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Research Article

Antidiabetic Effects of the Ethanolic Root Extract of *Uvaria chamae* P. Beauv (Annonaceae) in Alloxan-Induced Diabetic Rats: A Potential Alternative Treatment for Diabetes Mellitus

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Diabetes mellitus has been a menace to mankind from time immemorial. However, a natural product such as *U. chamae* P. Beauv (Annonaceae) offers alternative treatment for diabetes mellitus. The study aimed at evaluating antidiabetic activity of the ethanolic root extract of *U. chamae* in alloxan-induced diabetic rats. Diabetes was induced in Sprague Dawley rats after overnight fast with 150 mg/kg alloxan intraperitoneally. After 72 h, those with plasma glucose levels >200 mg/dl were classified as diabetic. Five diabetic rats in each group were treated daily for 14 days orally with 100, 250, and 400 mg/kg of the extract, glibenclamide (71 μg/kg) and pioglitazone (429 μg/kg), respectively, while another group was untreated. Control received 0.5 ml of *Acacia senegal*. Effects of extract on glucose, other biochemical, and hematological parameters were evaluated. α-amylase and α-glucosidase inhibitory activities of extract and its fractions were also evaluated. Percentage inhibition and IC_{50} values were determined. Diabetic control was achieved on the 7th day of the study with 100, 250, and 400 mg/kg of the extract showing glucose reduction of 72.14%, 78.75%, and 87.71%, respectively. The HDL-cholesterol levels of diabetic rats treated with extracts were significantly increased. Extract and its fractions caused α-amylase and α-glucosidase inhibition. Histologically, pancreas of diabetic rats treated with extract showed regenerated islet cells which were not seen in rats treated with glibenclamide and pioglitazone. This study showed that *U. chamae* has antidiabetic activity which may be through α-amylase and α-glucosidase inhibition and regeneration of pancreatic beta cells. Also, it may reduce the risk of cardiovascular disease by increasing HDL-cholesterol levels.

1. Introduction

Diabetes mellitus (DM) has been a threat to mankind from time immemorial, and it is now wreaking havoc disproportionately worldwide [1]. It is a public health problem acknowledged as one of the most important killer diseases and a prominent cause of death in low- and middle-income countries [2]. The life expectancy of diabetic patients is usually low compared to normal people [3]. DM is a noncommunicable disease in which there is a metabolic disorder of various etiologies described by sustained hyperglycemia with disorders of carbohydrate, fat, and protein metabolism following defects in insulin secretion, insulin action, or both [4]. It is caused by the destruction of pancreatic β-cells or dysfunctional β-cell and insulin resistance which results in hyperglycemia [5, 6]. Over time, diabetic patients with poor glycemic control undergo micro- and macrovascular complications including nephropathy, retinopathy, neuropathy, and cardiovascular diseases [7]. These complications increase their suffering and are the major sources of expenses for patients with diabetes as well as increasing the financial burden of nations [8, 9]. Above and beyond insulin are other therapeutic options for the treatment of type 1 diabetes which include transplantation of whole organ pancreas and

isolated islets, marred by both lack and quality of the donor's pancreas [10, 11]. However, numerous agents that are currently used for the treatment of type 2 diabetes are facing limited efficacy and tolerability [12]. For instance, sulfonylureas induce β-cell death in isolated rodent and human islets while glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors have potential risks for pancreatitis, pancreatic, and thyroid cancers [13–15]. Therefore, logical long-term solution to diabetic therapy is restoration of β-cells since β-cell deficiency underlies both type 1 and type 2 diabetes [16]. The restoration of deficient β-cell mass by transplantation from exogenous sources or by endogenous regeneration of insulin-producing cells would undoubtedly be a worthwhile therapeutic goal that will significantly ameliorate diabetes and its complications [17, 18]. Another approach to the treatment of diabetes is the application of medicinal plants with phytochemicals that cause beta-cell regeneration leading to normal blood glucose in animals and humans [19]. Many medicinal plants of African origin, such as *Momordica charantia* (bitter melon), *Cyclopia genistoides* (honeybush), and *Catharanthus roseus* (Madagascan periwinkle), are effective against various diseases including diabetes mellitus [20]. *Uvaria chamae* is one of such plants used traditionally to treat diabetes mellitus and other conditions such as bronchitis, gastroenteritis, amenorrhea, menorrhagia, abdominal pain, and wound healing [21–23]. It is a climbing medicinal plant that belongs to the family Annonaceae and is commonly found in West Africa, where it is known with different names by the Igbo, Hausa, Yoruba, Esan, and Igala natives of Nigeria as *Mmimi ohia*, *Kaskaifi*, *Oko oja*, *Ogholo*, and *Ayiloko*, respectively [24]. Several studies have confirmed that the bioactive compounds of *U. chamae* such as alkaloids, flavonoids, phenols, tannins, and terpenoids produce hypoglycemic, anti-inflammatory, antifungal, and antimalarial effects [24–27]. However, there is limited documentation on the potential use of *U. Chamae* in the treatment of diabetes mellitus. Therefore, this study aimed to evaluate antidiabetic effects of ethanolic root extract of *U. chamae* in alloxan-induced diabetic rats and its potential use in the treatment of diabetes mellitus.

2. Materials and Methods

2.1. Collection and Extraction of Uvaria chamae. The roots of the plants were collected in the Esan Central region of Edo state, Nigeria. They were identified and authenticated by Mr. T. K. Odewo, a taxonomist in the Department of Botany and Microbiology, Faculty of Science, University of Lagos, Nigeria. The voucher specimen numbered LUH 3572 was deposited in the institutional herbarium. The plant extraction was done using the methods described by Emordi et al. [23]. The ethanolic root extract of *U. chamae* (crude) was separated into chloroform, ethyl acetate, and ethanolic fractions via column chromatography.

2.2. Animals for the Experiment. The thirty-five animals used in this study were 6–8-week-old Sprague Dawley rats of either sex weighing 160 ± 20 g acquired from the Animal Center, College of Medicine, University of Lagos, Idi-Araba, Lagos State, Nigeria. They were placed into 7 groups of 5 rats and maintained under standard environmental condition (12/12 hr light/dark cycle) with free access to water and standard rodent diet (Pfizer Feeds Plc., Nigeria). The cage beddings and water bottles were cleaned daily, and the animals were allowed to adapt for two weeks to the laboratory conditions before the beginning of the experiment.

2.3. Ethical Considerations. The experimental protocol was approved by the Research grants and Experimentation Ethics Committee on animal use of the College of Medicine, University of Lagos, Lagos, Nigeria (with a protocol ID: RGEEC/21/2015). This was carried out in strict compliance with the National Research council guidelines on the care and use of laboratory animals [28].

2.4. Experimental Procedures

2.4.1. Induction of Diabetes. Except for the rats in group 1 (control), DM was experimentally induced in the animals of groups 2–7, after fasting them overnight by intraperitoneal administration of alloxan monohydrate dissolved in normal saline (150 mg/kg) [29]. Three days later, the blood glucose measurements were monitored with a glucometer, and the rats with plasma glucose greater than 200 mg/dl were labeled diabetic [23].

2.4.2. Animal Treatment. The treatment of the animals via oral route lasted for 14 days. Group 1 (normal control) received 0.5 ml (2% solution) of *Acacia senegal*. Groups 2 and 3 received 71 μg/kg of glibenclamide and 429 μg/kg of pioglitazone, respectively. Groups 4, 5, and 6, received 100, 250, and 400 mg/kg of the root extract of *U. chamae*, respectively, as determined by the outcome of the acute toxicity study by Emordi et al. [23]. Group 7 was not treated with the extract as it represented the diabetic control. During the treatment period, the weight of the animals and the fasting blood glucose (FBG) measurements were determined with a weighing scale and a glucometer (using the tail vein), respectively, every 2 days from the beginning of the treatment (day 1) to the last day of the experiment (15th day).

