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# Antidiabetic and antioxidant effects of *Croton lobatus* L. in alloxan-induced diabetic rats

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## ABSTRACT

**Background:** *Croton lobatus* contains a high amount of antioxidant phytochemicals that probably account for its wide use as food and medicine in the traditional communities of West Africa. **Methods:** The study evaluated the modulatory role of methanol extract of Croton lobatus leaf on alloxan-induced diabetes and associated cardiovascular complications. Male rats were randomly selected and assigned to one of six groups (A to F) of eight animals each: A (distilled water); B (corn oil); C (Alloxan); D (Alloxan + 100 mg kg-1 Croton lobatus); E: (Alloxan + 200 mg kg-1 C. lobatus); and F (Alloxan + 100 mg kg-1 glibenclamide). **Results:** Acute toxicity studies revealed no mortality of rats at the administration of different doses of extract up to the 5,000 mg kg-1 dose. Histology of the pancreas showed focal area of necrosis, and fatty infiltration in diabetic untreated rats, but these lesions were absent in pancreas of rats treated with *C. lobatus* extract. **Conclusion:** Methanol leaf extract of *C. lobatus* reduced arteriogenic risk factors, improved antioxidant status, restored the observable pathological lesions associated with experimental diabetes in rats, and thus offers a new therapeutic window as herbal therapy for the treatment of diabetes mellitus and associated cardiovascular complications.

KEY WORDS: Blood pressure, diabetes, electrocardiogram, oxidative stress

## INTRODUCTION

Diabetes mellitus ranked among the leading causes of death in developed countries and is one the most prevalent metabolic disorder in the world [1]. Although several etiologies have been implicated, defects in insulin secretion, insulin action, or both are often the primary characteristic of the disease [2]. Long-term complications of diabetes include coronary heart diseases, retinopathy, nephropathy, and foot ulceration [3]. Alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil), one of the commonly used drugs for the induction of experimental diabetes in rats, is a diabetogenic agent that selectively destroys pancreatic β-cells [4].

Oxidative stress, which reflects an imbalance between the systemic manifestation of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates [5], has been implicated in the pathogenesis of diabetes via the apoptosis of pancreatic β-cells, and insulin resistance respectively [6]. Hyperglycemia generates ROS which in turn cause damage to the cells in many ways [7]. The resulting oxidative stress mediated damage to the cells ultimately results in secondary complications associated with diabetes [8].

Many of the currently available therapeutic options for the management of diabetes mellitus have serious adverse effects including hypoglycemia, gastrointestinal disturbances,

liver toxicity, and heart failure [9]. Consequently, natural compounds, in recent time, have been exploited as feasible alternatives for the treatment of diabetes, and its complications, because they are generally considered to be less expensive and safe [10]. *Croton lobatus* (family Euphorbiaceae) is an erect, annual, herbaceous plant, often harvested from the wild for local use as food and medicine [11]. Phenolic substances including lignoids, proanthocyanidins, and flavonoids with highly potent antioxidant properties have been reported as the predominating phytochemicals of *C. lobatus* leaves [12]. Since cellular oxidative stress has been reported to play cardinal roles in the development of hyperglycemia-related tissue damage [13], this study was designed to evaluate the ameliorative effects of *C. lobatus* on alloxan-induced diabetes and associated cardiovascular derangements by determining the effects of the leaf extract on blood glucose level, blood pressure changes, electrocardiographic (ECG) abnormalities, lipid profile, and anti-oxidant status of alloxan-induced diabetic rats.

## MATERIALS AND METHODS

### Collection and Identification of Plant Material

Fresh mature leaves of *C. lobatus* were collected from Orogun area, Ibadan, and were identified and authenticated by a taxonomist at the herbarium in the Department of Botany, University of Ibadan, Ibadan. The voucher number UIH–22482 was assigned.

### Chemicals and Drugs

Alloxan was obtained from Sigma Chemical Co. (St. Louis, M.O., USA). Daonil® (glibenclamide) manufactured by Aventis Pharma Ltd. was purchased from Diadem pharmacy in Ojoo, Ibadan-Nigeria. Randox kits (Randox Laboratories Limited) for the determination of triglyceride (TG), cholesterol, and high-density lipoprotein (HDL) were purchased from Long Global Health Ltd., Lagos, Nigeria.

