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ANTIDIABETIC ACTIVITY OF THE METHANOL EXTRACT OF *HYPOESTES CANCELLATA* NEES (ACANTHACEAE) ON ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

Aim: This study evaluated the acute toxicity and antidiabetic activity of *Hypoestes cancellata*, a member of the Acanthaceae family.

Place and Duration of the Study: This study was conducted at the Department of Pharmacology and Toxicology, Ahmadu Bello University from March to April, 2023.

Methodology: The shade dried plant material was size reduced and macerated with 70% aqueous methanol to obtain the extract. Organization for economic co-operation and development (OECD) guideline 425 was used to carry out the acute toxicity study of the extract. Diabetes was induced in rats by administration of a single dose of alloxan monohydrate at a dose of 170mg/kg body weight of the rats and only rats with blood glucose levels 11.1mmol/L or above were selected for the study. The rats were randomly divided into five groups and a group of normal rats each consisting of six rats. All groups were treated daily for four weeks while blood glucose levels were determined weekly.

Results: At a limit dose of 5000mg/kg, the methanol extract of *H. cancellata* was found to be relatively safe. The methanol extract of *H. cancellata* at all doses significantly reduced blood glucose level of diabetic rats within the 28 days of treatment. Percentage reduction in blood glucose at day 28 of treatment for different groups were 34%, 71% and 61% for 250, 500 and 1000mg/kg body weight respectively. Treatment with methanol extract of *H. cancellata* produced a decrease in total cholesterol, high density lipoprotein, low density lipoprotein and triglycerides at various doses of the extract though statistically insignificant at P value ≤ 0.05.

Conclusion: The 70% methanol extract of *H. cancellata* significantly reduced blood glucose levels in alloxan induced diabetic rats within 28 days of treatment.

Keywords: Diabetes mellitus, *Hypoestes cancellata*, Alloxan-induced diabetes, Acanthaceae

INTRODUCTION

Diabetes is a group of metabolic disorders characterized by a persistent high blood glucose level caused by either deficiency in the secretion of insulin by the pancreas, lack of response to the secreted insulin by cells, or both commonly accompanied by disturbances in the metabolism of carbohydrates, fat and proteins [1]. It has been estimated that about 463 million adults are living with diabetes mellitus, which may increase to 700 million by 2045 [2]. Diabetes mellitus and its complications cause an estimated annual death of more than four

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million [3]. In Nigeria, diabetes is showing an enormous increase in prevalence. This presents a real challenge to health financing by governments and nongovernmental organizations [4]. Most of the conventional antidiabetic drugs in use have adverse effects such as severe hypoglycemia, lactic acidosis, idiosyncratic liver cell injury, permanent neurological deficit, digestive discomfort, headache and dizziness [5]. The traditional medicine systems of many cultures globally have employed medicinal plants in the treatment and management of diabetes mellitus [6]. Today, medicinal plants continue to play a significant role in the management of diabetes, especially in developing countries, where many people do not have access to conventional anti-diabetic therapies [6]. *Hypoestes cancellata* is a species of the savanna belt occurring in the forests of West and Central Africa [7].

MATERIALS AND METHODS

Plant Collection, Identification and Preparation

Hypoestes cancellata was collected from Kudendan, Chikun Local Government Area of Kaduna State. The plant was authenticated and identification at the Herbarium Section of the Department of Botany, Ahmadu Bello University, Zaria, with voucher number 1033. Thereafter, the plant material was shade dried and subsequently size-reduced. It was then stored in an air tight container.

In a stoppered glass jar, 1000g of the powdered plant material was extracted by maceration with 70% methanol at room temperature for 7 days with occasional shaking. The contents of the jar were then filtered and the filtrate evaporated to dryness on a thermostatic water bath at 60ºC.

Acute Toxicity

An acute toxicity study was conducted using the Organization for Economic Cooperation and Development (OECD) guidelines in rats (OECD Test Guideline 425, 2001). The limit dose test procedure was adopted to evaluate the acute toxicity of 70% methanol extract of *H. cancellata* via oral administration in rats. A total of 3 rats were used. Prior to treatment, rats were fasted overnight but allowed free access to water. One rat was dosed with 5000 mg/kg of the 70% methanol extract of *H. cancellata*. The rat was observed during the first 30 minutes and one hour post dosing. After 24 hours, two additional rats were dosed and observed for a period of 14 days for signs of toxicity (convulsion, hyperactivity, dullness, diarrhoea, tiredness, weakness and excessive urination) and mortality.