2.4.3. Sample Analysis. The blood was collected on the 15th day through ocular puncture into heparinized bottles for biochemical assays, ethylenediaminetetraacetic acid (EDTA) bottles for hematological assays, and plain bottles for insulin assay and the rats sacrificed. The blood samples with anticoagulants were centrifuged within five minutes of collection for 10 min at 4,000 g. By precipitation and modified enzymatic procedures from Sigma Diagnostics, the total cholesterol (TChol), triglyceride [30], and high density lipoprotein- (HDL-) cholesterol measurements were determined from the obtained plasma while the Friedewald equation was used to calculate low-density lipoprotein- (LDL-) cholesterol [31]. Also, creatinine and the enzymes

(aspartate aminotransferase [32], alanine aminotransferase (ALT), and alkaline phosphatase (ALP)). obtained from the plasma were evaluated using standard enzymatic assay methods [33]. Additionally, the plasma glucose, total protein, and albumin levels were determined using enzymatic spectroscopic methods [34].

2.4.4. Histological Studies. At the end of the experiment, the animals were sacrificed, and vital organs including the pancreas were harvested and fixed in 10% buffered formalin. The pancreatic tissue was processed using standard procedures as described by Grizzle et al. [35]. The tissue section was observed with a light microscope at a high magnification for histological changes and photomicrographs taken.

2.5. Determination of α-Amylase Inhibition. The determination of α-amylase inhibition by *U. chamae* was carried out according to the modified method by Kazeem et al. [36]. The mixture containing 200 μl of 0.02 M sodium phosphate buffer (pH 6.9), 20 μl of alpha-amylase, and 200 μl of the plant extract or its fractions in a concentration of 10–100 μg/ml was incubated for 10 minutes at 37°C, followed by addition of 200 μl of 1% starch solution in all the test tubes. The mixture was incubated for 15 min at 37°C. Addition of 400 μl dinitrosalicylic acid (DNS) reagent was used to terminate the reaction. The mixture was placed in a boiling water bath for 5 minutes, cooled, and diluted with 5 ml of distilled water, and the absorbance measured at 540 nm. The control samples were prepared without any plant extracts. The % inhibition was calculated according to the following formula:

$$\text{inhibition (\%)} = \text{Abs 540 (control)} - \text{Abs 540 (extract)} \times \frac{100}{\text{Abs 540 (control)}}. \quad (1)$$

The IC_{50} values were calculated by nonlinear regression analysis from the mean inhibitory values. Acarbose (STD = standard) was used as the reference α-amylase inhibitor. All tests were performed in triplicate.

2.6. Determination of the Type of α-Amylase Inhibition. The determination of the type of α-amylase inhibition by *U. chamae* and its fraction was done using the crude extract of *U. chamae* and its chloroform fraction that had the lowest IC_{50}. The experiment was carried out according to the modified method described by Kazeem et al. [36]. The extract and its chloroform fraction (250 μl of 5 mg/ml) were placed in two sets of test tubes and incubated with 250 μl of α-amylase solution, respectively, for 30 min at 25°C. In another set of tubes, α-amylase was incubated with 250 μl of phosphate buffer (pH 6.9). Then, 250 μl of starch solution at increasing concentrations (0.1–5.0 mg/ml) was added to the mixtures to start the reaction. The mixture was then incubated for 30 min at 25°C and then boiled for 5 min after addition of 500 μl of DNS to stop the reaction. The amount of reducing sugars released was determined spectrophotometrically. This was followed by its conversion to reaction velocities. The type of α-amylase inhibition by the crude extract and its chloroform fraction was determined by Lineweaver–Burk plot ($1/v$ versus $1/[S]$, where v is the reaction velocity and [S] is substrate concentration).

2.7. Isolation of α-Glucosidase from Rat's Small Intestine. The small intestine of male Sprague Dawley rat (180 g) was collected after sacrificing the animal. The intestine was thoroughly cleaned with normal saline, and epithelial layer (mucosal tissue) was collected by scraping the luminal surface firmly with a spatula. The mucosal scraping was homogenized in phosphate-buffered saline pH 7.4 containing 1 % triton X-100 and then centrifuged at 12000 rpm for 15 min. The supernatant fraction contained rat's small intestinal α-glucosidase. Butanol was added to the supernatant fraction 1 : 1 proportion and centrifuged at 15000 rpm for 15 min. The aqueous layer was dialyzed overnight against the same buffer. After dialysis, the concentrated enzyme was used as a crude α-glucosidase enzyme in the study [37].

2.8. Determination of α-Glucosidase Inhibition. The determination of α-glucosidase inhibition by *U. chamae* was carried out by using the modified method described by Kazeem et al. [36]. The isolated α-glucosidase (0.5 mg) from rat's small intestine was dissolved in 100 mM phosphate buffer pH 6.9. *p*-Nitrophenyl-α-D-glucopyranoside (pNPG) was used as the substrate. Plant extract and its fractions were used in the concentration ranging from 10–100 μg/ml. Different concentrations of the crude extract or its fractions, chloroform, ethyl acetate, and ethanol, and α-glucosidase, were mixed with 320 μl of 100 mM phosphate buffer pH 6.9 and incubated at 37°C for 10 minutes. Subsequently, the reaction was initiated by adding 50 μl of 3 mM pNPG and incubated for 20 mins. The reaction was terminated by adding 3 ml of 50 mM sodium hydroxide, and the absorbance was read at 410 nm. The control samples were prepared without any plant extract or the fractions. The % inhibition was calculated according to the following formula:

$$\text{Inhibition (\%)} = \text{Abs 410 (control)} - \text{Abs 410 (extract)} \times \frac{100}{\text{Abs 410 (control)}}. \quad (2)$$

The IC_{50} values were calculated by nonlinear regression analysis from the percentage inhibition. Acarbose was used as the control (the reference α-glucosidase inhibitor). All tests were performed in triplicate.

2.9. Determination of the Type of α-Glucosidase Inhibition. The determination of the type of α-glucosidase inhibition by *U. chamae* was assessed by using the crude extract and its ethanol fraction that had the lowest IC_{50}. This was done according to the modified method described by Kazeem

et al. [36]. The extract and ethanol fraction (50 μl of 5 mg/ml) were incubated with 100 μl of α-glucosidase solution, respectively, for 30 min at 25°C in two sets of tubes. In another set of tubes, α-glucosidase was incubated with 50 μl of phosphate buffer (pH 6.9). The reaction was started by addition 50 μl of pNPG at increasing concentrations (0.5–20 mM) to both sets of mixtures. The mixtures were then incubated for 10 min at 25°C, followed by addition of 500 μl of sodium bicarbonate to stop the reaction. The quantity of reducing sugars released was determined spectrophotometrically. This was followed by its conversion to reaction velocities. The type of α-amylase inhibition by the crude extract and its chloroform fraction was determined by Lineweaver–Burk plot ($1/v$ versus $1/[S]$, where v is the reaction velocity and [S] is the substrate concentration).

2.10. Analysis of Data. Analysis of data was carried out using GraphPad Prism 6 and SPSS version 22. GraphPad Prism was used for the diabetic study, and SPSS was used for the nonlinear regression analysis of α-amylase and glucosidase inhibitory activities. Nonlinear regression analysis was done with an R-square value of 0.9 and above and the IC_{50} values calculated from the regression analysis. The comparison of means of the groups was with one-way analysis of variance followed by Dunnett's post hoc test. The results were reported as mean ± SEM. The level of significance was set at $p < 0.05$.

