. represents the body weight at the start and end of the study

Effects of extracts on blood glucose:

The result indicates the plant extracts, *Dichanthium annulatum* (DA), *Hippeastrum vittatum* (HV), and *Saccharum benghalense* (SB), exhibited varying degrees of anti-diabetic effects as shown in Table 2 and Figure 2.

Table 1: Antidiabetic activity in STZ-induced diabetic rats

Blood Glucose Levels (mg/dl)			
S.No.	**Group**	**Before Dose**	**After Dose**
1.	**Normal**	150.10±4.00	170.25±6.21
2.	**Inducer**	487.60±4.10	395.00±6.90
3.	**Standard**	389.50±7.84*	209.3±2.72
4.	**DA 100mg/kg**	365.23±3.52	210.32±6.25**
5.	**DA 200mg/kg**	305.67±5.03*	198.67±8.14*
6.	**HV 100mg/kg**	384.65±3.02	266.00±5.44
7.	**HV 200mg/kg**	360.00±5.23	260.21±5.41
8.	**SB 100mg/kg**	350.11±3.12	240.21±8.65
9.	**SB 200mg/kg**	327.38±4.03**	232.09±7.84*

Values will be shown as MEAN±SD for n=6, evaluated with a one-way ANOVA followed by a Bonferroni test, *P<0.050.

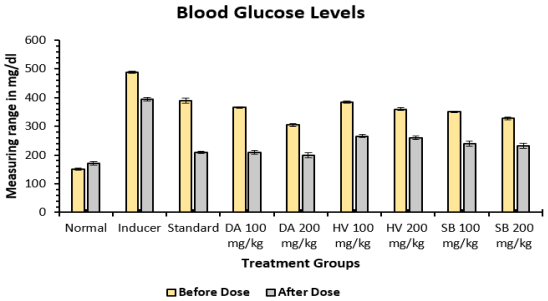

Fig.2. represents anti-diabetic activity in STZ-induced diabetic rats.

Biochemical analysis:

The result indicates the plant extracts, *Dichanthium annulatum* (DA), *Hippeastrum vittatum* (HV), and *Saccharum benghalense* (SB), exhibited varying degrees of biochemical parameters as shown in Table 3 and Figure 3.

Table 3: Biochemical Parameters in mg/dL

S. No.	Groups	Total Cholesterol (mg/dL)	HDL-cholesterol (mg/dL)	LDL-cholesterol (mg/dL)	VLDL (mg/dL)	Triglycerides (mg/dL)
1.	**Normal**	98.5 ± 3.2	52.4 ± 2.8	66.8 ± 2.1	19.3 ± 1.0	96.7 ± 5.2
2.	**Inducer**	178.6 ± 4.5	30.2 ± 2.3	142.4 ± 3.5	45.9 ± 2.7	229.6 ± 9.1
3.	**Standard**	112.8 ± 4.1*	47.9 ± 3.0*	67.6 ± 2.4*	27.3 ± 1.6	136.5 ± 7.0*
4.	**DA 100mg/kg**	126.3 ± 3.9	42.1 ± 2.7	138.2 ± 2.9	32.0 ± 1.9	160.2 ± 6.7
5.	**DA 200mg/kg**	108.4 ± 3.6*	49.3 ± 2.5*	83.9 ± 2.2*	25.2 ± 1.3	125.9 ± 6.1*
6.	**HV 100mg/kg**	130.1 ± 4.2	39.8 ± 3.1	153.2 ± 3.0	34.2 ± 2.1	171.3 ± 7.5
7.	**HV 200mg/kg**	115.6 ± 3.5	45.7 ± 2.6	89.7 ± 2.6	28.0 ± 1.5	140.2 ± 6.3*
8.	**SB 100mg/kg**	120.7 ± 3.8	41.5 ± 2.8	116.9 ± 2.5	30.7 ± 1.8	153.5 ± 6.8
9.	**SB 200mg/kg**	110.3 ± 3.7*	48.0 ± 2.9*	75.5 ± 2.3*	26.8 ± 1.4	134.1 ± 6.5*

Values will be shown as MEAN±SD for n=6, evaluated with a one-way ANOVA followed by a Bonferroni test, *P<0.050.

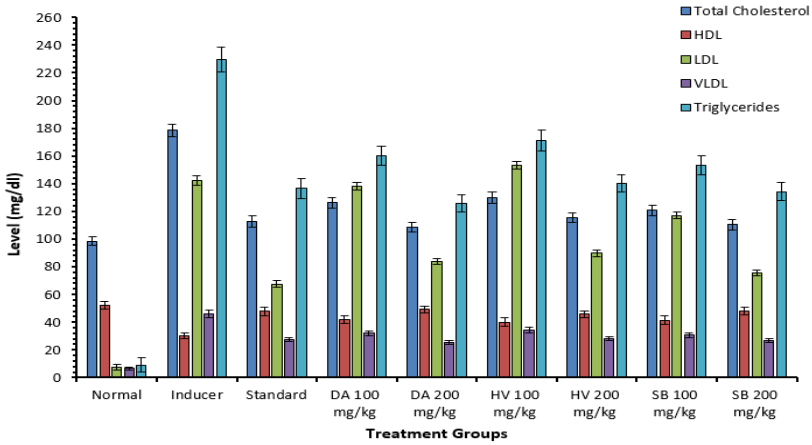

Fig. 3 represents biochemical parameters.