Experimental Animals

Wistar albino rats weighing 150–200g were obtained from the Animal House, Department of Pharmacology and Toxicology, Ahmadu Bello University, Zaria. The animals were maintained in a ventilated room, fed on standard animal feed and granted access to water. Ethical approval for the experimental protocols was sought from the Ahmadu Bello University Animal Use Ethics Committee with approval number ABUAUC/2023/145.

Induction of diabetes mellitus

Diabetes was induced by administration of a single dose of alloxan monohydrate in the rats at a dose of 170 mg/kg via the intraperitoneal route [8]. The rats were given a 10% glucose solution in place of drinking water for 24 h to prevent mortality due to initial hypoglycemia. The animals were given food and water and then observed over

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a period of 72 h. The determination of glucose concentration was done using test strips that follow the glucose oxidase principles. The animals with blood glucose levels ≥ 11.1mmol/L were considered diabetic and selected for the study.

Grouping

The normal and alloxan-induced diabetic rats were randomly divided into five groups of six rats each. The rats were treated orally daily for 28 days as follows:

Group I: Normal control group (Non diabetic) treated with normal saline (1ml/kg).
Group II: Diabetic control group treated with normal saline (1ml/kg).
Group III: Diabetic rats treated with 250mg/kg extract
Group IV: Diabetic rats treated with 500mg/kg extract
Group V: Diabetic rats treated with 1000mg/kg extract
Group VI: Diabetic rats treated with metformin 500mg/kg

Serum Biochemical Parameters

Serum biochemical parameters were analyzed by collecting blood samples from the jugular veins of anaesthetized rats on the 28th day of treatment. Total cholesterol (TC), triglycerides (TGs), low-density lipoprotein (LDL) and high-density lipoprotein (HDL), as well as liver and kidney function indicators were analyzed.

Histopathological Studies

Anesthetized animals were dissected; the pancreas, liver and kidney were excised and placed immediately into a 10% formalin solution in stoppered containers. The organs were then processed to dehydrate, clear and infiltrate the tissues using paraffin wax. This was then embedded to allow orientation of the specimen in a block form that can be sectioned. Thin sections of the specimens were made using a microtome, placed on a microscope slide and then stained using haematoxylin and eosin. The sections were examined microscopically for histopathological coparison.

RESULTS

Acute Toxicity

Oral administration of the methanol extract of *Hypoestes cancellata* at a limit dose of 5,000mg/kg did not produce any visible sign of toxicity in the rats over a period of 14 days. The LD_{50} in rats was therefore estimated to be above 5,000 mg/kg.

Antidiabetic Activity

Table 1 shows the effect of the methanol extract of *Hypoestes cancellata* on the blood glucose levels of alloxan-induced diabetic rats.

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Table 1: Effects of the methanol extract of *Hypoestes cancellata* on blood glucose of alloxan induced diabetic rats

Treatment	Blood Glucose (mmol/L)				
	Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Normal Control	6.72±1.36*	6.07±1.59*	6.10±2.92*	6.18±1.67*	5.43±1.67*
Diabetic Control	21.93±1.92	22.80±2.26	24.27±4.13	26.17±2.36	27.27±2.32
Metformin (500mg/kg)	23.33±1.92	24.27±2.26	21.37±4.13	18.00±2.36	8.00±2.32*
MCH (250mg/kg)	22.73±1.92	18.87±2.26	18.57±4.13	15.27±2.36	15.00±2.32*
MCH (500mg/kg)	20.70±1.67	14.78±1.95	15.38±3.57	10.15±2.04*	5.90±2.01*
MCH (1000mg/kg)	21.20±1.36	14.83±1.59	12.80±2.92	9.40±1.67*	8.30±1.64*

Values are expressed as mean ± standard error of mean (SEM)
*The mean difference is significant compared to the diabetic control at P≤0.05 using repeated measures ANOVA followed by Bonferroni post *hoc* test
MCH: Methanol Extract of *Hypoestes cancellata*

Serum Biochemical Parameters

Table 2 shows the effect of the methanol extract of *Hypoestes cancellata* on the lipid profile of alloxan-induced diabetic rats.