### Preparation of Methanol Leaf Extract of *C. lobatus*

The extraction of *C. lobatus* was carried out according to the method described by Iweala and Okeke [14]. The fresh leaves of the plant were air dried for 30 days and milled to powder using an electrical blender. 1 kg of the powdered leaves was macerated in 6 L of distilled methanol for 72 h with occasional stirring. The liquid extract obtained was filtered through a cotton wool and finally with Whatmann no.1 filter paper. The filtrate was passed through a pressure pump to ensure that no particles or residues were left in the filtrate. The filtrate was concentrated in a rotary evaporator (Stuart model RE300D) at a temperature of 45°C. The concentrated extracts were subsequently evaporated to dryness on a water bath at 50°C to obtain a sticky, dark green mass weighing 37.18 g which represents 3.72% yield, and kept refrigerated at 4°C until use.

### Experimental Animals

A total of 48 healthy male albino rats of the Wistar strain weighing between 100 and 160 g, obtained from the central animal house of the University of Ibadan, Ibadan, Nigeria, were used for the study. The animals were kept in groups of 8 per white plastic cage within the animal house to acclimatize for 2 weeks before the experiments with conditions of the animal housing facility, ambient temperature, standard environmental conditions of 12 h light and 12 h dark and adequate ventilation for 2 weeks. The rats were fed with standard rat diet (Ladokun Feeds, Ltd.) and clean water was provided *ad libitum*. All experimental protocols were conducted in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals.

### Acute Toxicity Test

Acute oral toxicity was carried out using the procedure described by the Organisation for Economic Cooperation and Development [15]. The test was performed using healthy albino rats of Wistar strain weighing between 83 and 118 g. The animals were divided into six groups of three rats each and administered 0, 50, 100, 200, 3000, 5000 mg/kg orally. Animals were observed after dosing 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 for a total of 14 consecutive days.

### Experimental Design

The animals were randomly selected and assigned into one of the six groups (A to F) of eight animals each: A (distilled water), B (corn oil), C (Alloxan), D (Alloxan + 100 mg/kg *C. lobatus*), E: (Alloxan + 200 mg/kg *C. lobatus*), and F (Alloxan + 100 mg/kg glibenclamide). Diabetes was induced in Groups C, D, E, and F by a single intraperitoneal injection of aqueous alloxan monohydrate at a dose of 100 mg/kg, after the rats were subjected to overnight fast, and fasting blood glucose was determined. 96 h after alloxan administration, blood glucose levels of the rats were determined with the aid of an Accu Chek® active digital glucometer using test strips. Animals with blood glucose level greater than or equal to 200 mg/dl were considered diabetic and selected for the study.

### Blood Pressure Measurement

Indirect blood pressure parameters (systolic, diastolic, and mean blood pressure) were determined in unanesthetized rats by tail plethysmography using an electrosphygnomanometer (CODA, Kent Scientific, USA). The average of at least nine readings, taken in the quiescent state, following acclimatization, was taken per animal.

### ECG

Standard lead II ECG was recorded in conscious rats using a 7-lead ECG machine (EDAN VE-1010, Shanghai, China) 24 h after the last administration of the extract. The machine was calibrated at 20 mm/mV paper speed and 50 mm/s paper speed. From the recorded ECGs, parameters such as heart rate (HR), P-wave duration, PR-interval, QRS duration, R-amplitude, QT segment, and Bazett's correction of the QT interval were determined.

### Blood Sample Collection and Preparation

At the end of the treatment period, blood was collected by retro–orbital venous puncture using micro-hematocrit capillary tubes into lithium heparinized bottles and taken to the laboratory for plasma preparation. Plasma was separated by centrifugation (G+M; Great Medical England centrifuge model 80-2) at 4000 revolutions per minutes for 15 min. The plasma obtained was collected by pipetting into a plain bottle using Pasteur pipette and refrigerated at 4°C until required for biochemical assays.