3. Results

3.1. Effect of the Root Extract of U. chamae on Blood Glucose in Alloxan-Induced Diabetes Mellitus. On day one to day three, the blood glucose measurements of diabetic rats not treated and those treated with the root extract of *U. chamae*, glibenclamide, and pioglitazone were significantly ($p < 0.05$) elevated compared to the control (Table 1). However, on the 7th day to the end of the study, the 15th day, there was no significance difference in the blood glucose measurements of diabetic rats treated with the root extract of *U. chamae* compared to the control (Table 1). The rats treated with 100, 250, and 400 mg/kg of the root extract of *U. chamae* on the 7th day showed a marked blood glucose reduction of 72.14%, 78.75%, and 87.71%, respectively (Table 1). Conversely, the reference drugs glibenclamide and pioglitazone had a plasma glucose reduction of 63.10% and 30.46%, respectively. On the 15th day, the rats treated with 100, 250, and 400 mg/kg of the root extract of *U. chamae* showed a significant glucose reduction of 79.11%, 78.56%, and 88.11%, respectively, compared to the 74% and 55.07% glucose reduction of glibenclamide and pioglitazone, respectively (Table 1).

3.2. Effect of the Root Extract of U. chamae on Lipids in Alloxan-Induced Diabetes Mellitus. Effect of the root extract of *U. chamae* on plasma lipids is summarized in Table 2. There was no significant difference among the plasma LDL-cholesterol, TChol, and TG measurements of the diabetic rats treated with the root extract of *U. chamae* compared to the control. The HDL-cholesterol measurements of the diabetic rats treated with the root extract of *U. chamae* were significantly ($p < 0.05$) elevated compared to the control. While the LDL-cholesterol measurements of the diabetic rats not treated were significantly ($p < 0.05$) elevated compared to the control.

3.3. Effect of the Root Extract of U. chamae on Other Plasma Biochemical Parameters. Effect of the root extract of *U. chamae* on the other biochemical parameters is shown in Table 3. The root extract of *U. chamae* caused no significant alteration in the plasma creatinine, urea, protein, albumin, ALT, AST, and ALP measurements of the diabetic rats compared to the control. However, the plasma creatinine measurements were significantly ($p < 0.05$) elevated in diabetic rats not treated.

3.4. Effect of the Root Extract of U. chamae on Body Weight. The effect of the root extract of *U. chamae* on the body weight of the rats is summarized in Table 4. The root extract of *U. chamae* at doses of 100 and 400 mg/kg caused significant ($p < 0.05$) reduction in the weights of the rats from the 5th to 15th day of the study while 250 mg/kg of the extract caused no significant change in the weights of the rats except on the 11th day when the reduction became significant ($p < 0.05$) compared to the control. The weights of the diabetic rats that were not treated reduced significantly ($p < 0.05$) from the 7th to the 15th day of the study.

3.5. Effect of the Root Extract of U. chamae on the Blood Components. The effect of the root extract of *U. chamae* on the blood components is presented in Table 5. The root extract of *U. chamae* caused no significant alteration in white blood cell (WBC), red blood cell (RBC), hemoglobin concentration (Hgb), packed cell volume (PCV), mean corpuscular hemoglobin [38], mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and platelet (PLT) measurements of the diabetic rats compared to the control. However, glibenclamide caused a significant ($p < 0.05$) elevation in the WBC counts of the group of diabetic rats compared to the control.

3.6. Histological Findings. Normal cytoarchitectural features of the pancreas were observed in tissue sections of the normal control rats with intact and defined islets of Langerhans surrounded by acinar cells (Figure 1). The tissue sections of diabetic rats treated with glibenclamide (71 μg/kg) (Figure 2) and pioglitazone (429 μg/kg) (Figure 3) showed no visible islet cells, respectively. However, regenerated islet cells were seen (Figures 4–6) in the tissue sections of the pancreas of the diabetic rats treated with the root extract of *U. chamae* (100, 250, and 400 mg/kg, respectively) whereas, there was distinct absence of islets of Langerhans in the diabetic rats not treated (Figure 7).

Table 1: Effect of the root extract of *U. chamae* on blood glucose (mg/dl) in alloxan-induced diabetes mellitus and percentage reduction in blood glucose (%).

Days	Control (2% acacia)	Glibenclamide (71 μg/kg)	Pioglitazone (429 μg/kg)	UC (100 mg/kg)	UC (250 mg/kg)	UC (400 mg/kg)	Diabetic untreated
0	86.75 ± 2.36	76.00 ± 3.22	83.33 ± 9.20	88.33 ± 3.76	71.33 ± 3.76	77.67 ± 4.26	65.33 ± 5.90
1	91.75 ± 1.93	357.7 ± 28.30*	352.3 ± 9.40*	359.0 ± 16.10*	337.3 ± 6.70*	501.7 ± 56.30*	264.0 ± 5.80*
3	82.00 ± 3.20	222.3 ± 51.40* (37.85)	239.0 ± 38.40* (32.16)	284.7 ± 22.40* (20.70)	270.7 ± 18.8* (19.75)	280.3 ± 8.30* (44.13)	272.0 ± 2.00* (−3.03)
5	84.00 ± 3.00	189.0 ± 48.80 (47.16)	173.7 ± 35.80 (50.70)	198.7 ± 64.30 (44.65)	94.0 ± 7.50 (72.13)	157.0 ± 61.10 (68.71)	282.3 ± 1.50* (−6.93)
7	78.25 ± 4.27	132.0 ± 16.80 (63.10)	245.0 ± 59.81* (30.46)	100.0 ± 11.68 (72.14)	71.67 ± 9.17 (78.75)	61.67 ± 1.86 (87.71)	288.7 ± 0.88* (−9.36)
9	90.25 ± 3.10	117.0 ± 11.90 (67.29)	275.3 ± 48.40* (21.86)	91.67 ± 13.60 (74.47)	70.33 ± 8.70 (79.15)	58.33 ± 6.40 (88.37)	297.7 ± 1.20* (−12.77)
11	84.75 ± 2.50	109.3 ± 10.30 (69.44)	202.3 ± 70.50 (42.58)	90.33 ± 9.20 (74.84)	73.0 ± 4.00 (78.36)	50.33 ± 7.40 (89.97)	305.3 ± 4.80* (−15.64)
13	83.50 ± 4.90	100.0 ± 7.50 (72.04)	145.7 ± 77.70 (58.64)	79.0 ± 10.70 (77.99)	68.33 ± 2.00 (79.74)	55.0 ± 7.60 (89.04)	312.7 ± 9.20* (−18.45)
15	79.75 ± 3.50	93.0 ± 5.60 (74.00)	158.3 ± 59.30 (55.07)	75.0 ± 10.00 (79.11)	72.33 ± 4.30 (78.56)	59.67 ± 6.40 (88.11)	318.7 ± 11.30* (−20.72)

*Significant difference ($p < 0.05$; $n = 5$) between the mean ± SEM of test groups vs. control. UC: *Uvaria chamae*.

Table 2: Effect of the root extract of *U. chamae* on lipids (mg/dl) in alloxan-induced diabetes mellitus.

Parameters	Control (2% acacia)	Glibenclamide (71 μg/kg)	Pioglitazone (429 μg/kg)	UC (100 mg/kg)	UC (250 mg/kg)	UC (400 mg/kg)	Diabetic untreated
TChol	151.3 ± 4.67	168.7 ± 17.4	172.3 ± 13.0	176.0 ± 5.0	163.0 ± 13.8	163.0 ± 1.8	179.3 ± 11.1
TG	51.33 ± 9.40	53.3 ± 6.8	57.7 ± 7.80	55.3 ± 5.90	43.3 ± 3.80	46.0 ± 5.03	65.7 ± 0.33
HDL	35.33 ± 0.88	47.33 ± 4.97	41.67 ± 4.97	44.67 ± 2.4*	50.0 ± 2.88*	57.67 ± 1.5*	33.67 ± 3.18
LDL	105.7 ± 0.60	110.7 ± 1.3	119.1 ± 1.03	120.3 ± 0.52	104.3 ± 0.12	96.1 ± 0.30	132.2 ± 0.30*

*Significant difference ($p < 0.05$; $n = 5$) between the mean ± SEM of test groups vs. control. UC: *Uvaria chamae*.

