

Hypoglycemic and Immunomodulatory Effects of Aqueous Leaf Extract of *Vernonia ampla* on Streptozotocin-Induced Diabetes in Wistar Rats

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Abstract

Diabetes mellitus is a metabolic syndrome of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrates, fats and proteins metabolisms resulting from defects in insulin secretion by pancreatic beta cells and insulin action or both. Chronic hyperglycemia is associated with long-term damage, dysfunction, and failure of different organs, including the kidneys. The present study aimed to determine the hypoglycemic and immunomodulatory effects of aqueous leaf extract of *Vernonia ampla* on streptozotocin-induced diabetes in Wistar rats. A total of 30 Wistar rats were randomly divided into 6 groups of 5 rats as follows: Normal control. Diabetic control: high fat diet, 35 mg/kg streptozotocin + distilled water. Reference control drug: high fat diet, 35 mg/kg streptozotocin + 500 mg/kg metformin. Aqueous extract 125 mg/kg: high fat diet, 35 mg/kg streptozotocin + 125 mg/kg aqueous extract. Aqueous extract 250 mg/kg: high fat diet, 35 mg/kg streptozotocin + 250 mg/kg aqueous extract. Aqueous extract 500 mg/kg: high fat diet, 35 mg/kg streptozotocin + 500 mg/kg aqueous extract. The body weights of animals in the diabetic control, significantly increased during the treatment as compared to the normal control $p < 0.001$ while groups administered different extract doses revealed an increase in body weight compared to the diabetic control $p < 0.05$. Administration of aqueous extract 125, 250 and 500 mg/kg significantly reduced elevated glucose levels as compared to the diabetic control $p < 0.01$. The group administered with 125 mg/kg increase creatinine levels and 250 mg/kg aqueous extract is remaining comparable to those of the diabetic control with urea concentrations. The diabetic control revealed significantly elevated levels of cytokines (TNF- α , IL-1 β and IL-10) as compared with the normal control group $p < 0.01$, despite the fact that, based on biological interpretation, IL-10 is not a pro-inflammatory marker. The

aqueous extract 125, 250 and 500 mg/kg significantly reduces the blood levels of cytokines: TNF- α , IL-1 β and modulates IL-10 with increasing dosage of extract as compared to diabetic control $p < 0.01$. IL-10 levels can vary over time. An increase may occur during the early phase of inflammation regulation, followed by a decrease once the inflammation has resolved. From these results, it is evident that aqueous extracts of *V. ampla* have hypoglycemic properties, immunomodulatory effects, and renal effects that appear to be dose-dependent.

Keywords

Diabetes, Hypoglycemia, Immune System, Streptozotocine, *Vernonia ampla*

1. Introduction

Diabetes mellitus is a metabolic syndrome of multiple etiologies characterized by chronic hyperglycaemia with disturbances of carbohydrates, fats and proteins metabolisms resulting from defects in insulin secretion by pancreatic beta cells and insulin action or both [1]. Chronic hyperglycemia inevitably leads to long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, reproductive organs, heart and blood vessels [2]. Diabetes represents one of the world's four major non communicable disease such as Cardiovascular diseases, cancers and chronic respiratory diseases that are of great public health concern in the healthcare system. It accounts for about 1.6 million deaths each year globally [3]. In 2015 in Nigeria alone, 1.56 million cases were reported including 105,091 deaths and about 95% of all cases reported were attributed to Type-2 diabetes mellitus [4]. In spite of advancements recorded to date on basic and clinical investigations into diabetes, a properly effective final remedy does not exist.

Diabetes is a serious, chronic disease that occurs either when the pancreas does not produce enough insulin (a hormone that regulates blood sugar, or glucose), or when the body cannot effectively use the insulin it produces [5]. Diabetes is of two main types: type 1 also called insulin dependent diabetes occurs when the pancreas is unable to produce enough insulin to metabolize the glucose circulating in the blood. The individual is dependent on continuous administration of insulin and hence the name insulin dependent diabetes. Type 2 also called insulin independent diabetes occurs when the pancreas produces enough insulin unfortunately, the body is unable to use this insulin to metabolize glucose, or when the body's cells produce no effect in the presence of insulin. The above situations result in high glucose levels in the blood, a condition known as hyperglycemia [6]. Type 2 diabetes is the most common form of diabetes, accounting for more than 90% of all diabetes cases worldwide. Type 2 diabetes used to occur nearly entirely among adults, but now occurs in children too. In type 2 diabetes, hyper glycaemia is the result, initially, of the inability of the body's cells to respond fully to insulin,

a condition termed insulin resistance. With the onset of insulin resistance, the hormone is no longer effective and, in the long term, causes an overproduction of insulin [6]. Over time, inadequate production of insulin can develop as a result of failure of the pancreatic beta cells to keep up with demand. Type 2 diabetes may have symptoms similar to those of type 1 diabetes but, in general, symptoms are much less dramatic and the condition may be completely symptomless [7]. Also, the exact time of the onset of type 2 diabetes is usually impossible to determine. As a result, there is often a long pre-diagnostic period and as many as one-third to one-half of people with type 2 diabetes in the population may be undiagnosed. If diagnosis is delayed for a long time, complications such as vision problems, slow-healing lower limb injuries, heart disease, or stroke may lead to diagnosis. In the chronic stage, diabetes results in chronic inflammation and renal failure [8]. Type 2 diabetes is a progressive disease over time, characterized by a decrease in insulin secretion over the long term. Introduction of oral hypoglycemic agents (OHA) will often be necessary in patients treated with diet and physical activity only, and further intensification with insulin might be needed as the illness progresses and OHAs are not sufficient to control glycaemia [9]. Poor control of diabetes can lead to damage of the kidney, which is a condition known as diabetic nephropathy.

