Available online on 15.02.2023 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Research Article

Antihyperglycemic Effects of Methanol Extract and Fractions of *Oxytenanthera abyssinica* Leaf (A. Rich.) Munro (Poaceae) in Alloxan-Induced Diabetic Albino Wistar Rats

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Article Info:

Article History:

Received 15 Nov 2022
Reviewed 02 Dec 2022
Accepted 16 Jan 2023
Published 15 Feb 2023

Cite this article as:

Atchrimi KS, Tougoma A, Omale S, Batawila K, Egesie GU, Odeh SO, Antihyperglycemic Effects of Methanol Extract and Fractions of *Oxytenanthera abyssinica* Leaf (A. Rich.) Munro (Poaceae) in Alloxan-Induced Diabetic Albino Wistar Rats, Journal of Drug Delivery and Therapeutics. 2023; 13(2):1-10

DOI: http://dx.doi.org/10.22270/jddt.v13i2.5841

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Abstract

The present study was carried out to investigate the antihyperglycemic effect of *Oxytenanthera abyssinica* leaf extract and fractions. Diabetes was induced in rats by administering a single dose of 150 mg/kg of alloxan monohydrate. Single doses of 100 and 200 mg/kg of methanol extract (Met-E), n-hexane fraction (nHex-F), ethyl-acetate fraction (EA-F) and methanol fraction (Met-F) were orally administered to diabetic rats. Met-E and Met-F were selected and administered at 400 mg/kg. The plasma glucose level was checked at 0, 1, 2, 4, 8, 12, and 24 h after treatment in acute effect study. Then once daily administration dose effect of 400 mg/kg of Met-E and Met-F was evaluated in chronic study for 28 days. The dose of 200 mg/kg of Met-E and Met-F significantly ($p<0.05$) decreased the plasma glucose level at 12th hour after treatment while their marked decrease effect at 400 mg/kg was observed at 4th hour after treatment. The repeated administration of Met-E showed a significant ($p<0.05$) decrease effect in plasma glucose level at the end of 1st, 3rd and 4th weeks while Met-F showed a significant ($p<0.05$) decrease effect at the end of 2nd, 3rd and 4th weeks. Treatment with Met-E and Met-F reduced pancreatic tissue deterioration compared to diabetic control. The present results showed that methanol extract and fraction of *Oxytenanthera abyssinica* exerted antihyperglycemic effect both in acute and chronic administration, which might be due to an amelioration of alloxan-induced pancreatic islet's tissue damages.

Keywords: Alloxan, *Oxytenanthera abyssinica*, *Antihyperglycemic effect, Pancreas, Methanol extract, Methanol fraction.*

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder caused by impairment in the ability of an organism to either produce insulin or being sensitive to its effect and thereby, unable to maintain glucose homeostasis. The disease is a 21st century pandemic with huge morbidity, mortality and economic burdens [1]. According to the World Health Organization (WHO), more than 451 million people were living with diabetes in 2017 worldwide and the disease rising could reach 693 million by 2045 [2]. An average of about 8.6 million new cases could be diagnosed each year along the next 28 years. With 69 % of diabetics, living in developing countries [3], recently about 80 % of worldwide death due to diabetes in low-and middle-income countries (LMICs) was reported [4]. According to the International Diabetes Foundation (IDF) report, LMICs bear 76.5 % of global undiagnosed diabetes cases while Africa alone accounts for 69.2 % of them [2]. Indeed, in most of Africa, people with DM might be increasingly diagnosed due to unhealthy nutritional and behavioural habits, e.g. lack of physical activity and "Western-style" diet with associated obesity [5]. The Chinese Centre for Disease Control and Prevention reported that individuals with

DM represent 7.3 % of the case-fatality rate of the 2.3% overall mortality due to Coronavirus disease 2019 (COVID-19) in 72,314 total cases [6]. The comorbidity of DM in the current burden of COVID-19 pandemic is much more alarming as the recent escalated increase in diabetes pandemic became a favourable field and exacerbated the infection risks, complications and deaths in COVID-19 patients [7] in the world. The incidence of COVID-19 in the United States might be fatal as those immune-compromised hosts who are diabetics represent 10.5 % (34.2 million) of the total population, in country [8]. DM is a huge public health challenge on a global scale. The lack of earlier diagnosis and unaffordability of conventional diabetic healthcare could be unfortunately the precursor of the high incidence of DM in LMICs. Thus, almost 70% of the global population prefer to apply traditional medicine resources in the management of DM [9]. Plant derivatives are rich sources of natural nutrients used since decades as diet and medicine to maintain health. Indeed, plants' secondary metabolites are bioactive compounds endowed with disease prevention and treatment in animals but most importantly in humans. As far as DM is concerned, investigating plant resources for their antidiabetic activities is undeniably needed. *Oxytenanthera abyssinica* (Poaceae), an endemic species of bamboo in Sub-Saharan Africa is widely used in African traditional medicine [10]. Previous studies revealed that the leave's extract possess antidiabetic activity [11, 12]. We also recently reported the antinociceptive activities of the leave's extract in rats. However, to the best of our knowledge, no research has been done on the antidiabetic activities of fractions from the leaves. In this study, leaf fractions and methanol extract of *O. abyssinica* were screened for their antihyperglycemic activities in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Chemicals and Drugs

Alloxan monohydrate, methanol, ethyl acetate and n-hexane (Sigma Aldrich Chemical Co., St. Louis, MO, USA), D-glucose, Sodium Chloride and Polysorbate 80 solution (BDH/Merck, Poole, UK); Glibenclamide: Daonil (Sanofi-Aventis, India), Accu-check glucometer active (Roche Diagnostics Co., Mannheim, Germany), Distilled water (ACEPRD, University of Jos, Jos, Nigeria).