Table 3: Effect of the root extract of *U. chamae* on other plasma biochemical parameters.

Parameters	Control (2% acacia)	Glibenclamide (71 μg/kg)	Pioglitazone (429 μg/kg)	UC (100 mg/kg)	UC (250 mg/kg)	UC (400 mg/kg)	Diabetic untreated
AST (U/L)	21.67 ± 4.26	30.33 ± 4.10	27.67 ± 4.63	23.0 ± 1.53	27.67 ± 2.03	26.67 ± 0.88	30.0 ± 1.16
ALT (U/L)	15.67 ± 1.33	26.67 ± 3.18	18.33 ± 4.49	19.33 ± 6.00	22.67 ± 3.18	24.33 ± 3.38	17.33 ± 2.03
ALP (U/L)	30.67 ± 5.70	30.67 ± 3.53	30.67 ± 4.18	21.00 ± 2.08	28.33 ± 6.49	28.00 ± 6.55	34.0 ± 6.66
Creatinine (mg/dl)	0.83 ± 0.03	1.00 ± 0.10	0.80 ± 0.10	1.03 ± 0.09	0.83 ± 0.03	0.80 ± 0.06	1.13 ± 0.03*
Urea (mg/dl)	38.00 ± 7.02	39.67 ± 7.05	33.67 ± 4.81	32.00 ± 1.16	26.67 ± 3.48	34.0 ± 3.22	44.67 ± 5.36
Protein (g/dl)	6.90 ± 0.62	5.97 ± 0.54	6.73 ± 0.38	6.47 ± 0.58	6.13 ± 0.59	6.70 ± 0.38	6.97 ± 0.09
ALB (mg/dl)	3.40 ± 0.35	2.83 ± 0.38	3.73 ± 0.32	3.23 ± 0.30	3.03 ± 0.20	3.53 ± 0.29	3.20 ± 0.20

*Significant difference ($p < 0.05$; $n = 5$) between the mean ± SEM of test groups vs. control. UC: *Uvaria chamae*.

Table 4: Effect of the root extract of *U. chamae* on body weight (g).

Days	Control (2% acacia)	Glibenclamide (71 μg/kg)	Pioglitazone (429 μ/kg)	UC (100 mg/kg)	UC (250 mg/kg)	UC (400 mg/kg)	Diabetic untreated
1	146.0 ± 0.57	124.0 ± 10.69	153.0 ± 4.04	139.3 ± 9.35	156.0 ± 6.81	124.7 ± 3.71	131.3 ± 4.18
3	144.7 ± 0.33	115.7 ± 4.80*	152.0 ± 5.29	135.3 ± 7.42	154.3 ± 7.54	123.3 ± 3.33	130.0 ± 5.29
5	140.7 ± 2.33	119.7 ± 4.91*	129.7 ± 1.86	111.7 ± 7.88*	143.7 ± 7.3	119.0 ± 2.65*	125.0 ± 2.89
7	144.0 ± 2.08	122.7 ± 5.90*	131.0 ± 2.65	122.7 ± 3.71*	143.7 ± 5.78	117.3 ± 1.76*	119.3 ± 4.26*
9	144.7 ± 2.67	118.3 ± 9.28*	127.3 ± 2.91	113.7 ± 5.81*	132.3 ± 6.69	114.7 ± 2.91*	107.3 ± 4.06*
11	145.0 ± 2.65	131.3 ± 7.69	127.3 ± 2.90	114.3 ± 3.48*	109.7 ± 4.84*	110.0 ± 2.65*	115.3 ± 4.33*
13	149.7 ± 3.18	132.0 ± 3.61*	141.3 ± 2.60	112.3 ± 1.45*	136.0 ± 5.69	107.0 ± 5.57*	109.3 ± 3.71*
15	155.0 ± 3.51	133.3 ± 1.76*	141.7 ± 2.85	103.7 ± 2.33*	137.3 ± 1.20	106.3 ± 8.95*	116.0 ± 5.51*

*Significant difference ($p < 0.05$; $n = 5$) between the mean ± SEM of test groups vs. control. UC: *Uvaria chamae*.

Table 5: The effect of the root extract of *U. chamae* on the blood components.

Parameters	Control (2% acacia)	Glibenclamide (71 μg/kg)	Pioglitazone (429 μ/kg)	UC (100 mg/kg)	UC (250 mg/kg)	UC (400 mg/kg)	Diabetic untreated
WBC ($\times 10^9$/L)	6.9 ± 0.47	11.9 ± 1.22*	9.6 ± 0.70	5.2 ± 0.36	6.4 ± 0.56	6.3 ± 0.64	9.17 ± 0.61
RBC ($\times 10^{12}$/L)	5.8 ± 0.30	5.2 ± 0.26	5.8 ± 0.50	5.5 ± 0.90	6.0 ± 0.48	5.8 ± 0.31	6.3 ± 0.62
Hgb (g/dl)	12.0 ± 0.6	10.0 ± 0.43	10.3 ± 0.79	10.0 ± 1.64	10.8 ± 0.8	10.0 ± 0.12	12.4 ± 1.69
PCV (%)	40.3 ± 3.38	29.9 ± 0.67	30.23 ± 2.10	32.0 ± 5.74	32.9 ± 2.64	32.8 ± 1.48	38.10 ± 5.29
MCV (fL)	70.5 ± 7.0	54.6 ± 2.24	56.0 ± 2.52	58.4 ± 2.17	55.3 ± 2.95	59.3 ± 4.33	60.2 ± 3.23
MCH (pg)	20.9 ± 1.29	19.3 ± 0.70	17.9 ± 0.23	18.4 ± 0.43	18.0 ± 0.94	18.5 ± 0.67	19.5 ± 0.92
MCHC (g/dl)	30.0 ± 1.30	33.3 ± 0.67	34.0 ± 0.35	31.3 ± 0.64	32.8 ± 0.03	32.2 ± 1.58	32.5 ± 0.30
PLT ($\times 10^9$/L)	918.7 ± 5.90	944.0 ± 25.74	958.0 ± 34.70	971.3 ± 8.99	967.7 ± 22.60	929.0 ± 17.04	955.0 ± 26.63

*Significant difference ($p < 0.05$; $n = 5$) between the mean ± SEM of test groups vs. control. UC: *Uvaria chamae*.

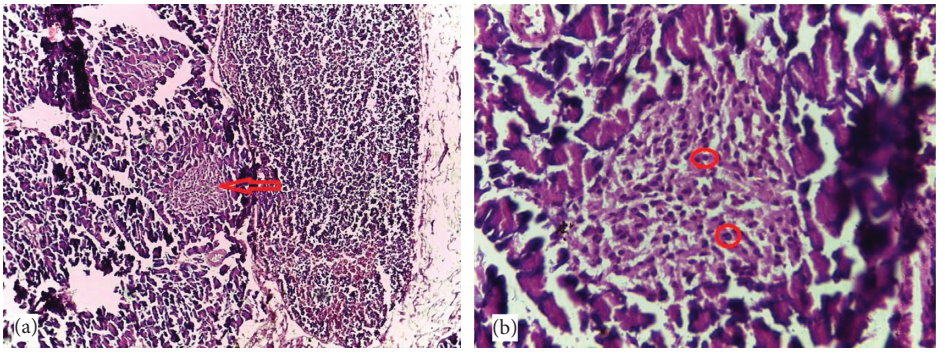

Figure 1: The tissue sections of control pancreas (H&E; (a) ×100; (b) ×400) show islets of Langerhans (a) and intact islet cells (b) and acinar cells with no remarkable alterations.