### Sacrifice of Animals and Relative Organ Weight Determination

At the end of the experimental period, 5 rats from each group were sacrificed by cervical dislocation. The abdomen was dissected using dissecting tools; the kidney, pancreas, liver, and heart were removed and weighed to determine their weight in relation to the total body weight of the animals [16].

### Histopathology of the Pancreas

The pancreas from each rat was removed immediately and preserved in a sample bottle containing 10% formalin solution. The pancreas was processed by the paraffin technique. Sections of 5 $\mu$m thickness were cut and stained by hematoxylin and eosin for histological examination.

### Assay for Plasma Lipid Profile

Cholesterol, TGs, and HDL tests were carried out using Randox kits. Very low-density lipoprotein cholesterol (VLDL-C) and LDL-C were estimated as described by Friedwald *et al*. [17].

### Determination of Markers of Oxidative Stress and Antioxidant in Plasma

Superoxide dismutase (SOD) was determined by measuring the inhibition of auto-oxidation of epinephrine at pH 10.2 at 30°C as described by Misra and Fridovich [18] with modification from Oyagbemi *et al*. [19]. Glutathione (GSH) Level was determined using Ellman's reagent. Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances according to the method of Varshney and Kale [20]. The MDA level was calculated according to the method of Adams and Sergi [21]. Hydrogen peroxide was generated by the method described by Wolff [22]. Nitric oxide (NO) was determined using standard protocol [23].

### Statistical Analysis

Analysis of variance was performed using the Minitab 16.0 statistical software program. Duncan Multiple Range Test and Fischer's pairwise comparison test were used for separation of statistically significant means. All the data were expressed as a mean ± standard deviation. $P < 0.05$ was considered significant.

## RESULTS

The median lethal dose for the *C. lobatus* extract was estimated to be greater than 5000 mg/kg, since visible adverse effects and mortality were not recorded at this dose. The body weight of the diabetic untreated rats decreased significantly ($P < 0.05$) compared with the control and treated groups of rats [Table 1], significant differences were not recorded in the relative organ weight of rats [Table 2]. The blood glucose level of diabetic untreated rats significantly increased ($P < 0.05$) when compared with control rats, but administration of 100 mg/kg and 200 mg/kg *C. lobatus* extract, significantly decreased ($P < 0.05$) the blood glucose level from hyperglycemic to normoglycemic levels, similar to those of the glibenclamide-treated rats [Table 3].

There was no significant difference ($P > 0.05$) in the diastolic and mean arterial blood pressures of the diabetic untreated groups of rats when compared with the control group, but the systolic blood pressure significantly increased ($P < 0.05$) compared with the control [Table 4]. The extract at 100 mg/kg caused a significant decrease ($P < 0.05$) in the systolic, mean arterial blood pressure, and pulse pressure of rats when compared with those of the diabetic untreated group of rats. There was no significant difference in the HR, P-wave duration (P-Dur), PR interval (PR-Int), QRS, QTc and Ramp of diabetic untreated rats (Group C) when compared with the control, but there was a significant difference in QT duration of diabetic

Table 1. Percentage body weight gain of alloxan-induced diabetic rats

| Groups weeks | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| Week 0 | 123.70±7.09 | 124.30±3.21 | 108.50±2.12 | 107.50±3.54 | 100.00±11.31 | 113.00±4.24 |
| Week 2 | 151.00±10.58 | 144.00±10.58 | 95.00±4.04 | 104.00±3.21 | 118.50±4.95 | 136.50±6.36 |
| %age weight gain (%) | 18.1 | 13.88 | −14.2 | −3.4 | 15.61 | 17.2 |

Table 2. Relative organ weight of alloxan-induced diabetic rats

| Groups organ | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| Kidney | 0.65±0.19 | 0.72±0.09 | 1.05±0.11 | 0.94±0.06 | 0.85±0.08 | 0.79±0.08 |
| Liver | 4.17±0.52 | 4.00±0.99 | 5.00±0.39 | 3.34±2.61 | 3.94±0.55 | 5.51±2.32 |
| Pancreas | 0.23±0.12 | 0.29±0.09 | 0.32±0.03 | 0.27±0.10 | 0.19±0.06 | 0.23±0.09 |
| Heart | 0.22±0.06 | 0.37±0.07 | 0.38±0.01 | 0.37±0.09 | 0.41±0.06 | 0.38±0.16 |