Collection of plant material

Oxytenanthera abyssinica fresh leaves were collected from Kpéwa, Togo in December and authenticated by Professor Atsu K. GUELLY in Botany and Plant Ecology Laboratory, Faculty of Sciences, University of Lomé, Togo. A voucher specimen of Herbarium accession No: TOGO15189, was deposited in the herbarium of the Department of Botany. The leaves were thoroughly washed; shade dried under 20°C air-conditioned temperature in the Physiology-Pharmacology Laboratory, Faculty of Sciences, University of Lomé, Togo and then powdered.

Extract and fractions preparation

To prepare the extract, 500 g of the leaf's powder was mixed with 2.5 L of 80 % methanol and soaked in a glass jar. The mixture was kept macerating under periodic agitation for 72 h at room temperature and filtered**.** The fractionation was carried out on another 500 g of the leave's powder using solvent with increased polarity in an exhaustive and successive fractionation procedure. The powder was soaked in 2.5 L of n-hexane and regularly agitated during 72 h. The defatted material was freed from residual n-hexane at room temperature and extracted with Ethyl acetate and methanol successively. Each step of the extraction and fractionation was repeated twice. The filtrates obtained were pooled together and concentrated under reduced pressure in the Buchi rotavapor R-200. Methanol extract (Met-E), n-Hexane fraction (nHex-F), Ethyl-acetate fraction (EA-F), and Methanol Fraction (Met-F) obtained were collected, weighed and stored at -4°C till further use.

Phytochemical analysis

The preliminary screening for the presence of phytochemicals such as: flavonoids, tannins, alkaloids, terpenoids, steroids, cardiac glycosides, saponins, carbohydrates, anthocyanins was carried out on the methanol crude extract and fractions using different methods as described by Evans [13] and Harborne [14].

Animals

Male Albino Wistar rats of 4 weeks age were purchased and housed in the animal experimental unit of University of Jos. They were kept at room temperature under 12 h light/dark cycle in a clear and dry cage, and served standard pellet diet and water for 6 weeks*.* The matured and healthy rats weighing between 170-190 g were selected and used in the experiments. Approval for the study was obtained from the ethical committee animal experimental unit of University of Jos, Nigeria (reference number: UJ/FPS/F17-00379).

Oral acute toxicity: lethality (LD_{50}) tests

The oral median lethal dose **(**LD_{50}**)** of the Met-E was evaluated as described by Lorke [15]. The study was conducted into two successive phases on healthy rats. The animals were kept fasting for 16 h with free water access before the test. In the first phase, three groups of three rats each were orally administered 10, 100 and 1000 mg/kg body weight (b.w.) of Met-E respectively. The rats were observed over 24 h for signs of toxicity and possible death. Three other groups of three rats each were orally administered 1600, 2900, 5000 mg/kg body weight of Met-E respectively based on the outcomes of the first phase and were monitored for the same period as in phase one for toxicity signs and death.

Diabetes induction

Male albino Wistar rats (10 weeks) after 24h fasting and baseline plasma glucose level checked, were injected a single intraperitoneal dose of 150 mg/kg b.w. of alloxan monohydrate (ALX) freshly prepared in normal saline. Thirty minutes after the injection, animals were served 10% glucose solution and then 5% glucose solution was made available to them until the following day. Accu-Check Active glucometer (Roche, Germany) was used to check the plasma glucose level 72h after injection. Rats with plasma glucose level equal or greater than 200 mg/dL were selected as diabetics and crosschecked two weeks later before being included in the study.

Experimental design of antihyperglycemic test

All the treatments were administered to the rats by gastric intubation using a force-feeding needle and the blood samples were then collected from the tail-tip of each rat at different times to evaluate the plasma glucose level using the glucose oxidase method.

Effect of single dose oral administration of the extract and different fractions

Seven groups of five rats each were used in the study. The antihyperglycemic effect of Met-E, Met-F, EA-F and nHex-F was tested at 100 and 200 mg/kg b.w. Group 1: normoglycemic untreated rats were given 2 ml/kg b.w. of 0.5 % tween 80 (normal control); Group 2: diabetic untreated rats were also given 2 ml/g b.w. of 0.5 % tween 80 (diabetic control). Group 3-6 were treated with 100 or 200 mg/kg b.w. of Met-E, n-Hex-F, EA-F, Met-F respectively while the rats in Group 7 received Glibenclamide at a dose of 10 mg/kg and served as standard. The effect of the treatment on plasma glucose levels were recorded by interval of time (0, 1, 2, 4, 8, 12 and 24 h) in normoglycemic, diabetic treated and untreated rats. The treated rats' plasma glucose levels were compared to the one of diabetic control rats. Based on the results, Met-E and Met-F were selected and their antihyperglycemic effects were evaluated at 400 mg/kg b.w.

Antihyperglycemic effect of oral daily administration of Met-E and Met-F

Diabetes mellitus is a chronic disease and diabetes health care lasts usually the entire patient's life. The experiment was conducted on five groups of five rats each following the previous procedure described for a single oral administration. It involved repeated administration of 400 mg/kg b.w. of the extract and fractions once a day during 4 weeks and the plasma glucose levels were checked at the end of each week during the evaluation. The effect of Met-E and Met-F on pancreas histology was evaluated at the end of the four weeks.