Table 2: Effects of the methanol extract of *Hypoestes cancellata* on the lipid profile of alloxan-induced diabetic rats

mMol/L	**Normal Control**	**Diabetic Control**	**Metformin (500mg/Kg)**	**MCH (250mg/Kg)**	**MCH (500mg/Kg)**	**MCH (1000mg/Kg)**
TC	3.97±0.79	4.4±0.29	3.85±0.25	3.95±0.25	4.07±0.38	4.50±0.32
HDL	1.24±0.11	1.35±0.04	1.32±0.09	1.29±0.02	1.33±0.03	1.32±0.04
LDL	1.68±0.47	1.82±0.36	1.15±0.09	1.40±0.23	1.58±0.39	1.89±0.39
TG	1.29±0.06	1.39±0.17	1.39±0.08	1.26±0.04	1.16±0.05	1.24±0.08

Values are expressed as mean ± Standard Error of mean (SEM)
There was no statistically significant difference compared to diabetic control using one-way ANOVA followed by Bonferroni post *hoc* test

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Keys: MCH: Methanol Extract of *Hypoestes cancellate*, TC: total cholesterol, HDL: high density lipoprotein, LDL: low density lipoprotein, TG: triglyceride

Table 3: Effects of the methanol extract of *Hypoestes cancellata* on hepatic and renal function of alloxan-induced diabetic rats

	Normal Control	Diabetic Control	Metformin (500mg/kg)	MCH (250mg/kg)	MCH (500mg/kg)	MCH (1000mg/kg)
AST(U/L)	5.67±0.33	7.67±0.88	6.00±3.00	4.00±1.00	7.00±0.58	6.67±1.76
ALT(U/L)	7.33±1.20	5.33±2.40	11.00±5.00	6.50±2.50	6.00±1.53	7.67±1.45
ALP(U/L)	33.33±3.84	42.33±6.44	30.50±2.50	36.50±4.50	34.00±6.66	38.67±6.89
T.BIL (mmol/L)	7.67±1.45	10.33±2.95	10.50±2.50	7.00±1.00	8.00±1.53	5.67±1.67
C.BIL (mmol/L)	2.93±0.52	3.07±0.47	3.30±0.30	2.05±0.25	2.40±0.40	1.99±0.71
UREA (mmol/L)	2.57±0.23	3.13±0.45	3.10±0.10	2.70±0.20	3.23±0.45	2.73±0.35
CREATININE (µmol/L)	690±52.31	690±15.87	720±90.00	648±72.00	666±68.15	600±21.63

Values are expressed as mean ± standard error of mean (SEM)
There was no statistically significant difference compared to diabetic control one-way ANOVA followed by Bonferroni post *hoc* test
MCH: methanol extract of *Hypoestes cancellate*, AST: aspartate transaminase, ALT: alanine transaminase, ALP: alkaline phosphatase, T.BIL: total bilirubin, C.BIL: conjugated bilirubin

Histopathology of organs

The histological examination of the sectioned pancreas (Plate I) revealed normal islet (NI) in the normal control group (A), intense islet necrosis (IN) in the diabetic control group (B), moderate islet necrosis in 250mg/kg (C) and slight islet necrosis in 500mg/kg (D), 1000m/kg (E) extract treatment groups and metformin group (F).