Researchers have shown that cytokines such as IL-1 β , tumor necrosis factor alpha (TNF- α) and interferon gamma (IFN- γ) are cytotoxic to beta cells in vitro [10] in addition TNF and IFN- γ stimulate HLA antigen expression and may therefore facilitate the recognition of beta cell antigens by cells of the immune system; TNF and IFN- γ also induce the synthesis of intracellular adhesion molecules by beta cells [11], while IL-1 and IL-6 are capable of regulating insulin secretion and thus modulating the expression of antigens associated with the secretory capacity of beta cells [12].

Many medications have been discovered and are used against diabetes. Some of these drugs manifest different side effects and due to their cost, they are not easily accessible to all people. For this reason, research is still currently going on to provide drugs which are more cost effective and more accessible to the population [13]. A large number of plant extracts have been studied for their hypoglycemic and/or sugar-reducing properties; most of these extracts have shown therapeutic effects. Despite the many studies already carried out, there still remain some plants which are currently being used to treat diabetes traditionally but the mechanism has not yet been tested scientifically. Some medicinal plants such as *Vernonia amygdalina* have been tested for antidiabetic effects and the results show positive.

Vernonia ampla, a medicinal plant belonging to the same family, has been used for its antifungal effects, but little and almost nothing has been done regarding diabetes. Gas chromatography-mass spectrometry analyses of extracts of roots, stem bark and leaves of *Vernonia ampla*, as well as the study of their phytochemical properties, revealed the presence of twenty-six peaks on the chroma-

togram [14]. Fifteen chemical compounds were identified in the root including: 2,4-Hexadiene, 2,3-dimethyl-, Octadecane, 1,2 benzenedicarboxylic acid, butyl,2-ethylhexyl ester, Dichloroacetic acid, heptadecyl ester, 1H-Naphtho [(2,1-b) pyran, 3-ethenyl]do decahydro-3,4a,7,7,10a-pentamethyl-, [3S-(3.alpha.,4a.alpha.,6a.beta.,10a.alpha.,10b.beta.)], Kaur-16-ene, Cis-13-octadecenoic acid, methyl ester, Trans-13-octadecenoic acid, Cis-vaccenic acid, Cis-13-octadecenoic acid, Oleic acid and 9-Octadecenoic acid. Six were found in the stem bark including: Caffeine, Hexadecanoic acid, methyl ester, 2-Cyclopenten-1-one, 2,3-dimethyl and 9,12-Octadecadienoic acid. Five were identified in the leaf of the plant including Pentadecanoic acid, Hexadecylpentyl ether and Methyl stearate. The principal phyto-constituents of the plant are oxalate, phytates, tannins, saponins, flavonoids, cyanogenic glycosides [15], alkaloids, terpenes, anthraquinone, steroid, coumarins, lignans, xanthonenes, edotides and sesquiterpenes and phenol. The present study aimed to determine the hypoglycemic and immunomodulatory effects of aqueous leaf extract of *Vernonia ampla* on streptozotocin-induced diabetes in Wistar rats.

2. Materials and Methods

2.1. Plant

2.1.1. Plant Collection

Freshly harvested leaves of *Vernonia ampla* were identified by local residents. The information gathered included the vernacular name (wild bitter leaf), parts used (root, leaves and stem bark), quantity used (7 to 10 leaves in a cup of water), and the ailments treated (anorexia; ascariis infestation, typhoid, breast cancer and promoting the uptake and utilization of glucose by muscle and liver cells of the human body). The leaves of *V. ampla* were collected in the Bamenda III subdivision, Mezam division, North West region, Cameroon, area called mile 4 Nkwen (6° 14' 5" North and 9° 25' 57" East) in September 2023, between 5 pm and 6 pm. The plant was harvested at sunset because heat from the sun causes some ingredients such as flavanoids to dissociate and reform in the absence of the heat; therefore early hours or late hours are preferable for harvesting the plant [16]. The plant was carried to the University of Bamenda. The plant samples were provided to an acknowledged taxonomist for botanical authentication. A sample was carried to the national Herbarium Yaounde-Cameroon, where it was further confirmed (*Vernonia ampla*: D 750 specimen no 41863 SRFCam). Leaves of the plant were harvested and washed using tap water and air dried away from the sun [16]. The dried leaves were then ground into fine homogenous powder using electrical mill followed by sieving through mesh sieve and stored at room temperature waiting extraction.