Pancreas histopathology

Pancreatic tissues of diabetic (treated and untreated) and normoglycemic rats were collected immediately after animals were euthanized by decapitation under ethyl ether anaesthesia and fixed in 10 % formalin. Tissues were processed, paraffin embedded and sections were then stained in Harris' haematoxylin and counterstained in 1 % aqueous eosin [16, 17]. The sections were examined under light microscope for treatment's effects on tissue's morphology.

Statistical data analysis

GraphPad Prism software version 8.0.1 (San Diego, California USA) was used for data analysis. Differences between groups were analysed using two-way analysis of variance (ANOVA) followed by Dunnett's *t*-test for multiple comparisons. The results are expressed as mean ± SEM (n=5) and differences between groups were considered significant at $p<0.05$.

RESULTS

Preliminary phytochemical screening

The phytochemical screening of *Oxytenanthera abyssinica* leaf fractions and extract revealed the presence of various bioactive groups as shown in table 1.

Acute toxicity and lethal test

The rats survived the various doses administered in phase 1 (10; 100; 1000 mg/kg b.w.) as well as in phase 2 (1600; 2900 and 5000 mg/kg b.w.) and any unusual appearance or behaviour was not observed. Therefore, no mortality or any other toxicity signs were recorded with oral dose up to (5000 mg/kg) of *Oxytenanthera abyssinica* leaf extract.

Antihyperglycemic effect of single oral administration

None of the treatments with Met-E or fractions (nHex-F, EA-F and Met-F) showed significant ($p>0.05$) reduction effect in plasma glucose level at 100 mg/kg b.w. in alloxan induced-diabetic rats compared to the DC. A significant ($p<0.01$) increase of 158.54 % and 151.82 % in plasma glucose level was observed in nHex-F and EA-F treated diabetic rats respectively at 8th hour after administration (Fig 1). At 200 mg/kg b.w. the Met-E and Met-F have both induced significant ($p<0.05$; $p<0.01$) reduction effects of 42.88 and 34.1 % in plasma glucose level respectively at 12th hour after administration. However, the dose of 200 mg/kg of nHex-F and EA-F has in contrast exacerbated an increase in plasma glucose level in diabetic rats. The significant ($p<0.0001$) effect induced by nHex-F and EA-F was still observed at 8th hour after administration with an increase of 320.36 and 237.25 % in plasma glucose level respectively (Fig 2). At a dose of 400 mg/kg b.w., the both selected Met-E and Met-F have significantly ($p<0.0001$; $p<0.001$) showed a decrease effect of 62.29 and 45.7 % in plasma glucose level respectively at 4th hour after administration. Glibenclamide (10 mg/kg b.w.) induced a significant ($p<0.01$; $p<0.0001$) decrease effect of 56.56 and 82.06 % in plasma glucose level after the 2nd and 4th hours respectively after administration (Fig 3).

Table 1: Phytoconstituent in extract and fractions of *Oxytenanthera abyssinica*

Phytoconstituents	Met-E	nHex-F	EA-F	Met-F
Flavonoids	+++	-	+	+++
Tannins	++	-	+	+++
Alkaloids	+++	-	++	+++
Anthocyanins	-	-	-	-
Saponins	+	-	++	+++
Terpenoids	+	+++	++	-
Steroids	++	+	+++	+
Carbohydrates	++	+	+	+
Cardiac-glycosides	++	+++	+++	++
Yield	**9.1%**	**3.78 %**	**0.86%**	**5.26%**

Absent (-); Trace (+); Less abundant (++) Abundant (+++)

Antihyperglycemic effect of repeated oral administration

The effect of once daily administration of Met-E and Met-F on plasma glucose level in diabetic rats was evaluated each week (7 days) during 4 weeks (28 days). At the end of the 1st week, a significant (*p<0.01*) reduction of 44.72 % in plasma glucose level was exhibited in diabetic rats treated with Met-E and Glibenclamide-treated rats showed a significantly (*p<0.01*) reduction effect of 64.91 % compared to DC. Nevertheless, the decrease effect in plasma glucose level induced by Met-F treatment was not significant (*p>0.05*) at the end of the first week. In contrary, at the end of the 2nd week, Met-F treated animals showed a significant (*p<0.0001*) reduction effect of 71.35 % in plasma glucose level and Glibenclamide significantly (*p<0.0001*) showed 64.88 % of reduction effect while the effect of the Met-E at this period was not statistically significant (*p>0.0001*) compared to DC. At the end of the 3rd and 4th Week, all the treated diabetic rats displayed a marked reduction effect in plasma glucose level. Met-E showed significant (*p<0.0001* and *p<0.001*) reduction effects of 31.41 and 58.17 % in plasma glucose level at the end of 3rd and 4th Week. Met-F-treated rats significantly (*p<0.001*) displayed 57.86 and 58.61 % of reduction effect at the end of the 3rd and 4th Week successively. Glibenclamide at the two last weeks also exhibited a significant (*p<0.0001*) reduction of 54.14 and 67.76 % in plasma glucose level (Table 2).