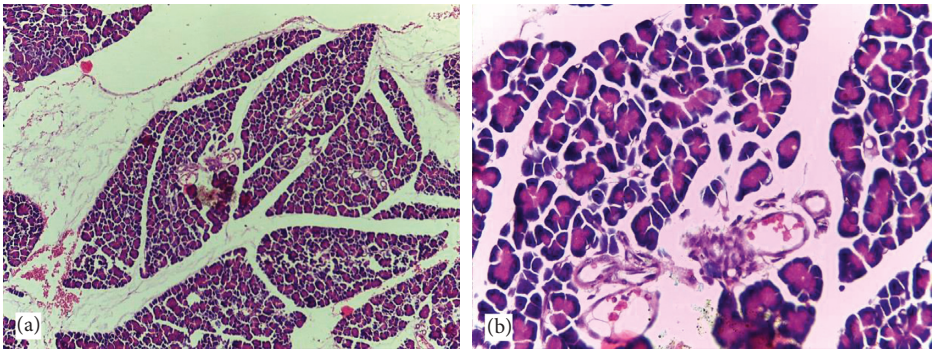

Figure 2: The tissue sections of the pancreas of diabetic rat treated with glibenclamide (71 μg/kg) (H&E; (a) ×100; (b) ×400) showing no islet cells.

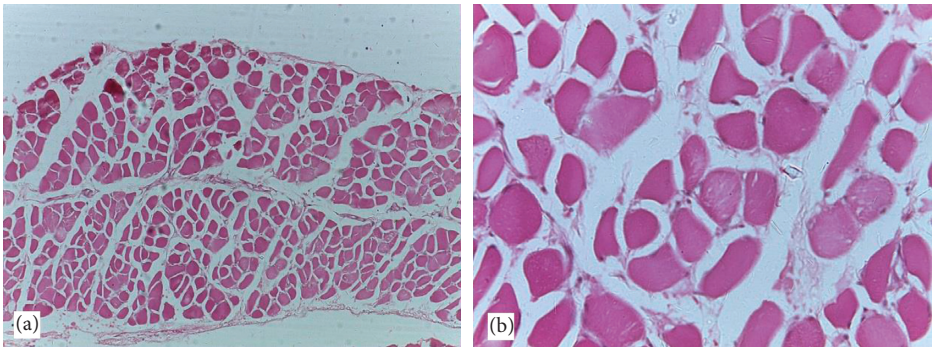

Figure 3: Tissue sections of the pancreas of diabetic rat treated with pioglitazone (429 μg/kg) (H&E; (a) ×100; (b) ×400) showing no distinct islet cell regeneration in the tissue cross section.

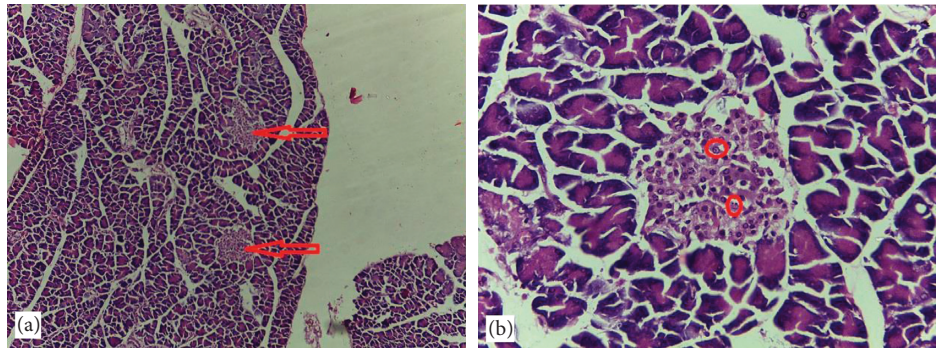

Figure 4: The islets of Langerhans (a), red arrow and regenerated islet cells (b), encircled in the tissue sections of the pancreas of diabetic rat treated with the root extract of *Uvaria chamae* (100 mg/kg) (H&E; (a) ×100; (b) ×400).

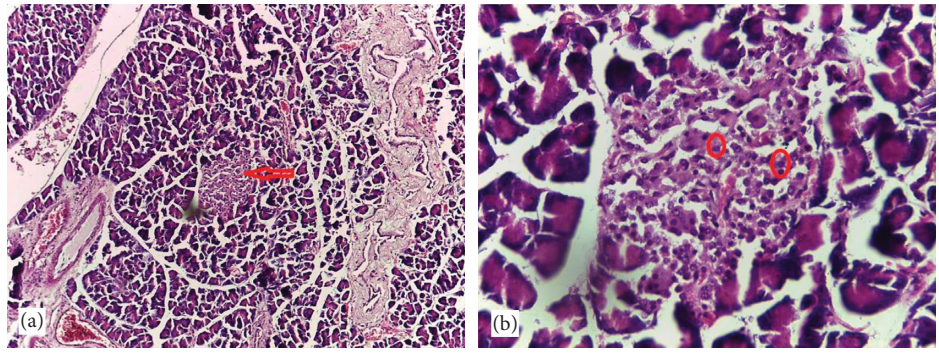

Figure 5: The islets of Langerhans (a), red arrow and regenerated islet cells (b), encircled in the tissue sections of the pancreas of diabetic rat treated with the root extract of *Uvaria chamae* (250 mg/kg) (H&E; (a) = ×100; (b) = ×400).

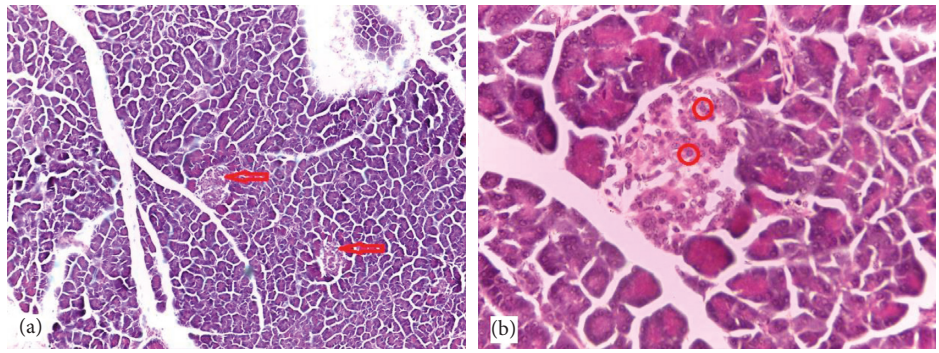

Figure 6: The islets of Langerhans (a), red arrow and regenerated islet cells (b), encircled in the tissue sections of the pancreas of diabetic rat treated with the root extract of *Uvaria chamae* (400 mg/kg) (H&E; (a) = ×100; (b) = ×400).

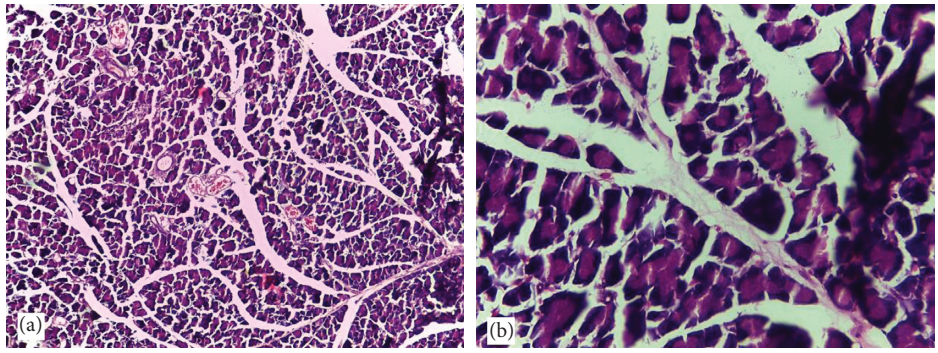

Figure 7: The distinct absence of islets of Langerhans in the tissue sections of the pancreas of diabetic rat not treated (H&E; (a) ×100; (b) ×400).