2.1.2. Extraction of Aqueous Extract of *Vernonia ampla* Leaves

The aqueous extract of dried plant leaves was made by macerating in 1.8 liter of distilled water 180 grams of leaf powder (initial weight) for seventy-two (72) hours,

then filtered using Wattman's No1 filter paper [17]. The obtained filtrate was evaporated at 40°C to dryness for 72 hours in a thermostat oven (DHG-9101-15APEC) in biochemistry laboratory, University of Bamenda. A dark brown solid extract was obtained, weighted and recorded (48 g), the extract yield calculated. It was stored in tight-labelled bottle in a refrigerator at 4°C until further use.

$$\text{Extract yield} = \frac{\text{final weight}}{\text{initial weight}} \times 100 \text{ therefore } \frac{48 \text{ g}}{180 \text{ g}} \times 100 = 26.67\%$$

Based on information provided by local herbalists, doses of 125, 250 and 500 mg/kg body weight (bw) were selected as follow: Herbalists macerate approximately 10 leaves (25 g) of *Vernonia ampla* in 350 mL of drinking water and consume this daily to treat diabetes. They consume two cups of that liquid solution for a person weighing approximately 70 kg. Therefore, a maceration of 25 g of powdered *Vernonia ampla* leaves in 350 mL of distilled water was carried out for three hours, stirring every 30 minutes [17]. After filtration and evaporation, 1.143 g of extract was obtained. This mass of extract is assumed to be consumed by an adult of 70 kg bodyweight. Many authors explained the formula for determining the therapeutic dose in humans [18]. Researchers have developed a formula for the relationship between therapeutic doses in humans and therapeutic doses in animal species [19]. These equations are as follows:

$$\text{HTD}(\text{mg/kg}) = \frac{\text{Weight of the extract consumed}}{\text{Bodt weight}}$$

where: HTD = Human therapeutic dose

$$\text{RED}(\text{mg/kg}) = \text{HTD} \times \frac{\text{Human Km}}{\text{Rat Km}}$$

where: RED = Rat equivalent dose, Km = Species standard factor with Human Km = 37 and Rat Km = 6.

Therefore: HTD = 1.43 g/70 kg = 20.42 mg/kg and RED = 20.42 × 37/6 = 125 mg/kg.

After obtaining this RED, we multiplied the value obtained each time by 2 to find the doses of 125, 250 and 500 mg/kg.

2.2. Animals

2.2.1. Ethical Approval

Animals were handled in accordance with the ethical guidelines of the Cameroon National Veterinary Laboratory as referenced by the approval and head central on the 10/01/2023 No 004/23CCS/MINPIA/RD-NW/DD-MELSSV.

2.2.2. Experimental Animals

Thirty health female of Wistar albino rats (*Rattus rattus*), at the age of 5 to 7 weeks (90 to 120 g) were obtained from a breeder in Mile 4 Nkwen Bamenda-Cameroon and were taken to the animal house of the Faculty of Science, University of Bamenda. They were kept for two weeks before initiation of the experiments because transportation of laboratory rodents unavoidably causes stress. Researchers must pro-

vide a sufficient acclimatization period to allow for the (re-)stabilization of parameters [20]. The animals were caged in six (6) plastic cages of dimension (45.5 cm of diameter and 22.5 cm of high). The rats were maintained at ambient temperature in 12 hrs light and dark cycle. They were fed water and standard diet (30% weed brown, 30% soya beans, and 40% maize) and to ensure that the new substances were completely administered to the rats and the desired dose was in their bodies food was withdrawn 18 hours before initiation of experiments.

2.2.3. The Statistical Model for Repeated Body-Weight Measurement

Body weight was measured repeatedly in the same rats at weeks 1, 2, 3, and 4. Therefore, body weight data were analysed using a repeated-measures ANOVA, with time (weeks 1 - 4) as the within-subject factor and treatment group as the between-subject factor. The group $\times$ time interaction was included to determine whether changes in body weight over time differed among treatment groups. Baseline differences between groups were also considered in the analysis. The assumption of sphericity was assessed using Mauchly's test; when this assumption was violated, the Greenhouse-Geisser correction was applied.

2.2.4. Diabetes Induction

The animals were divided into 6 groups of 5 animals each [21]. Groups 2 to 6 were administered a high fat diet for a period of 4 weeks to induce obesity and insulin resistance [22]. Diabetes was confirmed 24 hours after streptozotocin (STZ) injection. Fasting blood glucose was measured using a glucometer (BGMs), after a 12-hour fast with free access to water. A blood glucose level > 200 mg/dL was considered indicative of hyperglycemia. After administration of the high fat diet, the rats (group 2 - 6) were administered 35 mg/kg body weight of streptozotocin (STZ) intraperitoneally to further induce type 2 diabetes by destruction of pancreatic beta cells. The high fat diet was composed of 25% margarine, 25% ground nut meal, 15% ground nut oil with 35% of the animal feed or standard diet (corn flour, soya beans, corn and weed brown) [22].