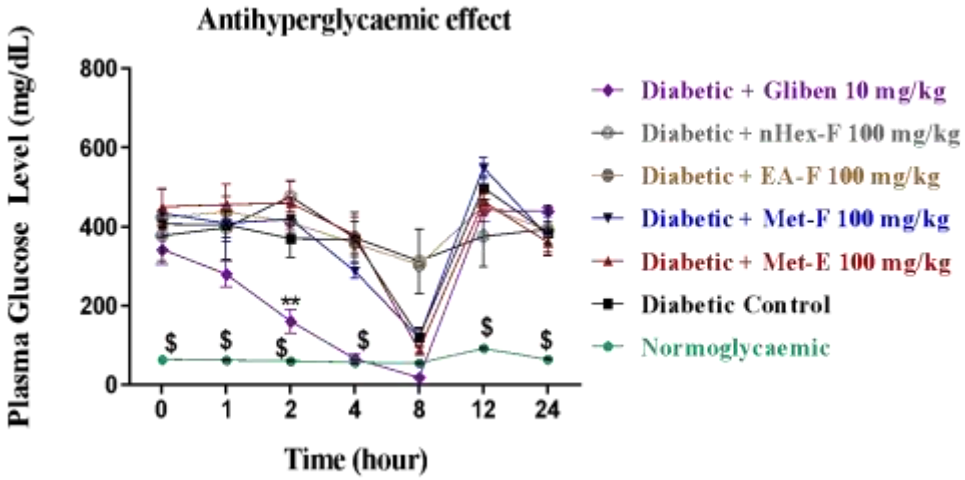

Figure 1: Antihyperglycemic effect of a single oral administration of 100 mg/kg b.w. of Met-E, Met-F, EA-F or nHex-F of *Oxytenanthera abyssinica* and Glibenclamide (10 mg/kg b.w.) in alloxan-induced diabetic rats.

The values expressed are mean ± SEM (n=5). ***p<0.01, ***p<0.001* and *$p<0.0001* (treated compared to diabetic control).

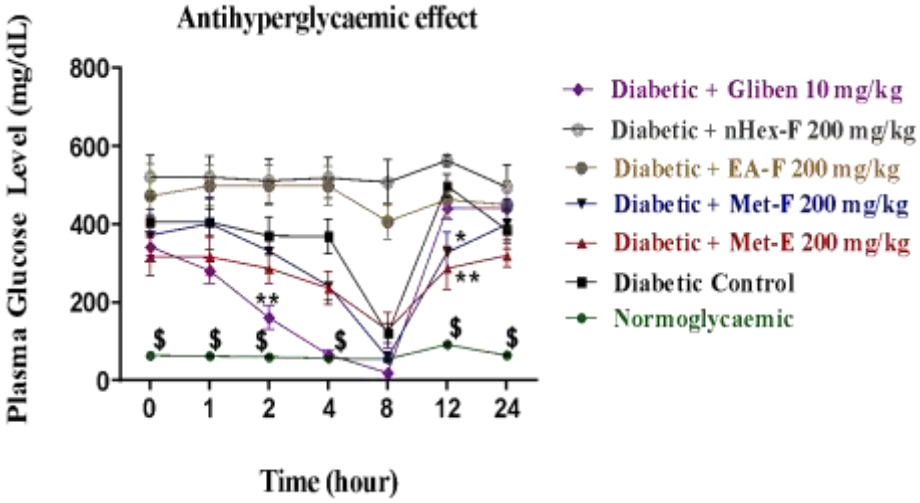

Figure 2: Antihyperglycemic effect of a single oral administration of 200 mg/kg b.w. of Met-E, Met-F, EA-F or nHex-F of *Oxytenanthera abyssinica* and Glibenclamide (10 mg/kg b.w.) in alloxan-induced diabetic rats.

The values expressed are Mean ± SEM (n=5). **P<0.05, **P<0.01* and *$P<0.0001* (treated compared to diabetic control).

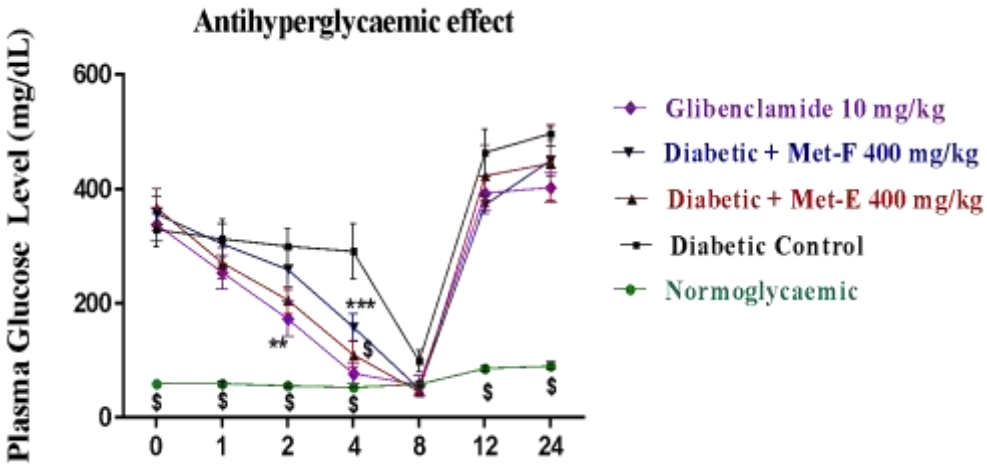

Figure 3: Antihyperglycemic effect of a single oral administration of 400 mg/kg b.w. of Met-E and Met-F of *Oxytenanthera abyssinica*, and Glibenclamide (10 mg/kg b.w.) in alloxan-induced diabetic rats.

The values expressed are Mean ± SEM (n=5). ***p<0.01, ***p<0.001* and *$p<0.0001* (treated compared to diabetic control).