Superscript (a) indicate significant increase compared with groups A or B at $P<0.05$

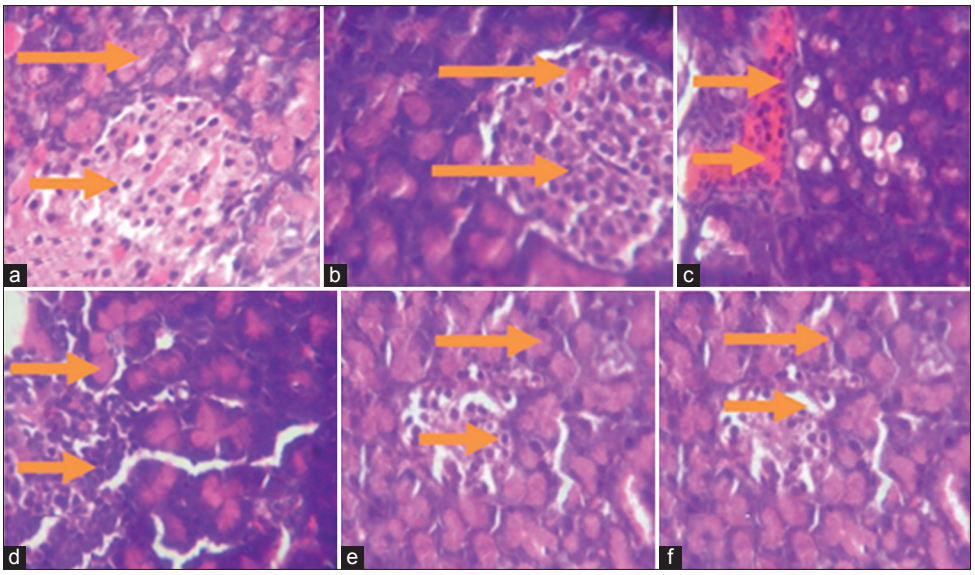


**Figure 1:** Effect of *Croton lobatus* leaf extract on pancreas in alloxan–induced diabetic rats. Histology of the pancreas following induction of diabetes mellitus and treatment with *C. lobatus* leaf extract in rats. (a and b) normal controls with normal Islet; (c) Untreated diabetic group with congestion of vessels, and focal area of necrosis, and fatty infiltration; (d) Diabetic treated with 100 mg/kg *C. lobatus* with moderate congestion of vessels, islets appear normal but few and small in size; (e) Diabetic treated with 200 mg/kg with mild congestion of vessels and normal islets, and normal exocrine acini containing zymogen granules; (f) Diabetic treated with gilbenclamide shows congestion of vessels, normal islets, and normal exocrine acini containing zymogen granules. Histologic slides were stained with Hematoxylin and Eosin (×400)

Table 3. Effect of *Croton lobatus* leaf extract on blood glucose of alloxan-induced diabetic rats

| Groups days | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| Day 1 | 45.60±8.85 | 44.60±5.81 | 231.50±11.39[a] | 156.20±7.50[b] | 165.00±4.90[b] | 170.00±4.26[b] |
| Day 2 | 65.80±10.06 | 62.00±7.07 | 242.00±1.81[a] | 140.50±3.67[b] | 171.60±2.75[b] | 126.80±1.63[b] |
| Day 3 | 69.40±7.50 | 81.69±11.30 | 255.00±2.05[a] | 134.00±1.89[b] | 70.50±3.10 [b] | 121.50±9.04[b] |
| Day 4 | 72.20±11.40 | 70.80±11.80 | 253.00±2.00[a] | 89.50±5.06 [b] | 129.80±4.78[b] | 79.80±10.90[b] |
| Day 5 | 63.80±4.87 | 61.40±4.39 | 255.05±2.00[a] | 145.00±10.48[b] | 147.80±4.69[b] | 62.00±12.52[b] |
| Day 6 | 58.00±5.61 | 57.60±8.02 | 250.00±2.05[a] | 177.50±5.37[b] | 131.00±1.25[b] | 60.80±1.73[b] |
| Day 7 | 68.00±11.20 | 62.20±15.40 | 256.00±2.23[a] | 169.80±4.56[b] | 142.00±4.32[b] | 11.30±4.38 [b] |
| Day 9 | 56.80±7.95 | 66.40±9.32 | 270.50±17.54[a] | 173.30±10.79[b] | 130.30±2.90[b] | 84.3±5.50 [b] |
| Day 11 | 102.60±14.86 | 94.00±15.43 | 279.30±4.81[a] | 179.80±4.58 [b] | 124.00±7.54[b] | 95.30±4.37[b] |
| Day 12 | 70.40±17.23 | 60.80±16.65 | 284.00±8.37[a] | 210.80±5.49 [b] | 115.00±5.47[b] | 93.80±4.90 [b] |
| Day 14 | 63.00±14.14 | 59.40±18.63 | 297.80±7.62[a,d] | 204.30±4.22[b,d] | 119.80±2.25[b,c] | 112.80±6.54[b,c] |