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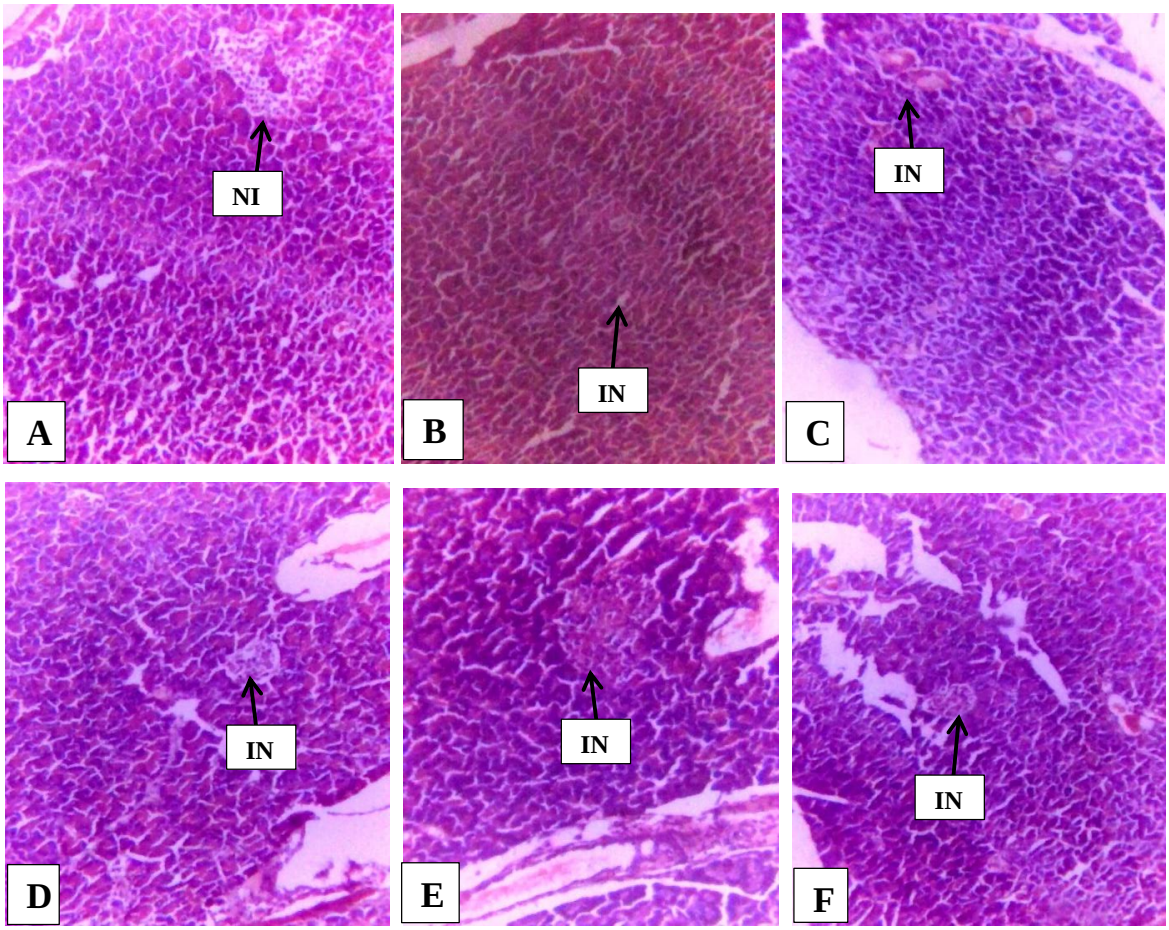

Plate I: Photomicrograph of the pancreas of treted rats (×400)

The histological examination of the sectioned liver (Plate II) revealed normal hepatocytes (NH) in the normal control group (A) and 1000m/kg extract group (E), slight hepatic necrosis (HN) in the diabetic control (B), 250mg/kg (C) and 500mg/kg (D) extract treatment groups and slight vascular congestion (VCN) in the metformin group (F)