Pancreas histology

The study was carried out to investigate the effect of the treatments on diabetic rats. Pancreas of rats in the healthy normoglycemic group showed a normal histological structure demonstrated by the presence of islet cells illustrated by their normal characteristic (faint pink staining), presence of red blood cells within the tissue and clearly stained secretary acinar and centroacinar cells (Fig4). In contrast, in the diabetic untreated rat (DC), alloxan administration has caused marked deteriorations in the pancreatic tissue architecture in form of pancreatic tissue necrosis associated with loss of islet cells and infiltration of massive inflammatory cells, severe atrophy of secretory acini making the centroacinar pronounced and the interlobular septal appeared clear (Fig 5). Met-E clearly prevents further damage induced by alloxan administration as demonstrated by the presence of pancreatic islets within the pancreatic tissue and an interwoven network of secretory acini and centroacinar cells (Fig 6). Met-F also clearly has induced a progressive reverse to normal tissue architecture as observed by the presence of an interwoven network of secretory acini (black arrows) and centroacinar cells (white arrows). Few red blood cells (black arrowheads) are seen within the blood vessels. However, the section of the pancreas tissue figured out did not show islet cells (Fig 7). Glibenclamide did not show any reverse effect on alloxan-induced pancreatic tissues damaged. Thus, tissue necrosis, inflammatory cell infiltration, pancreatic islets' loss and atrophied secretory acini were observed (Fig 8).

Table 2: Antihyperglycemic effect of repeated oral administration of Met-E and Met-F from *Oxytenanthera abyssinica* leave

Treatment group	Before	During treatment			
		Week 1	Week 2	Week 3	Week 4
Normoglycemic	100.20 ± 4.95	85.80 ± 4.88	99.00 ± 3.67	94.00 ± 2.50	103.40 ± 3.10
Diabetic Control	395.00 ± 22.66	382.40 ± 20.82	515.80 ± 34.37	595.20 ± 2.57	547.00 ± 7.38
Diabetic + Met-E 400 mg/kg	374.20 ± 30.93	211.40 ± 49.23**	373.40 ± 59.06	187.00 ± 43.02$	318.20 ± 55.97***
Diabetic + Met-F 400 mg/kg	381.80 ± 30.96	246.20 ± 49. 27	147.80 ± 22.95$	344.40 ± 90. 95***	320.60 ± 779.51***
Diabetic + Gliben 10 mg/kg	287.80 ± 52.63	134.20 ± 49.81***	181.20 ± 39.89$	273.00 ± 57.55$	176.40 ± 44.31$

The values (mg/dL) are expressed as mean ± SEM (n=5). Data were analyzed by two-way ANOVA followed by Dunnett's multiple comparison test. ***p<0.01, ***p<0.001* and *p<0.0001* (treated compared to Diabetic Control).

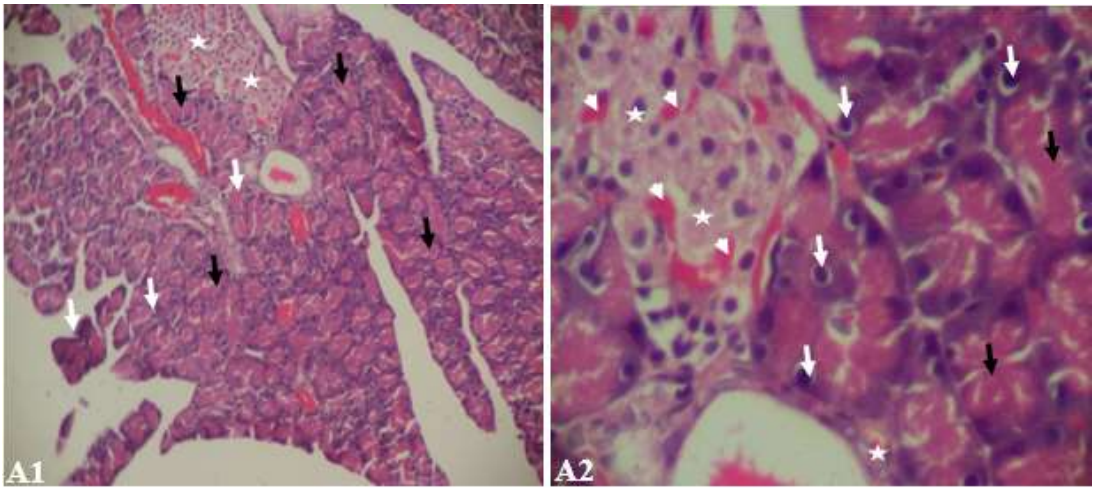

Figure 4: Photomicrograph of pancreatic tissue in normoglycemic rats. Normal architecture of the pancreatic cells demonstrated by clearly stained secretory acini (black arrows) and centroacinar cells (white arrows). The islet cells (white stars) are present and red blood cells (white arrowheads) are seen within the islet tissue.