2.2.5. Animal Treatment

The rats that did not develop the expected hyperglycemia were excluded prior to assignment to treatment groups. Thirty female rats were fed with standard diet. The animals were treated as follows [21]: Normal control, received distilled water (5 mL/kg) and standard diet (corn flour, soya beans, and wheat brown). STZ-induced diabetes was established over seven consecutive days, while the induction of obesity was continued. Diabetic control, received high fat diet and STZ (35 mg/kg) and distilled water (5 mL/kg). Reference control drug, received high fat diet and STZ (35 mg/kg) and metformin (500 mg/kg). AE 125 mg/kg, received high fat diet and STZ (35 mg/kg) and aqueous extract (125 mg/kg). AE 250 mg/kg, received high fat diet and STZ (35 mg/kg) and aqueous extract (250 mg/kg). AE 500 mg/kg, received high fat diet and STZ (35 mg/kg) and aqueous extract (500 mg/kg).

2.2.6. Sample Collection

At the end of the experiment, the animals were anesthetized and blood was collected by jugular vein puncture [23]. The blood was collected in assay tubes without anti-coagulant. The samples were centrifuge at 3000 rpm for 20 minutes. After centrifuging, the serum was collected into Eppendorf tubes for storage. The serum samples were stored at a temperature of -20°C for subsequent analysis.

2.3. The Statistical Model for Repeated Glucose Measurement in Diabetic Period

Blood glucose levels were measured repeatedly in the same rats on days 1, 2, 3, 4, 5, 6, and 7. Therefore, glucose data were analyzed using a repeated-measures ANOVA, with time (days 1 - 7) as the within-subject factor and treatment group as the between-subject factor. The group $\times$ time interaction was included to determine whether changes in blood glucose levels over time differed among treatment groups. Baseline glucose values were considered to account for initial differences between groups.

2.4. Analysis

2.4.1. Serum Urea Analysis

Principle: Endpoint colorimetry using the Dialab kit allowed the measurement of serum urea [24]. Urease enzyme hydrolysis urea to ammonia and carbon dioxide. The NH_3 so formed reacts with alkaline hypochloride and sodium salicylate in the presence of sodium nitroprusside to form a coloured chromophore.

Procedure: Serum samples (for urea) were analyzed using a biochemistry machine Randox Monaco after samples were centrifuged for 3 minutes at 3000 rpm using a centrifuge and 150 - 200 μl serum was separated using a micropipette into sample cuvettes. A software program in which all identification parameters are entered is connected to a spectrophotometer. When the identification number has been entered, the machine is opened and the filled sample cuvette is inserted into the respective sample path of the disk of the machine. The machine is covered and the start button is pressed which runs for 8 minutes. The machine can process multiple samples simultaneously. The Randox Monaco machine uses the principle of spectrophotometry based on Beer Lambert's Law which states that "The intensity of the light absorbed is directly proportional to the concentration of the analyses present in the solution". The machine measures the intensity of light at specific wavelengths between 340 - 800 nm.

2.4.2. Serum Creatinine Analysis

Principle: The assay is based on the reaction of creatinine with an alkaline solution of sodium picrate to form a red complex [25]. The red colour is proportional to the concentration of creatinine in the sample. The intensity of the colour which is proportional to the concentration of creatinine is assessed through photometry and results recorded.

Procedure: Serum samples (for creatinine) were analyzed using the same pro-

cedure as above for urea.

2.4.3. The Insulin Tolerance Test

A glucose and insulin tolerance test was performed after treating the rats with the aqueous extract. This was to assess the effect of the aqueous extract in improving the sensitivity of the animals to insulin action.

Recombinant human insulin was used, and the dose was 0.75 IU/kg. The fasting duration was 5 hours, with free access to water during the fast. Baseline blood glucose (T_0) was measured prior to insulin injection, and subsequent measurements were taken at 15, 30, and 45 minutes post-injection. A rat with blood glucose > 200 mg/dL is considered diabetic. Insulin sensitivity was measured by glucose disappearance rate within 15 minutes. The insulin tolerance test (ITT) value was calculated using these four data points, following the method developed and described by other researchers [26]. The Calculation of glucose disappearance rate (KITT). The approach used consists of calculating the slope of the logarithmic regression of blood glucose levels over time. The following formulas were used:

$$b = \frac{n \sum (X \times \ln(Y)) - (\sum X)(\sum \ln(Y))}{n(\sum x^2) - (\sum x)^2}$$

where X = Time (min); Y = Glucose (mg/dL); $\ln$ = Natural logarithm; b = Slope; Σ = Sum; n = 4.

$$\text{KITT} = (-b) \times (-100)$$

where b = slope; KITT = blood glucose disappearance rate (%/min).

$$T_{1/2} = \ln k / -b$$

where k = substance depletion constant (0.693); b = slope; $T_{1/2}$ = half-life.