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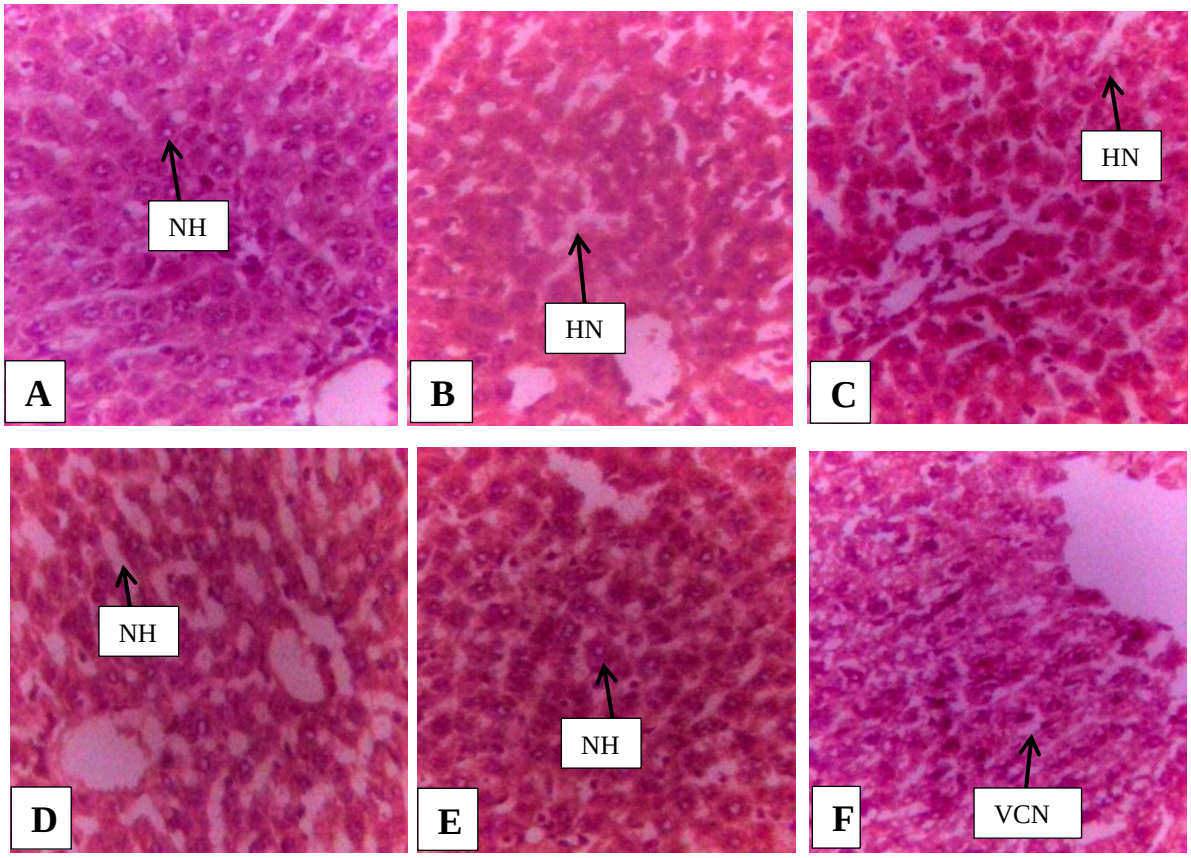

Plates II: Photomicrograph of the liver of treated rats (×400)

The histological examination of the sectioned kidney (Plate III) revealed normal tubules (T) and glomerulus (G) in the normal control group (A), intense tubular necrosis (TN) in the diabetic control group (B), moderate tubular necrosis and tubular atrophy (TA) in 250mg/kg extract group (C) slight tubular necrosis and glomerular necrosis (GN) in 500mg/kg extract group (D), slight tubular necrosis in 1000mg/kg extract group (E) and metformin group (F).