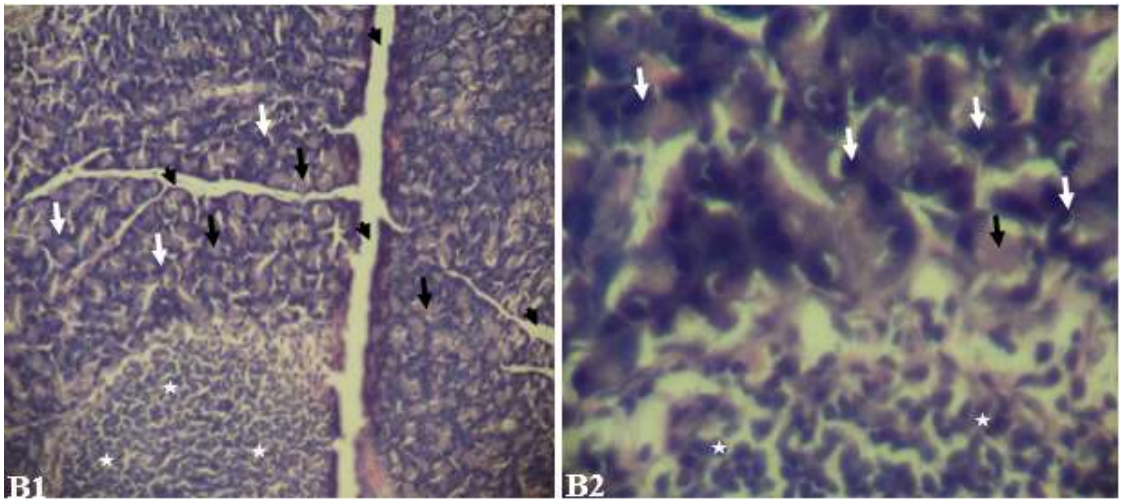

Figure 5: Photomicrograph of pancreatic tissue in diabetic untreated rats. Massive tissue degeneration and massive presence of inflammatory cells (white stars). Pancreatic islets are lost while the secretory acini (black arrows) are severely atrophied making the centroacinar cells (white arrows) more pronounced and the interlobular septal appeared clear. H&E: B1 X100; B2 X400.

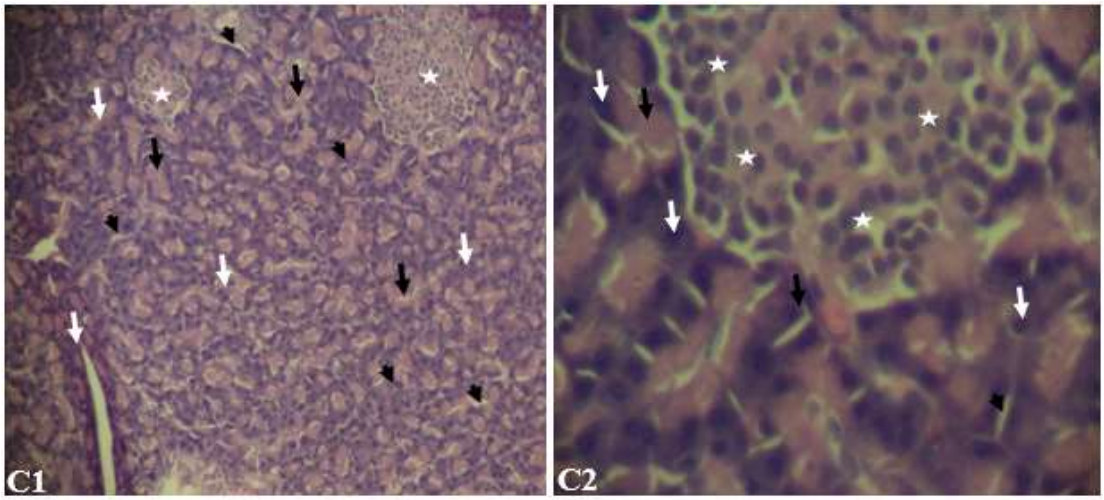

Figure 6: Photomicrograph of pancreatic tissue in Met-E-treated diabetic rats. A reversed to normal tissue morphology is observed as demonstrated by the presence of an interwoven network of secretory acini (black arrows), centroacinar cells (white arrows) and pancreatic islets (white stars). Black arrowheads indicate interstitial spaces. H&E: C1 X100; C2 X400.

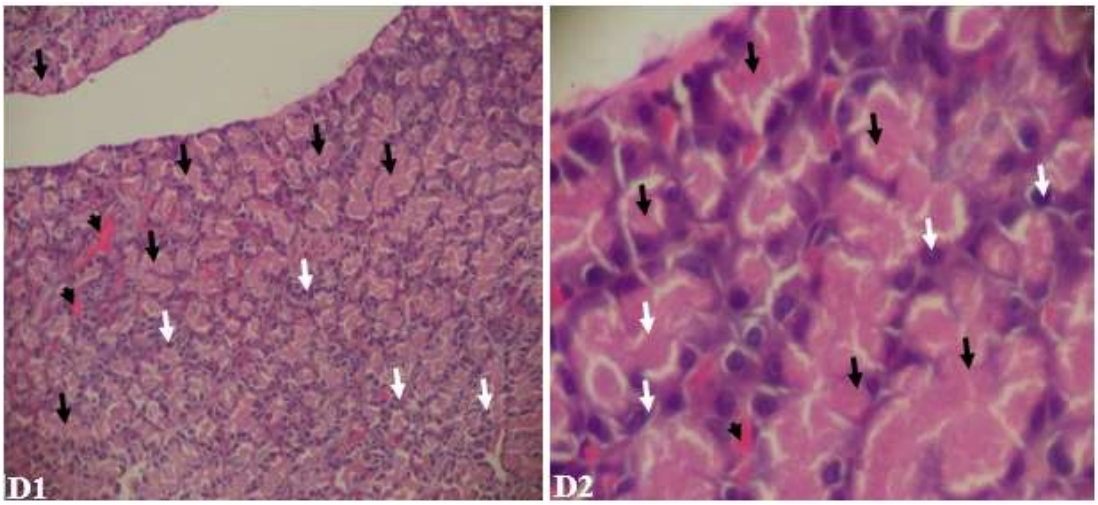

Figure 7: Photomicrograph of pancreatic tissue in Met-F-treated diabetic rats. A reversed to normal tissue architecture is observed as demonstrated by the presence of an interwoven network of secretory acini (black arrows) and centroacinar cells (white arrows). Few red blood cells (black arrowheads) are seen within the blood vessels. H&E: D1 X100; D2 X400.