2.4.4. Assessment of the Anti-Inflammatory Properties

Specific ELISA kits (cantine) allowed the measurement of interleukin 1 beta (IL-1 β), tumor necrosis factor alpha (TNF- α) and interleukin 10 (IL-10). The ELISA sandwich was used and the experiment was carried out in the laboratory of biological sciences of the University of Buea. Briefly, the plates were sensitized by putting 20 μ L of a specific antibody and incubated for 18 hrs at a -5°C . After the incubation, 20 μ L of serum samples were added into various wells. After 10 minutes, the wells were washed and emptied. After washing and drying, 20 μ L of the conjugate antibody was added into each well and after 2 hrs at room temperature, 100 μ L of substrate was then added into the each well and the reaction was stopped after 30 mins by adding 20 μ L of the stop solution and the results read using an ELISA machine at a wavelength of 450 nm.

2.5. Histological Studies

To study the kidney sections under microscope, 10% formalin was used for the fixation of collected kidney samples [27]. Later, samples were passed via many proceedings of fixation, sectioning and stain. In fixation, kidney samples of all ex-

perimental groups were kept in 10% neutral formalin. After fixation, tissues have been dehydrated in different percentages of alcohol (75%, 95% and 100% absolute), embedded in paraffin block and serially sectioned (5 μ m size) using a microtome. Kidney sections were stained with Mayer hematoxylin and eosin. streptozotocin induced diabetes was observed using microscope (Zeiss, Hallherbermoos, Germany).

2.6. Statistical Analysis

All data were presented as mean $\pm$ S.E.M of five rats ($n = 5$). Statistical Software; SPSS version 20, was used to assess the effect of the aqueous extract on the body weight and glucose levels. Difference between means was assessed by one-way and two-way Analysis of Variance (ANOVA), followed by Bonferroni post-test using Graph pad prism version 8.0.1.244 to analyze the effects of the aqueous extract on urea, creatinine and cytokine concentrations in the blood. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of the Aqueous Extract of *V. ampla* Leaves on Body Weight (g) in High Fat Diet and STZ-Induced Diabetic Rats

The body weight of rats significantly increased with time from week 1 all through the treatment across all the groups ($p < 0.01$). The body weight in the normal control showed a significant increase at week 4 (91.80 to 126.20 g) as compared with the diabetic control ($p < 0.01$). The body weights in the groups treated with aqueous extract 125, 250 and 500 mg/kg showed a significant increase (158.60, 143.60, and 144.00 g respectively; $p < 0.01$) compared to the diabetic control (118.20 g) at week 4 (Table 1).

Table 1. Effect of the aqueous extract of *V. ampla* leaves on body weight (g) in high fat diet and STZ-induced diabetic rats.

Treatment	Duration of treatment (weeks)				F _(3;15)
	1	2	3	4	
Normal control	91.80 $\pm$ 1.39 ^B	107.80 $\pm$ 1.36 ^B	122.40 $\pm$ 1.66 ^B	126.20 $\pm$ 1.93 ^B	88.96***
Diabetic control	114.20 $\pm$ 2.08 ^a	118.00 $\pm$ 3.08 ^a	117.40 $\pm$ 2.32 ^a	118.20 $\pm$ 1.86 ^a	25.83ns
Reference control drug	118.20 $\pm$ 1.16 ^c	138.40 $\pm$ 3.30 ^c	149.60 $\pm$ 2.77 ^c	153.40 $\pm$ 2.96 ^c	21.68***
AE 125 mg/kg.	125.20 $\pm$ 13.34 ^A	130.40 $\pm$ 5.30 ^c	152.00 $\pm$ 13.30 ^{Ac}	158.60 $\pm$ 13.86 ^c	12.36***
AE 250 mg/kg.	111.80 $\pm$ 2.61 ^{ca}	122.00 $\pm$ 4.76 ^{Aa}	138.20 $\pm$ 2.96 ^d	143.60 $\pm$ 5.19 ^d	11.35***
AE 500 mg/kg.	112.20 $\pm$ 1.16 ^{ca}	126.60 $\pm$ 2.84 ^{Aa}	135.80 $\pm$ 2.96 ^d	144.00 $\pm$ 4.01 ^d	34.89***
F _(3;15)	3.88*	7.84***	3.14*	3.12*	

The Values are expressed as mean $\pm$ SEM of 5 rats per group. The capital letters of the alphabet represent the change within the group while small letters represent changes across the groups. Same letters indicate non-significant difference and different letters indicate significant difference across groups. *represent significant change with time across the groups ($p < 0.05$). ***represents very highly significant change with time across the groups ($p < 0.001$). AE = Aqueous extract; F = point on table F that we can find the corresponding F value.

3.2. Effect of the Aqueous Extract of *V. ampla* Leaves on Glucose Levels (mg/dL) in High Fat Diet and STZ-Induced Diabetic Rats

The results indicated a very significant rise (228.14 to 264.60 mg/dL) in the glucose of the diabetic control compared to the normal control at $p < 0.01$. The groups treated with the various concentrations of the aqueous extract (125 mg/kg, 250 mg/kg and 500 mg/kg) have a very significant reduction (78.40, 81.60, and 84.40 mg/dL respectively) in the blood glucose levels ($p < 0.01$) compared with the diabetic control (264.60 mg/dL). The administration of Metformin in reference control drug and the highest concentration of 500 mg/kg aqueous extract shown similar significant ($p < 0.01$) reduction when compared with the diabetic control (Table 2).