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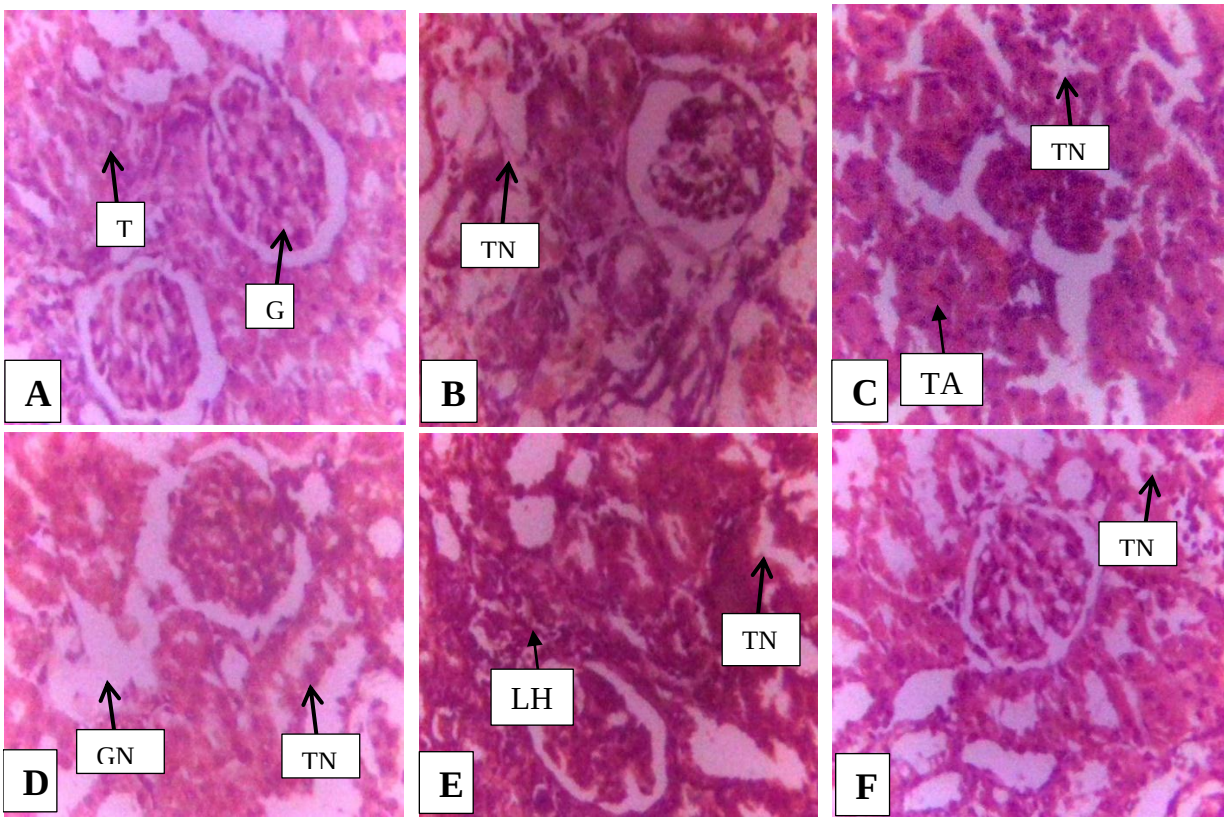

Plate III: Photomicrograph of the kidney of treated rats (×400)

DISCUSSION

Administration of the methanol extract of *Hypoestes cancellata* at a limit dose of 5000mg/kg showed no sign of toxicity in rats indicating that the plant is relatively safe. The methanol extract of *H. cancellata* significantly reduced the blood glucose levels in alloxan-induced diabetic rats at all doses of the extract when compared to the diabetic control group. The 1000mg/kg dose showed a steadier blood glucose reduction pattern than the other doses and metformin in this study. On day 28 of treatment, 250, 500 and 1000mg/kg body weight showed reduction in blood glucose levels of 34, 71 and 61% respectively in relation to the initial blood glucose levels (Day 0) of the groups. Metformin 500mg/kg body weight showed a 66% reduction in blood glucose level at day 28 relative to day 0.

Induction of diabetes by administration of alloxan increased the total cholesterol (TC), high density lipoproteins (HDL), low density lipoproteins (LDL) and triglycerides (TG) levels. However, treatment with the methanol extract of *H. cancellata* produced a decrease in TC, HDL, LDL and TG at various doses of the extract administered. TC, HDL, LDL and TG were all increased in diabetic rats compared to normal rats. In diabetic condition there is an increased release of fatty acids which leads to the production of ketone bodies and triglycerides thereby leading to hyperglyceridemia [9]. Likewise, lipoproteins are a vital component in the development of atherosclerosis, and their

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progression increases the risk of myocardial infarction [10].

Histopathological examinations of the organs of the treated rats suggest that the methanol extract of *H. cancellata* has the potential to reduce the intensity and/or reverse the damage caused by alloxan on the pancreas, liver and kidney. Alloxan is known to destroy beta cells of islets in the langaherns of pancreas [11] and also causes nephrotoxic alterations [12] as well as morphological and ultrastructural changes in the liver [13] and these can be seen in the diabetic untreated group.

CONCLUSION

The findings reported in this study suggest that 70% methanol extract of *H. cancellata* may contain novel bioactive principles that possess anti-diabetic activity.

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