Superscript (a) indicate significant increase compared with groups A or B at $P<0.05$, Superscript (b) indicate significant decrease compared with group C at $P<0.05$, Superscript (c) indicate significant decrease compared with day 1 of treatment at $P<0.05$, Superscript (d) indicate significant increase compared with day 1 of treatment at $P<0.05$

Table 4. Effect of *Croton lobatus* leaf methanol extract on blood pressure of alloxan-induced diabetic rats

| Groups parameters | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| Systolic (mmHg) | 128.50±22.61 | 131.30±3.01 | 146.80±18.10[a] | 100.00±13.80[b] | 129.00±13.67[b] | 122.70±13.80[b] |
| Diastolic (mmHg) | 109.70±23.89 | 105.00±4.43 | 109.60±26.93 | 88.80±8.41[b] | 116.80±11.86[a] | 111.70±14.43[ab] |
| MAP (mmHg) | 115.50±22.40 | 113.50±3.73 | 121.60±24.03[a] | 92.00±5.10[b] | 120.50±10.41 | 86.70±19.30[b] |
| Pulse Pressure (mmHg) | 7.30±0.58 | 26.30±3.83 | 37.20±9.31[a] | 2.50±1.00[b] | 10.00±1.00[b] | 11.00±2.65[b] |

Superscript (a) indicate significant increase compared with groups A or B at $P<0.05$, Superscript (b) indicate significant decrease compared with group C at $P<0.05$

untreated rats when compared with when compared with the control [Table 5].

There was a significant increase ($P < 0.05$) in the levels of total cholesterol (TC), TG and VLDL-C, but low HDL-C in diabetic untreated rats when compared with the control rats. Rats treated with *C. lobatus* extract showed a significant decrease ($P < 0.05$) in TC, TG, and VLDL-C when compared with the diabetic untreated counterparts, but the levels of these parameters in the extract treated groups were similar to those of the control and glibenclamide treatment group [Table 6].

The extract of *C. lobatus* significantly increased ($P < 0.05$) the level of SOD in a dose-dependent manner, compared with the diabetic untreated group of rats [Table 7]. There was a significant decrease ($P < 0.05$) in serum NO level of diabetic

Table 5. Effect of *Croton lobatus* leaf extract on electrocardiographic parameters of alloxan-induced diabetic rats

| Groups parameters | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| H/R (/min) | 269.0±60.3 | 256.0±19.92 | 220.3±12.01[a] | 205.0±20.88[a] | 219.3±14.47[a] | 246.7±40.67 |
| P-Dur (ms) | 20.7±1.53[b] | 25.0±3.61 | 24.7±3.21 | 26.7±3.51 | 25.0±3.61 | 24.7±3.21 |
| PR-Int (ms) | 46.0±9.54 | 44.0±4.00 | 50.7±4.16[b] | 53.0±4.36[b] | 50.0±5.29[b] | 52.0±1.73[b] |
| QRS (ms) | 17.0±3.46 | 14.3±1.15 | 12.7±0.58 | 15.3±1.53 | 16.3±7.77 | 16.0±3.61 |
| QT-Dur (ms) | 58.7±11.7 | 62.0±4.58 | 82.0±5.57[b] | 79.0±12.8[c] | 103.0±24.5[c] | 88.3±7.57 |
| QTc (ms) | 125.0±37.5 | 128.0±6.43 | 157.0±15.1[b] | 146.0±26.6[d] | 196.0±42.1[c] | 177.0±8.02[c] |
| Ramp (mV) | 0.4±0.24 | 0.4±0.08 | 0.6±0.15 | 0.4±0.09 | 0.5±0.15 | 0.4±0.12 |