Table 2. Effect of the aqueous extract of *V. ampla* leaves on glucose levels (mg/dL) in high fat diet and STZ-induced diabetic rats.

Treatment	After fat diet	One week after STZ	One week of treatment	After three weeks of treatment	Before sacrifice	F _(6,30)
	Duration of treatment (weeks)					
	0	1	2	3	4	
Normal control	101.40 ± 4.98 ^B	100.12 ± 2.13 ^B	96.40 ± 1.50 ^B	99.60 ± 21.12 ^B	88.80 ± 2.65 ^B	0.88 ^{ns}
Diabetic control	99.40 ± 3.04 ^B	228.14 ± 1.6 ^a	242.20 ± 5.95 ^a	266.60 ± 4.47 ^A	264.60 ± 2.44 ^a	16.82 ^{***}
Reference control drug	95.00 ± 3.2 ^a	132.21 ± 1.3 ^c	118.60 ± 4.68 ^c	90.60 ± 2.40 ^c	80.60 ± 0.74 ^c	16.26 ^{***}
AE 125 mg/kg	93.40 ± 3.56 ^a	129.47 ± 3.4 ^a	119.60 ± 3.83 ^c	94.20 ± 10.01 ^b	78.40 ± 3.63 ^A	11.98 ^{***}
AE 250 mg/kg	97.20 ± 1.59 ^{Ba}	134.85 ± 1.62 ^{ac}	121.80 ± 1.99 ^A	87.60 ± 1.29 ^a	81.60 ± 2.78 ^c	89.57 ^{***}
AE 500 mg/kg	84.20 ± 0.86 ^b	131.23 ± 2.63 ^{ac}	119.80 ± 1.59 ^{Ac}	93.40 ± 0.51 ^b	84.40 ± 1.21 ^c	121.74 ^{***}
F _(6,30)	3.67*	2.89**	6.99***	2.76*	54.01***	

The Values are expressed as mean ± SEM of 5 rats per group. The capital letters of the alphabet represent the change within the group while small letters represent changes across the groups. Same letters indicate non-significant difference and different letters indicate significant difference across groups. *represent significant change with time across the groups ($p < 0.05$). **represent highly significant change with time across the groups ($p < 0.01$). ***represent very highly significant change with time across the groups ($p < 0.001$). STZ = Streptozotocin; AE = Aqueous extract; F = point on table F that we can find the corresponding F value.

3.3. Effect of the Aqueous Extract of *V. ampla* Leaves on the Variations of Glucose (mg/dL) Uptake after Administration of Insulin Following High Fat Diet and STZ-Induced Diabetic Rats

The diabetic control group showed the highest half life and lowest glucose disappearance rate (KITT) after administration of insulin ($p < 0.001$) as compared to the normal control. The administration of *Vernonia ampla* leave extract (125 mg/kg, 250 mg/kg and 500 mg/kg) significantly half life and increase the KITT after administration of insulin, ($p < 0.001$) compared to diabetic control (Table 3). The

rats treated with Metformin (35.40 mg/dL) in reference control drug and the highest dose of the plant extract (500 mg/kg) showed a significant increase of KITT (1.01 and 2.12%/min) upon administration of insulin ($p < 0.001$) as compared to the diabetic control (0.02%/min).

Table 3. Effect of the aqueous extract of *V. ampla* leaves on the variations of glucose (mg/dL) uptake after administration of insulin following high fat diet and STZ-induced diabetic rats.

Treatment	0 min	15 mins	30 mins	45 mins	KITT %/min	T _{1/2} (min)	F _(3;15)
Normal control	170.60 ± 3.63 ^{bc}	177.6 ± 2.21 ^c	160.40 ± 1.12 ^c	167.20 ± 0.93 ^B	-	-	92.13***
Diabetic control	235.5 ± 2.44 ^a	240.6 ± 1.69 ^a	242.4 ± 1.77 ^a	235.2 ± 2.02 ^a	0.02 ^b	796.33 ^b	71.41*
Reference control drug	201.80 ± 1.21 ^{bc}	212.00 ± 0.51 ^{Bb}	207.60 ± 0.51 ^b	217.20 ± 1.14 ^{Ab}	1.01 ^a	15.92 ^{Aa}	471.05**
AE 125 mg/kg	264.60 ± 0.74 ^c	235.40 ± 1.03 ^c	233.80 ± 0.98 ^c	228.60 ± 0.81 ^{Bc}	0.29 ^a	233.35 ^b	65.65***
AE 250 mg/kg	207.60 ± 2.78 ^c	200.20 ± 2.67 ^b	213.20 ± 2.48 ^b	211.60 ± 2.23 ^b	0.32 ^a	49.77 ^c	28.21***
AE 500 mg/kg	245.5 ± 2.65 ^B	250.6 ± 2.86 ^B	242.4 ± 1.81 ^B	255.2 ± 1.80 ^B	2.12 ^{Bc}	7.58 ^B	439.19***
F (3;15)	54.01**	81.29***	138.80***	154.25***	158.25***	164.25***	

The Values are expressed as mean ± SEM of 5 rats per group. The capital letters of the alphabet represent the change within the group while small letters represent changes across the groups. Same letters indicate non-significant difference and different letters indicate significant difference across groups. *represent significant change with time across the groups ($p < 0.05$). **represent highly significant change with time across the groups ($p < 0.01$). ***represent very highly significant change with time across the groups ($p < 0.001$). STZ = Streptozotocin; AE = Aqueous extract; KITT = glucose disappearance rate; T_{1/2} = Half life; F = point on table F that we can find the corresponding F value.