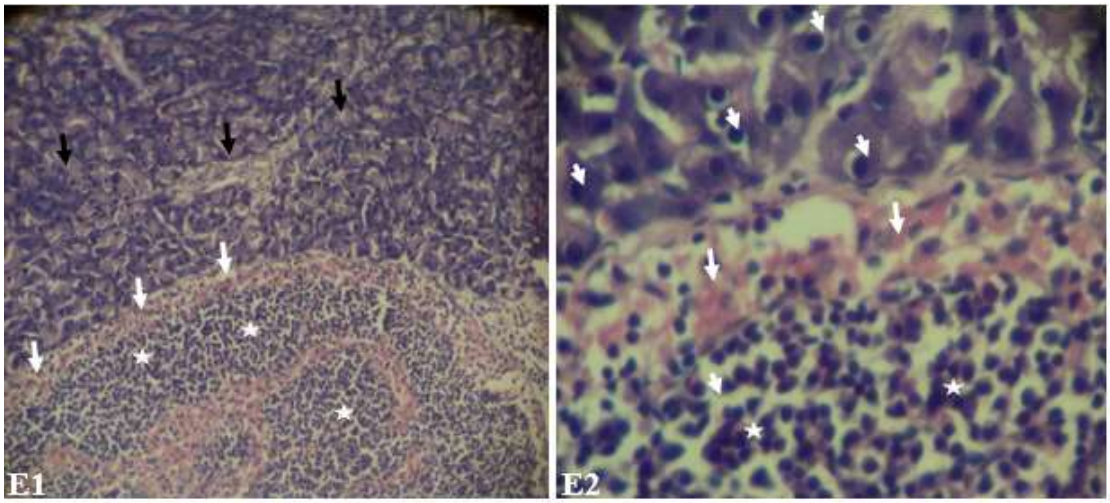

Figure 8: Photomicrograph of pancreatic tissue in Glibenclamide-treated rats. Tissue degeneration and portions of the degenerated tissues were taken over by inflammatory cells (white stars) within the tissue. A mild collagination of the pancreas was noted by the deposition of collagen fibres (white arrows). Pancreatic islets are lost and the secretory acini (black arrows) are greatly reduced (white arrowheads). H&E: E1 X100; E2 X400.

DISCUSSION

Medicinal plants and their derivatives as alternative antidiabetic therapy have become a focus in research as they have been used for decades throughout the world in the management of diabetes conditions, and are believed to be with minimal or no side effects. A few reports are available on the antidiabetic effect of *Oxytenanthera abyssinica* [11, 12]. In light of that, this present study was designed to examine the antihyperglycemic activity of methanol extract as well as some fractions of *O. abyssinica* leaf in alloxan-induced diabetes. The phytochemical screening carried out on the extract and fractions revealed the presence of different phytochemicals such as flavonoids, tannins, alkaloids, cardiac-glycosides, carbohydrates, steroids, terpenoids and saponins, and a marked of total absence of anthocyanins (Table 1). This result is similar to the previous reports on hydroalcoholic extract [18], ethanolic extract [12] and methanol extract [10] phytoconstituents of the plant leaf collected from different areas. The presence of the varieties of bioactive compounds might confirm the importance of the traditional use of the plant leaves in the treatment of various diseases in Togo [19, 20 ,21]. The diabetogenic agent used has produced significant increase in blood glucose level to an approximative average of 380 mg/dL by selectively destroying the pancreatic insulin secreting ß-cells and causing diabetes close to type 2 diabetes of humans. The antihyperglycemic effect of the methanol extract (Met-E), n-hexane fraction (nHex-F), Ethyl-acetate fraction (EA-F) and methanol fractions (Met-F) were evaluated in the diabetic rats after 16 hours of fasting. A chronic starving similar to recurrent severe hypoglycaemia generally leads to central neuroglycopenia and death mediated by numerous cardiac arrhythmias through sympathoadrenal response [22]. Thus, after the 8th hour during the evaluation, as an equivalent of a total 24h starvation period, animals were fed to avoid a risk of death during experimentation. The antihyperglycemic effects of the treatment were assessed at different doses: 100, 200 and 400 mg/kg over 24 h in acute effect study and at 400 mg/kg in a