3.7. Effect of the Root Extract of U. chamae on Insulin Secretion. The root extract of *U. chamae* caused a dose-dependent increase in insulin secretion with a marked increase in insulin concentration in the group of rats treated with 400 mg/kg of the extract compared to glibenclamide, pioglitazone, and diabetic untreated (Figure 8). However, there was no significant difference in insulin secretion in the diabetic rats treated with the extract compared to the control.

3.8. IC_{50} Values of α-Amylase and α-Glucosidase Inhibition. The summary of the calculated IC_{50} values from the nonlinear regression analysis is shown in Table 6. The chloroform fraction of *U. chamae* had the most effective inhibition of α-amylase with an IC_{50} value of −246.3 μg/ml. However, the ethanolic fraction had the most effective inhibition of α-glucosidase with the IC_{50} value of −44.53 μg/ml followed by the crude extract of *U. chamae* with the IC_{50} value of 15.29 μg/ml.

3.9. α-Amylase Inhibition. The summary of α-amylase inhibition by the crude extract of *U. chamae* and its fractions, ethyl acetate, chloroform, ethanol, and the reference drug (acarbose), is shown in Figure 9. The crude extract of *U. chamae* and its fractions caused a concentration-dependent inhibition of α-amylase. The chloroform and ethanolic fractions were more potent inhibitors of α-amylase.

3.10. The Type of α-Amylase Inhibition. The type of α-amylase inhibition by the root extract of *U. chamae* and its chloroform fraction using Lineweaver–Burk plot showed that both the crude extract of *U. chamae* and its chloroform fraction exhibited a noncompetitive mode of inhibition (Figures 10 and 11), respectively.

3.11. α-Glucosidae Inhibition. The summary of α-glucosidase inhibition by the root extract of *U. chamae* and its fractions, ethyl acetate, chloroform, ethanol, and the reference drug (acarbose), is presented in Figure 12. The crude extract of *U. chamae* caused a significant ($p < 0.05$) increase in the inhibition of α-glucosidase compared to the reference drug, acarbose. The ethanolic fraction was more potent than the other fractions.

3.12. Type of α-Glucosidase Inhibition. The type of α-glucosidase inhibition by the root extract of *U. chamae* and its ethanol fraction using Lineweaver–Burk plot showed that the crude extract and its ethanol fraction exhibited a competitive (Figure 13) and noncompetitive (Figure 14) type of inhibition, respectively.

4. Discussion

Diabetes mellitus was previously considered a disease of trivial importance to world health but is now regarded as a major public health challenge in the 21st century [39]. It is a disease characterized by chronic hyperglycemia in

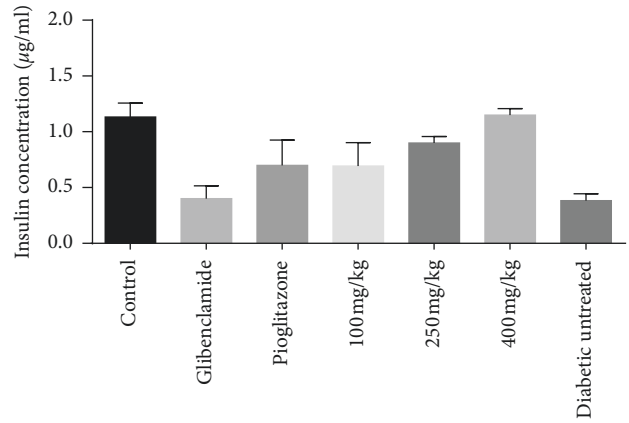

Figure 8: The increased insulin concentration from the serum of rats treated with the extract compared to the standard drugs (glibenclamide and pioglitazone).

Table 6: IC_{50} values of α-amylase and α-glucosidase inhibition.

Extract/fractions	IC_{50} (μg/ml)	
	α-Amylase	α-Glucosidase
Uvaria chamae	40.64	15.29
Ethyl acetate	57.52	34.38
Chloroform	−246.3	43.99
Ethanol	10.96	−44.53
Acarbose	3.12	15.89

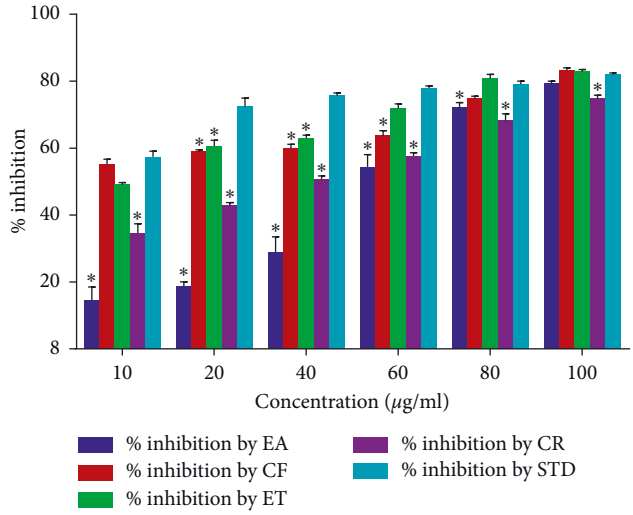

Figure 9: α-Amylase inhibition by *U. chamae* and its fractions. EA: ethyl acetate; CF: chloroform; ET: ethanol; CR: crude extract of *U. chamae*; STD: standard drug (acarbose). ($n = 3$) $^*p < 0.05$ vs standard (STD).

postprandial and fasting state with the risk of developing complications of the eyes, kidneys, peripheral nerves, heart, and blood vessels [40, 41]. These complications can be prevented by ensuring that the blood glucose measurements are within satisfactory limits [42]. Therefore, an important way of controlling diabetes mellitus is by the use of agents that reduce postprandial hyperglycemia by

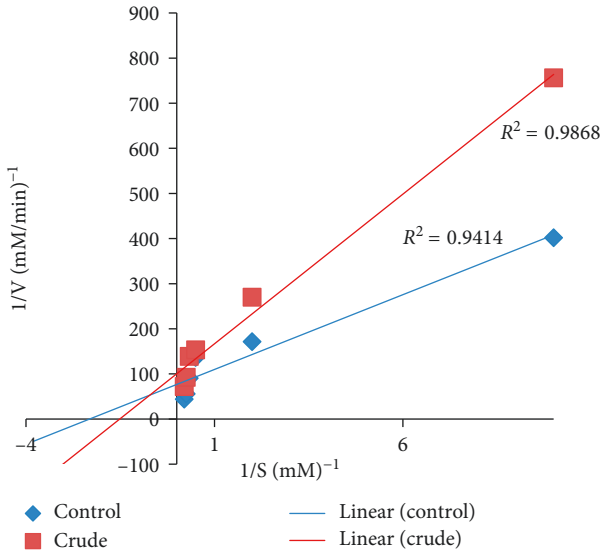

Figure 10: Noncompetitive α-amylase inhibition by *U. chamae*.