3.4. Effect of the Aqueous Extract of *V. ampla* Leaves on Serum Urea Concentration of High Fat Diet and STZ-Induced Diabetic Rats

The urea concentration in the diabetic control significantly increased as compared to that of the normal control ($p < 0.001$). Administration of the 250 mg/kg of the aqueous extract significantly increased the urea concentrations compared to the normal control group ($p < 0.001$). The same group of 250 mg/kg of the aqueous extract has no significant difference compared to the diabetic control (**Figure 1**). The reference control drug (metformin) showed a significant difference in urea concentrations as compared to diabetic control ($p < 0.001$).

3.5. Effect of the Aqueous Extract of *V. ampla* Leaves on Serum Creatinine Levels in High Fat Diet and STZ-Induced Diabetic Rats

There is a significant difference between normal control and the diabetic control groups ($p < 0.001$). It is noticed that treatment with 125 mg/kg of extract significantly increased serum creatinine levels compared to the diabetic control ($p < 0.001$). The aqueous extract (250 mg/kg and 500 mg/kg) showed a significant ($p < 0.001$) difference when compared with the diabetic control (**Figure 2**).

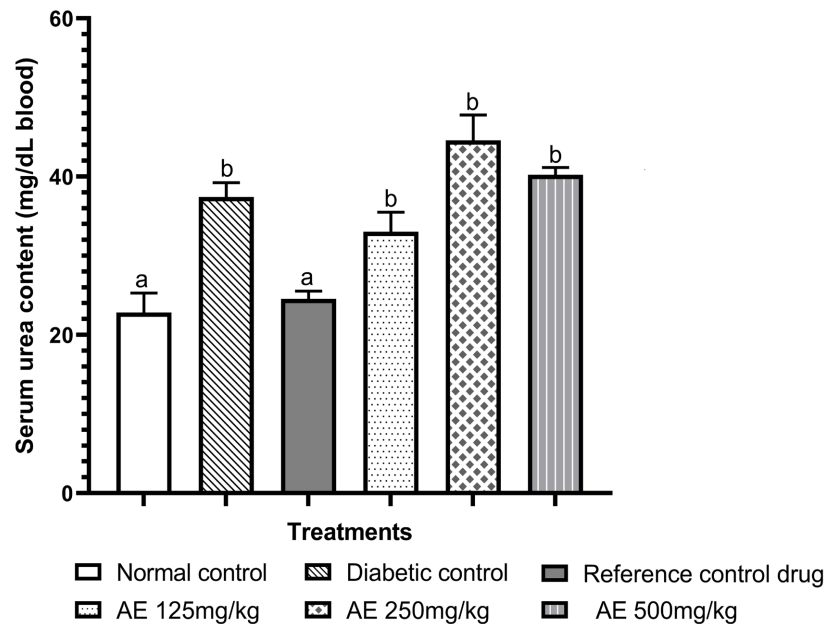


Figure 1. Effect of the aqueous extract of *V. ampla* leaves on serum urea concentration of high fat diet and STZ-induced diabetic rats. The histograms represent means $\pm$ SEM of 5 rats per group. The small letters represent changes across the groups. Same letters indicate non-significant difference and different letters indicate significant difference across groups. STZ = Streptozotocin; AE = Aqueous extract.