chronic effect study over 28 days. At 100 mg/kg, the methanol extract and the different fractions treated rats failed to exhibit a significant (*p>0.05*) reduction in plasma glucose level at the different times of the evaluation throughout the 24 h period (Fig 1). The hypothesis of the treatment-induced lowering effect in plasma glucose level seemed clearly not to be met with nHex-F and EA-F as, rather than reducing the plasma glucose level, they induced a noticeably significant (*p<0.01*) increase of glycaemia in diabetic rats at 8th hour (starving pick) after administration. Interestingly, the 200 mg/kg dose of both Met-E and Met-F have significantly (*p <0.01*; *p <0.05*) reduced the plasma glucose level at 12th hour of the evaluation, while on another hand, the treatment with both EA-F or nHex-F resulted in significant (*p <0.0001*) high increase in plasma glucose level which was tangible at 8th hour during the study period. Thus, the administration of nHex-F and EA-F would probably lead to an increase of gluconeogenesis or decrease of glucose uptake. These results suggested that the antihyperglycemic effect of the leaf might be endowed with bioactive compounds soluble in a polar solvent (methanol). Pandikumar, Babu, & Ignacimuthu [23] have previously demonstrated that even up to a dose of 200 mg/kg, Ethyl acetate and n-hexane bioactive soluble compounds from *Begonia malabarica* were not capable to induced hypoglycemic effect. It was reported similarly to our results that alloxan diabetic rats treated with methanol extract of *Gongronema latifolium* leaf showed significant decrease in blood glucose level whereas n-hexane and chloroform fraction did not exhibit any decrease in blood glucose level before the end of 24 hours [24]. Our results also support previous reports that concentration of active compounds of leaves extracted in polar solvents may elicit antihyperglycemic effects [25, 26]. Considering together the significant reduction effect of the plasma glucose level with 400 mg/kg of Met-F and Met-E at the 4th hour (Fig 3), and with 200 mg/kg at 12th hour (Fig 2), it could be assumed that they may act by stimulating β-cells recovery and insulin secretion [27, 28, 29, 30] or reducing carbohydrates absorption at relatively lower dose [11, 12]. The antihyperglycemic effect of aqueous seed extract of C*itrullus colocynthis* through β-cell regeneration has also been reported [31]. Though ß-cells regeneration is still not well understood, this hypothesis demonstrated in various studies (Mziaut et al. [32]; Saunders et al.[27]) can progressively be considered as an important mechanism which could be involved in the anti-diabetic properties of some plant extract [31, 33, 34, 35]. The presence of alkaloids and phenolic compounds (tannins, flavonoids) in methanol extract and fraction of *O. abyssinica* leave could be responsible of their antihyperglycemic effect while the special the terpenoids add to cardiac glycoside could play an important role in n-hexane and Ethyl acetate opposite effect. The abundance of terpenoids in nHex-F and EA-F against an absence in Met-F shows that the available terpenoids in *O. abyssinica* leave might be mostly made of non-polar terpenes conjugated with fatty acids or non-volatile terpenes and then much better extracted by very non-polar organic solvent such as n-hexane [36]. Plant-derived terpenoids like artemisinin from *Artemisia annua* are known for their various medicinal properties [36, 37]. However, the present study revealed that the ones present in *O. abyssinica* leave seem not to be beneficial in the management of diabetes. Glibenclamide (10 mg/kg b.w.) used as positive control demonstrated at 2th and 4th hours a significant (*p <0.01*; *p <0.0001*) decrease effect in plasma glucose level after administration. The daily treatment with 400 mg/kg of methanol extract and fraction showed that Met-E-treated animals exhibited a significant (*p<0.01; p<0.001* and *p<0.0001*) decrease in plasma glucose level at the first, third and fourth week respectively after treatment and Met-F-treated animals showed a significant (*p<0.0001*; *p<0.001*) decrease in plasma glucose level from the second to the fourth week respectively. The highest effect of methanol extract observed at the end of 1st week of the chronic daily treatment, revealed that all the treatments including Glibenclamide are yet to induce a stable balance of glucose homeostasis to the normal glycemic level. Yet, the gradual slowdown in plasma glucose level observed with Met-F with better activity at the end of 2nd week (71.35%) compared to Glibenclamide (64.88%), all compared to DC could be beneficial in promoting adjustment of glucose homeostasis and to avoid abrupt physiological reactions. In this study, the histopathological observation of diabetic untreated rats showed a complete pancreatic islet cell lost and severe atrophy of the secretory acini (Fig 5) after six weeks of alloxan injection compared to normal pancreatic tissue (Fig 4). The methanol extract of *Oxytenanthera abyssinica* treated rats demonstrated a recovery effect characterised by a normal tissue morphology with clear pancreatic islets, interstitial spaces, and interwoven network of secretory acini as well as Centro acinar cells (Fig 6). Met-E then might induce an antihyperglycemic effect by enhancing the proliferation of the surviving β-cells through stimulation of β-cells regeneration. A recovery to normal tissue architecture with well-irrigated pancreatic cells with reduced inflammation was observed in diabetic rats treated with methanol fraction. Meanwhile, islets cells were yet to be identified (Fig 7). This shows that the antihyperglycemic effect in Met-F treated animals would rather be mediated through sustainability over some survived β-cells and stimulation of insulin synthesis and release. This present pancreas histopathological *ex-vivo* study evidenced the previous reports of antidiabetic effect of *O. abyssinica* leave extracts mediated by antioxidant activity of the active compounds [10, 11, 12]. Further works would be needed to evaluate the variations of the antihyperglycemic effect of Met-E or Met-F in a greater long-term study and to investigate in-depth their mechanism of actions. The Glibenclamide treated rats did not reverse tissue degeneration nor induced anti-inflammatory activity. Therefore, loss of pancreatic islets was observed and the secretory acini are greatly reduced (Fig 8). Glibenclamide triggers insulin secretion through inhibition of pancreatic β-cells ATP-sensitive K^+ channel and increase of free intracellular Ca^{2+} level [38]. The decreased effect of plasma glucose level showed that the insulin secretory activity of the few β-cells is improved by Glibenclamide action.

CONCLUSION

The present study has demonstrated a general safety of *Oxytenanthera abyssinica* leave extract in oral administration and revealed that predominantly, the bioactive compounds soluble in polar solvents could be responsible for the antihyperglycemic effects of the leave in experimental diabetes model. Thus, the folk use of the plant's leaves in diabetes management may be validated by this study. However, the availability of the needed bioactive compounds and the efficacy of the antihyperglycemic property would be greatly influenced by the polarity of the chosen solvent during the preparation. Further investigation should be done to confirm the plant safety and efficacy in long term use beyond four weeks and the active substances in Methanol extract and fraction could be isolated and characterised.

ACKNOWLEDGEMENTS

Our heartfelt gratitude goes to the Department of Human Physiology, Africa Centre Excellence in Phytomedicine Research

and Development (ACEPRD) and Animal experimental Unit of University of Jos for their various supports in terms of laboratory space, some reagents provided and facilities made available to us during the work.

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