Superscript (a) indicate significant decrease compared with groups A or B at $P<0.05$, Superscript (b) indicate significant increase compared with groups A or B at $P<0.05$, Superscript (c) indicate significant increase compared with group C at $P<0.05$, Superscript (d) indicate significant decrease compared with group C at $P<0.05$

Table 6. Effect of *Croton lobatus* leaf extract on lipid profile in alloxan-induced diabetic rats

| Groups parameters | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| TC (mg/dL) | 28.00±2.45 | 21.90±11.70 | 52.30±13.50[a] | 23.10±7.28[b] | 11.00±3.60[b] | 27.40±9.48[b] |
| TG (mg/dL) | 39.60±23.60 | 48.40±24.00[c] | 128.00±34.70[a] | 55.60±29.70[b] | 80.50±19.60[b] | 74.10±25.80[b] |
| HDL (mg/dL) | 32.60±9.19 | 42.00±11.30 | 16.20±13.10[c] | 31.60±8.53[d] | 34.90±10.10[d] | 26.20±1.07[d] |
| VLDL (mg/dL) | 7.90±4.72 | 9.70±4.79 | 25.50±6.95[a] | 11.10±5.93[b] | 16.10±3.91[b] | 14.80±5.15[b] |
| LDL (mg/dL) | 12.50±10.51 | 29.70±19.98 | 14.10±16.68 | 19.60±10.09 | 39.90±10.73[d] | 13.60±12.71[b] |

Superscript (a) indicate significant increase compared with groups A or B at $P<0.05$, Superscript (b) indicate significant decrease compared with group C at $P<0.05$, Superscript (c) indicate significant decrease compared with groups A or B at $P<0.05$, Superscript (d) indicate significant increase compared with group C at $P<0.05$

Table 7. Effect of *Croton lobatus* leaf extract on oxidative stress markers in alloxan-induced diabetic rats

| Groups parameters | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| SOD units/mg protein | 107.00±4.24 | 82.70±15.50 | 96.90±9.47[a] | 89.70±0.99 | 122.00±2.53[b] | 112.00±5.18[b] |
| Nitric Oxide µmole/L | 1.04±0.04 | 0.70±0.26 | 0.40±0.02[c] | 0.40±0.17[c] | 0.80±0.23[d] | 0.80±0.03[d] |
| GSH µmole/mg protein | 64.00±0.35 | 63.50±0.35 | 13.10±2.30[a] | 63.90±2.30[b] | 64.10±0.18[b] | 66.10±0.88[c] |
| $H_2O_2$ µmole/mg protein | 12.00±0.71 | 10.50±0.35 | 12.50±0.35 | 11.90±0.18 | 11.90±0.53 | 10.80±0.35 |
| MDA µmole/mg protein[e] | 0.50±0.01 | 1.40±0.27 | 13.90±2.97[a] | 0.60±0.30[b] | 0.50±0.07[b] | 0.60±0.15[b] |

Superscript (a) indicate significant increase compared with groups A at $P<0.05$, Superscript ([b]) indicate significant decrease compared with group C at $P<0.05$, Superscript (c) indicate significant decrease compared with groups A or B at $P<0.05$, Superscript (d) indicate significant increase compared with group C at $P<0.05$

untreated animals when compared with the control, but *C. lobatus* extract at 200 mg/kg significantly increased the level of this parameter in rats. Similarly, the reduced GSH level in the diabetic untreated rats significantly decreased when compared the control group, but rats treated with the 100 and 200 mg/kg dose of *C. lobatus* had a significantly elevated level of GSH compared with the diabetic untreated group [Table 7]. There was no significant difference ($P > 0.05$) in $H_2O_2$ of the diabetic group of rats when compared with the control, and with the rats treated with *C. lobatus* extract and glibenclamide. However, the diabetic untreated rats showed a significant increase ($P < 0.05$) in the level of malondialdehyde (MDA) compared with control rats. Administration of the extract of *C. lobatus* at 100 and 200 mg/kg significantly decreased ($P < 0.05$) the MDA level compared with the diabetic untreated rats.