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Histopathology:

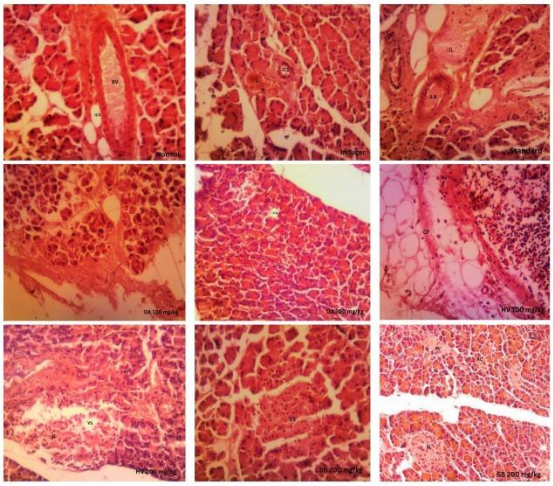

Fig. 4. Pancreatic histology in all groups that showed islet of Langerhans (IL), blood vessel (BV), interlobular duct (ILD), intercalated duct (ICD), arteriole (A), interlobular artery (ILA), acinar cell (AC), vascular stroma (VS), and collagen fibers (CF).

DISCUSSION:

The key objective of the investigation was to determine the effectiveness of plant extracts from *Dichanthium annulatum*, *Hippeastrum vittatum*, and *Saccharum benghalense* in managing diabetes induced through Streptozotocin (STZ). The study involved multiple test groups, including a normal control, an inducer group, a standard treatment group (Glibenclamide), and test groups receiving different doses of the plant extracts. The results demonstrated that STZ administration effectively induced hyperglycemia in the experimental rats, as evidenced by the significantly elevated blood glucose levels in the inducer group. The diabetic rats showed a decrease in body weight over the study period, which is a common symptom associated with diabetes due to the breakdown of proteins and fats as an alternative energy source. The administration of glibenclamide (standard drug) significantly lowered blood glucose levels, confirming its efficacy as a reference anti-diabetic agent.

Among the plant extracts, *Dichanthium annulatum* (DA), *Hippeastrum vittatum* (HV), and *Saccharum benghalense* (SB) exhibited varying degrees of anti-diabetic effects. The higher doses of DA (200 mg/kg), HV (200 mg/kg), and SB (200 mg/kg) demonstrated a more significant reduction in blood glucose levels compared to their respective lower doses. The DA extract, particularly at a higher dose, showed a glucose-lowering effect analogous to that of the standard product, suggesting a promising anti-diabetic potential. This may be attributed to bioactive compounds present in the extracts that enhance insulin secretion, improve glucose uptake, or protect pancreatic β-cells from STZ-induced damage.

Statistical analysis confirmed that the observed differences in glucose levels and body weight were significant ($P < 0.05$), reinforcing the reliability of the findings. The reduction in glucose levels across different groups suggests that these plant extracts may exert their effects through mechanisms such as insulin sensitization, β-cell regeneration, or inhibition of carbohydrate metabolism enzymes.

Additionally, there was an enormous rise in total cholesterol, low-density lipoproteins, and very low-density lipoproteins, as well as triglycerides, when the inducer group was compared to the normal control. On the other hand, when comparing the inducer group to the normal control, the total amount of high-density lipoproteins declined significantly. The result indicates the plant extracts, *Dichanthium annulatum* (DA), *Hippeastrum vittatum* (HV), and *Saccharum benghalense* (SB), exhibited varying degrees of biochemical parameters. The higher doses of DA (200 mg/kg), HV (200 mg/kg), and SB (200 mg/kg) demonstrated a more significant effect than the lower dose.

Histopathological examination of pancreatic tissues further supported the biochemical findings. The pancreatic sections from diabetic rats (inducer group) revealed degeneration of the islets of Langerhans, indicating severe pancreatic damage due to STZ toxicity. In contrast, the test groups treated with plant extracts, particularly at higher doses, exhibited improved pancreatic architecture, with visible regeneration of islet cells and preservation of acinar structures. This suggests that the plant extracts not only helped in glucose regulation but also provided a protective effect on pancreatic tissues.

In conclusion, this study highlights the potential of *Dichanthium annulatum*, *Hippeastrum vittatum*, and *Saccharum benghalense* as natural anti-diabetic agents. The findings suggest that these plant extracts, particularly at higher doses, could be explored further for their therapeutic efficacy in diabetes management. Future studies should focus on isolating the active compounds responsible for the observed effects, exploring their mechanisms of action, and evaluating their long-term safety and efficacy in clinical settings.

CONCLUSION:

The current research assessed the evaluation of antidiabetic activity of *Dichanthium annulatum*, *Saccharum benghalense* and *Hippaestrum vittatum* ethanolic extracts against streptozotocin-induced diabetic rats. These positive results validate the traditional usage of these plants for managing

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diabetes and reveal that they may be used as natural resources to create novel antidiabetic treatments. To identify the active ingredients, understand their precise mechanisms of action, and evaluate their safety and effectiveness in future clinical trials, further research is necessary.

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CONFLICT OF INTEREST:

The authors state that they possess no competing interests.

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