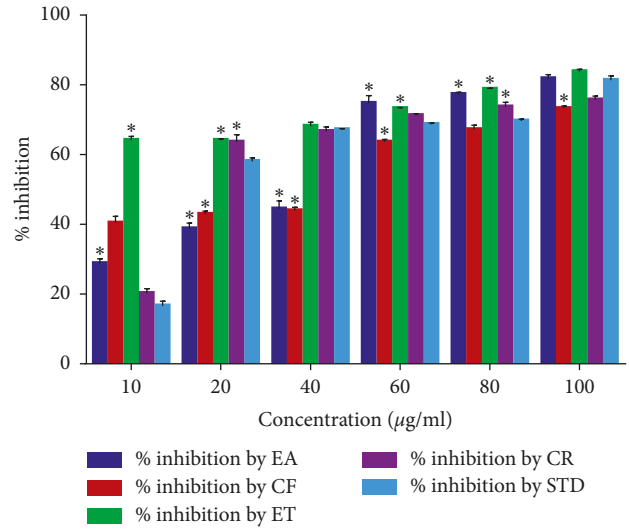

Figure 12: α-Glucosidae inhibition by *U. chamae* and its fractions. EA: ethyl acetate; CF: chloroform; ET: ethanol; CR: crude extract of *U. chamae*; STD: standard drug (acarbose). ($n = 3$) $^*p < 0.05$ vs standard (STD).

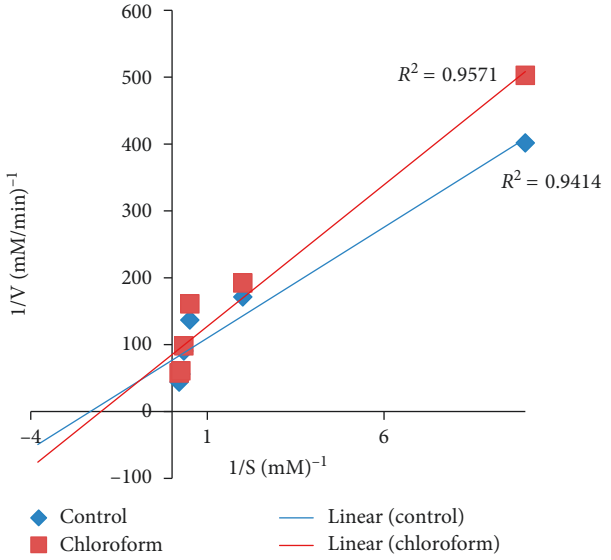

Figure 11: Noncompetitive α-amylase inhibition by chloroform fraction.

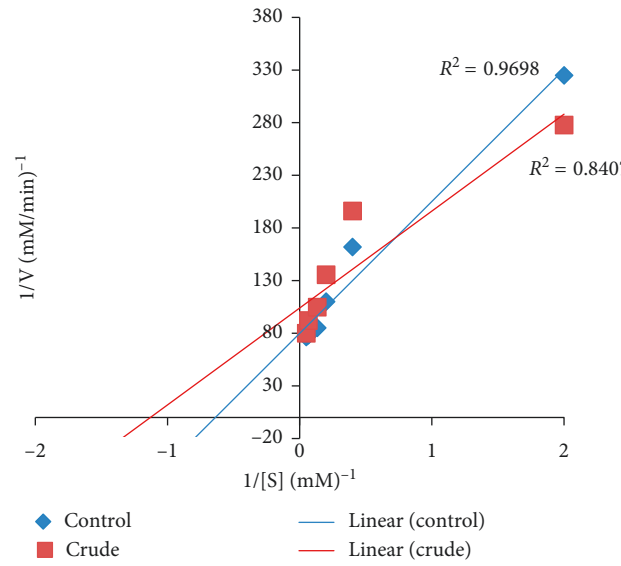

Figure 13: Competitive α-glucosidase inhibition by the crude extract.

suppressing hydrolysis of carbohydrate [43]. The findings of this study revealed that the blood glucose levels of the diabetic rats treated with *U. chamae* were comparable to the normal control. Nevertheless, diabetic control was achieved on the 7th day with a marked glucose reduction of 72.14%, 78.75%, and 87.71% following the administration of 100, 250, and 400 mg/kg of the root extract of *U. chamae*, respectively. This glucose control was sustained till the end of the study. The reference drugs glibenclamide and pioglitazone had a plasma glucose reduction of 63.10% and 30.46%, respectively, on the 7th day. The antidiabetic activity of *U. chamae* may be from the inhibition of α-amylase and α-glucosidase. These are enzymes responsible for breaking α, 1, 4 bonds in complex carbohydrate [44]. The inhibition of these enzymes delays the breakdown of carbohydrate which leads to reduced blood glucose [45, 46]. The findings of this study indicated that *U. chamae* and its fractions ethyl acetate, chloroform, and ethanol caused α-amylase and α-glucosidase inhibition. The α-amylase inhibition of *U. chamae* and the chloroform fraction were noncompetitive. Noncompetitive inhibitors decrease turnover numbers rather than reduction of the proportion of enzyme molecules that are bound to the substrate [47]. Therefore, *U. chamae* as an α-amylase inhibitor decreased the conversion of polysaccharides and disaccharides to glucose in a given unit of time. The

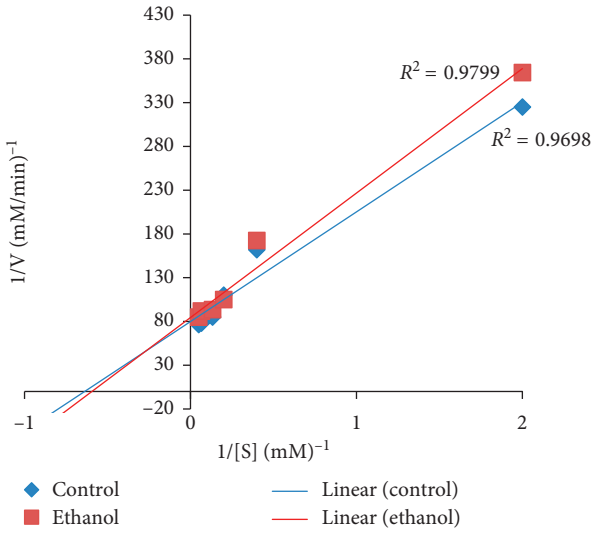

Figure 14: Noncompetitive α-glucosidase inhibition by the ethanolic fraction.

α-glucosidase inhibition of *U. chamae* and its ethanolic fraction were competitive and noncompetitive, respectively. A competitive inhibitor diminishes the rate of catalysis by reducing the proportion of enzyme molecules bound to a substrate [47]. Consequently, *U. chamae* as a competitive inhibitor of α-glucosidase may reduce absorption of glucose from the small intestine as glucose liberation from disaccharides is reduced. Therefore, the capacity of plant extracts to control the release and absorption of glucose is fast becoming an attractive therapeutic option in the treatment of diabetes mellitus [48]. The antidiabetic activity of *U. chamae* may also be from the presence of the secondary metabolites such as flavonoids, alkaloids, and tannins present in the plant [24]. Nevertheless, altered β-cell function and decreased β-cell mass may contribute to the defects in insulin release which is vital to the etiology of diabetes. These defects cause a progressive increase in glucose levels, with deterioration of glycemic control [6, 48, 49]. *U. chamae's* ability to cause increased insulin secretion from the regenerated islet cells may also have been responsible for the antidiabetic activity of the plant. Studies have shown that replacement of pancreatic beta cells may restore blood glucose and holds the key to the cure of diabetes [50, 51]. Consequently, *U. chamae* by its ability to cause regeneration of the beta cells of the pancreas may possibly play a role as a potential therapeutic option for diabetes mellitus. Although this result may look inspiring, further studies are still required to measure quantitatively the beta cell mass. It should be stated however that dyslipidemia arising from diabetes mellitus is a risk factor for coronary heart disease [52]. The findings of this study showed that treatment of the diabetic rats with the root extract of *U. chamae* caused a significant elevation in the HDL-cholesterol with no significant alteration in the plasma LDL-cholesterol, TChol, and TG levels. The hyperlipidemia associated with diabetes mellitus is reduced by limited absorption of free fatty acids and free cholesterol following inhibition of pancreatic lipase and pancreatic cholesterol esterase [53, 54]. The high levels of plasma HDL-cholesterol prevent risk of developing cardiovascular disease [55, 56]. Nonetheless, long-term complications of diabetes emanate from sustained chronic hyperglycemia [57, 58]. This study revealed that the plasma creatinine levels of the diabetic rats untreated were significantly increased. This may be an indication of renal impairment in this group of rats [59]. However, the plasma creatinine and urea of diabetic rats treated with the root extract of *U. chamae* were normal suggesting that *U. chamae* is not nephrotoxic. Hepatotoxicity is marked by profound elevations in the plasma levels of liver enzymes (ALT, AST, and ALP) and at times reduced plasma total protein and albumin levels [60, 61]. These liver enzymes are used to screen for hepatobiliary disease and identify the liver damage from abuse of drugs or substances [62]. AST and ALT are also released into the plasma in large quantities whenever there is damage to the liver and heart [31]. Nevertheless, there were no significant alterations in the plasma AST, ALT, ALP, and other hepatic function parameters such as total protein and albumin in the diabetic rats that received the root extract of *U. chamae* indicating that *U. chamae* is not hepatotoxic and cardiotoxic. Studies have shown that intentional weight loss in diabetic patients may improve glycemic control and reduce cardiovascular disease and mortality [63, 64]. The root extract of *U. chamae* may be of value to diabetic patients that are obese as it causes weight loss. The hematological parameters provide vital information regarding the status of bone marrow activity and intravascular effects such as hemolysis and anemia [65]. The findings of this study revealed that there was no significant difference on the hematological parameters of the diabetic rats treated with the root extract of *U. chamae* suggesting that *U. chamae* did not cause anemia and thrombosis nor suppressed the immune system. However, an increase in WBC count caused by glibenclamide is suggestive of boost immunity.