Histology of the pancreas showed focal area of necrosis, and fatty infiltration in diabetic untreated rats, but these lesions were absent in pancreas of rats treated with C. lobatus extract (Figure 1).

## DISCUSSION

In general, medicinal plants are believed to be safe, but a number of these therapeutic agents have been reported to cause specific organ damage in man and animals [24]. For instance, hepatotoxicity, nephrotoxicity, thrombocytopenia, and genotoxicity have been associated with the use of a number of medicinal plants [25]. The non-observance of mortality in rats even at the extremely high dose of 5000 mg/kg suggests a wide safety margin for the extract of *C. lobatus* used in this study.

In this study, the induction of diabetes mellitus in rats by alloxan was manifested by the significant reduction ($P < 0.05$) in the blood glucose level of rats administered 100 mg/kg alloxan monohydrate alone compared with those of the control group of rats [Table 3]. Further, the hyperglycemic state observed in rats became increasingly severe with increasing number of days as depicted by the significant elevation in the blood glucose level on the 14$^{th}$ day following induction of diabetes compared with the first day of therapy [Table 3]. However, the extract of *C. lobatus* showed a significant inhibition of alloxan-induced diabetes mellitus and exerts a potent anti-hyperglycemic effect as demonstrated by the significant decrease ($P < 0.05$) in blood glucose with the 100 and 200 mg/kg *C. lobatus* leaf extract treated groups, similar to those treated with the sulfonylurea glibenclamide, albeit, the 200 mg/kg dose showed higher effect over the 2-week treatment period. Observations in this study are similar to the reports of Okokon *et al.* [26] who reported

significant hypoglycemic activity for *Croton zambesicus* in alloxan-induced diabetes mellitus.

The antidiabetic activities of medicinal plants have been ascribed to their phytochemical constituents [27] that may delay the development of diabetic complications and regulate the associated metabolic abnormalities through a variety of mechanisms [28]. For instance, different polysaccharides of *Ganoderma lucidum* have been reported to increase plasma insulin levels in both normal and glucose-loaded mice, with accompanying decreased hepatic glycogen content due to a modulation of glucose metabolizing enzymes in the liver [29]. Moreover, the naturally occurring phenolic compounds, flavonoids, and alkaloids are reported to inhibit the activities of glucosidase, a key enzyme in the metabolism of glucose [30]. It is probable that the anti-diabetic effects of *C. lobatus* observed in this study is due to a stimulation of insulin secretion or improvement in digestion along with a reduction in blood sugar level, and inhibition of the breakdown of starch to glucose by inhibiting alpha-glucosidase activity [31]. Diabetes mellitus has been known to be associated with weight loss [32]. The weight loss in diabetic untreated rats, as observed in this study, may be due to dehydration and ineffective metabolism of carbohydrate, protein and fat [33]. However, the increase in body weight of diabetic rats treated with plant extract suggests a potent inhibition of diabetes by *C. lobatus* leaf extract.

Observation of a significantly elevated blood pressure in the untreated diabetic group of rats relative to the control and *C. lobatus* treated group [Table 4] probably is a manifestation of one of the complications of a persistent hyperglycemic state. Hyperglycemia causes changes in vascular structure and function by decreasing NO bioavailability and increasing the production of hydrogen peroxide in vascular endothelium [34]. From the result of this study, there was a significant decrease ($P < 0.05$) in NO levels in diabetic untreated rats, whereas the administration of *C. lobatus* extracts to diabetic rats increased the level of NO and in a dose-dependent manner [Table 7]. Since NO is a potent vasodilator, decreased bioavailability or biosynthesis of NO may potentiate the development of the hypertensive state [35].

Diabetes mellitus has been associated with cardiovascular complications of tachycardia, shortening of QT intervals and QRS as well as shortened activation time of ventricular myocardium [36]. Lee *et al.* [37] reported a prolongation of QT interval with hypoglycemia, and this was associated with increased risk of ventricular arrhythmias. Increased free radical (superoxide anion) activity can potentially elevate blood pressure by inactivating NO and consequently increase systemic vascular resistance [38]. Conversely, enhanced activity of NO has been found to play a key role in the reduction of vascular resistance and blood pressure that are elevated in hypertensive subjects [39]. Observations of this study on blood pressure changes of rats are similar to the findings of Paez *et al.* [40] who reported a prevention of the development of hypertensive state following the administration 200 mg/kgethanol leaf extract of *Croton schiedeanus* in NO deficit-induced hypertension, and attributed the effect to the flavonoid, diterpenoid, and fenibutanoid metabolites of *Croton* species that exert a vasodilatory effect by modulating the NO/cyclic guanosine monophosphate pathway. The blood pressure lowering effects of *C. lobatus* extracts, as observed in this study, may be due to direct or indirect action of constituent polyphenols that have been reported to decrease blood pressure by increasing endothelial NO bioavailability via their antioxidant action, and their capacity to activate vascular endothelial NO synthase [41].

The significantly elevated ($P < 0.05$) levels of TC, TG, VLDL-C, and low high HDL-cholesterol (HDL-C) observed in the diabetic untreated rats compared with those treated with *C. lobatus* suggest positive modulatory role of extract in lipid metabolism probably due to a rapid mobilization of TG and consequent increased plasma free fatty acids levels. Hypercholesterolemia is a common complication of diabetes mellitus and elevated VLDL-C TG reduces levels of cardioprotective HDL-C, with attending consequence of a reduction in antioxidant activities [42]. The results of this study corroborate earlier reports that most hypoglycemic plants have potentials of ameliorating diabetes-associated abnormalities of lipid metabolism *in vivo* [43]. The high level of HDL-C in diabetic rats treated with extract is advantageous because HDL-C transports fat molecules (such as cholesterol and TGs) out of arterial vasculature, and thus prevents cardiovascular diseases [44].

Furthermore, in this study, a significant decrease ($P < 0.05$) was observed in the activity of SOD of the diabetic untreated group of rats, compared with those of the control. The decreased SOD activity may be due to high level of free radicals with decreased antioxidant defense mechanisms [45]. The significantly increased SOD level in diabetic rats following the administration of *C. lobatus* extract suggests a positive modulatory role for the extract in the amelioration of the induced oxidative stress. Reduced GSH normally plays the role of a direct intracellular free-radical scavenger through interactions with free radicals [46]. The observed reduction in GSH level in diabetic untreated rats may be a result of the increased utilization of GSH due to oxidative stress [47]. MDA has been documented as a primary biomarker of free radical mediated lipid damage and oxidative stress [48]. The MDA level in diabetic rats treated with *C. lobatus* extract significantly decreased when compared with diabetic untreated rats [Table 7]. Medicinal plants with high flavonoid content have been reported to decrease MDA level in plasma, and thus provide protection against many chronic diseases by virtue of their free radical scavenging properties [49].

Pancreatic Islets are especially vulnerable to oxidative stress-mediated injuries because the antioxidant defense system of the pancreas is considerably weaker than those of other tissues [50]. In this study, histopathological evaluation revealed focal area of necrosis of islet cells in untreated diabetic rats. However, pancreatic islet cells of diabetic rats treated with *C. lobatus* leaf methanol extracts appear normal. This suggests an inhibition of the toxic mechanisms involved in the alloxan-induced, oxidative stress-mediated destruction of pancreatic Islet cells by the antioxidant rich, flavonoid-containing *C. lobatus*

extract inducing increased antioxidant enzyme activity, and consequently preserving Islet cells integrity [51].

## CONCLUSION

This study shows that *C. lobatus* possesses blood glucose lowering effects, antihypertensive effect, as well as antioxidant and free radical scavenging properties. Therefore, further investigation on the different phytochemical constituents may be beneficial for the treatment and management of diabetes mellitus and its complications, and provide a safer and cheaper alternative to the currently available anti-diabetic drugs.

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