5. Conclusion

The study demonstrated the antidiabetic effects of *U. chamae* which may be through α-amylase and α-glucosidase inhibition and increased insulin secretion from the regenerated pancreatic beta cells. The plant also showed a cardioprotective effect via an increase in HDL-cholesterol levels.

Abbreviations

DM: Diabetes mellitus
FBG: Fasting blood glucose
EDTA: Ethylenediaminetetraacetate
TChol: Total cholesterol
TG: Triglyceride
HDL: High-density lipoprotein
LDL: Low-density lipoprotein
AST: Aspartate aminotransferase
ALT: Alanine aminotransferase
ALP: Alkaline phosphatase
H&E: Haematoxylin and Eosin
DNS: Dinitrosalicylic acid

Abs: Absorbance
IC_{50}: Concentrations of extracts or fractions resulting in 50% inhibition of enzyme activity, v: reaction velocity
[S]: Substrate concentration
SPSS: Statistical Package for the Social Sciences
ANOVA: Analysis of variance
SEM: Standard error of mean
WBC: White blood cell
RBC: Red blood cell
Hgb: Hemoglobin
PCV: Packed cell volume
MCH: Mean cell hemoglobin
MCV: Mean corpuscular volume
MCHC: Mean corpuscular hemoglobin concentration
PLT: Platelet
EA: Ethyl acetate
CF: Chloroform
ET: Ethanol
CR: Crude extract
STD: Standard (acarbose).

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

EJE, AEO, and OIA conceived this study. EJE and IOI carried out the experiments. EJE performed the statistical analysis. EJE drafted the manuscript. The authors read and approved the final manuscript.

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Supplementary Materials

In the supplementary material, the nonlinear regression analysis was used to calculate the (IC_{50}: concentrations of extracts or fractions resulting in 50% inhibition of enzyme activity) IC_{50} values of the fractions and the crude extract of *U. chamae* against the enzymes (α-amylase and α-glucosidase). In the study, the lower the IC_{50} values, the more potent the fraction is in inhibiting the enzymes. Therefore, we are more likely to focus on evaluating the fractions with the lowest IC_{50} in future investigation. Details of results showing the different IC_{50} values are sufficiently indicated in the main manuscript. (*Supplementary Materials*)

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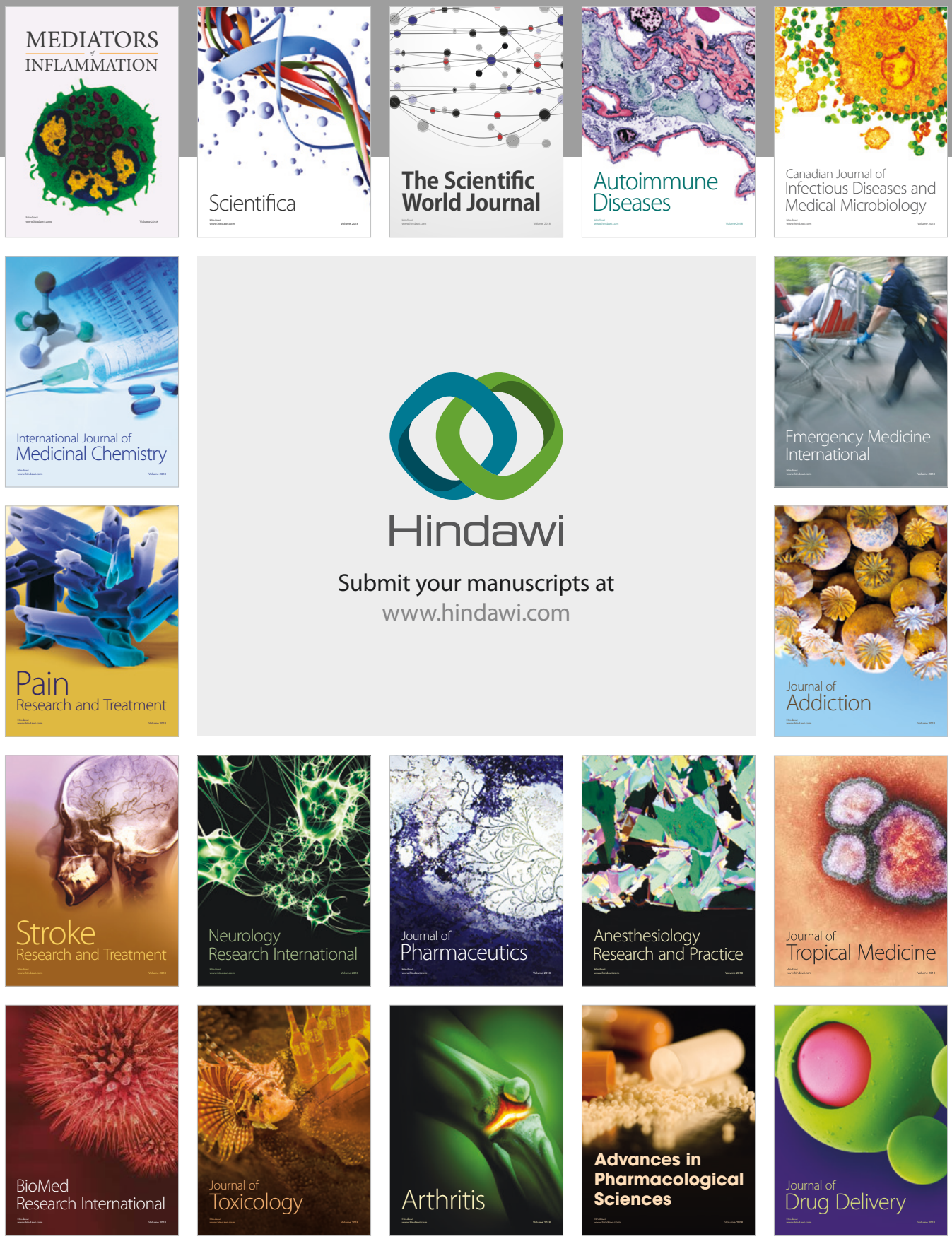
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