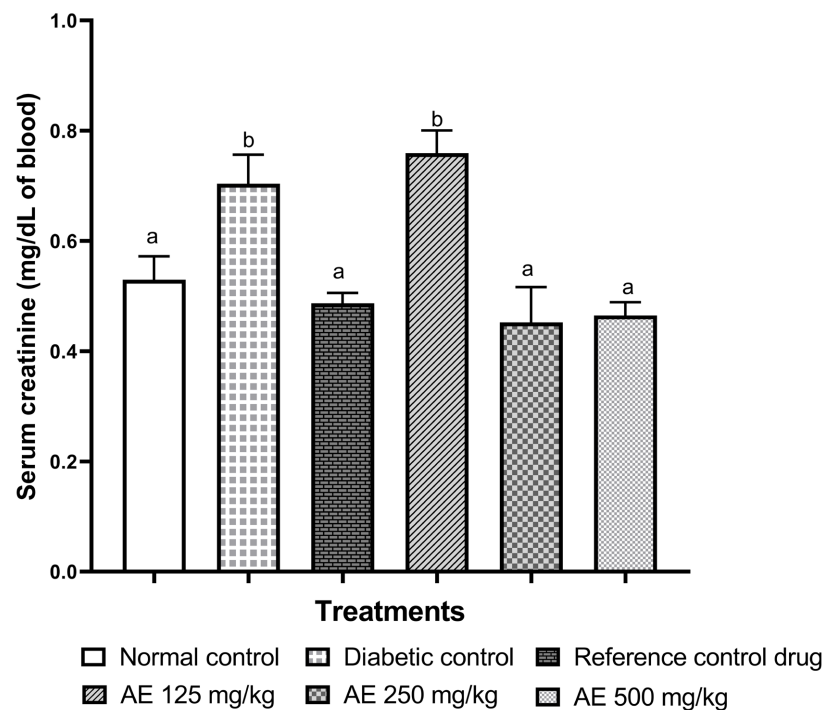


Figure 2. Effect of the aqueous extract of *V. ampla* leaves on serum creatinine levels in high fat diet and STZ-induced diabetic rats. The histograms represent means $\pm$ SEM of 5 rats per group. The small letters represent changes across the groups. Same letters indicate non-significant difference and different letters indicate significant difference across groups. STZ = Streptozotocin; AE = Aqueous extract.

3.6. Effect of the Aqueous Extract of *V. ampla* Leaves on the Concentration of Tumour Necrosis Factor-Alpha (TNF- α) in the Serum of High Fat Diet and STZ-Induced Diabetic Rats

The diabetic control presented a significantly high concentration of TNF- α compared to the normal control ($p < 0.01$). Reference control drug and treatment with 500 mg/kg of aqueous extract showed similar concentration of TNF- α compared to the normal control. The aqueous extract (125 mg/kg, 250 mg/kg and 500 mg/kg) significantly ($p < 0.01$) decreased concentrations of TNF- α when compared to the diabetic control (Figure 3).

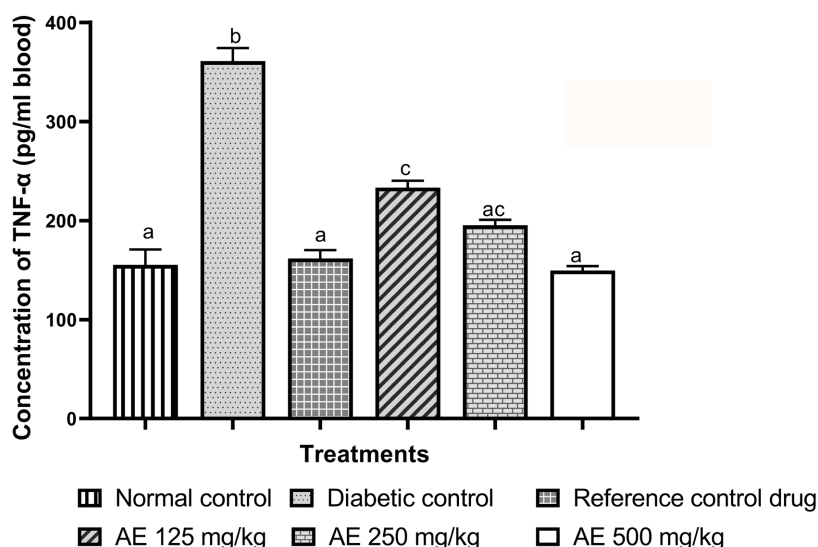


Figure 3. Effect of the aqueous extract of *V. ampla* leaves on the concentration of tumour necrosis factor-alpha (TNF- α) in the serum of high fat diet and STZ-induced diabetic rats. The histograms represent means $\pm$ SEM of 5 rats per group. The small letters represent changes across the groups. Same letters indicate non-significant difference and different letters indicate significant difference across groups. STZ = Streptozotocin; AE = Aqueous extract.

3.7. Effect of the Aqueous Extract of *V. ampla* Leaves on the Concentration of Interleukin 1 Beta (IL-1 β) in the Serum of High Fat Diet and STZ-Induced Diabetic Rats

The results showed a decrease in the concentration of IL-1 β in a dose dependent manner in the groups receiving the aqueous extract 125, 250 and 500 mg/kg compared to the diabetic control ($p < 0.01$).

The results revealed a non-significant difference in concentrations of IL-1 β between the normal control and the reference control drug, and the group receiving the highest dose (500 mg/kg) of the extract (Figure 4).

3.8. Effect of the Aqueous Extract of *V. ampla* Leaves on the Concentration of Interleukin 10 (IL-10) in the Serum of High Fat Diet and STZ-Induced Diabetic Rats

The results showed no significant difference in IL 10 concentrations in the normal

control compared to the reference control drug. The diabetic control shown a significantly increased of IL-10 ($p < 0.01$) when compared to the normal control. All diabetic groups treated with the aqueous extracts 125, 250 and 500 mg/kg showed a significant decrease in blood concentrations of IL 10 when compared to the diabetic control group ($p < 0.01$). There is no significant difference between the concentrations of IL 10 in the reference control drug compared to group treated with 500 mg/kg of aqueous extract (**Figure 5**).

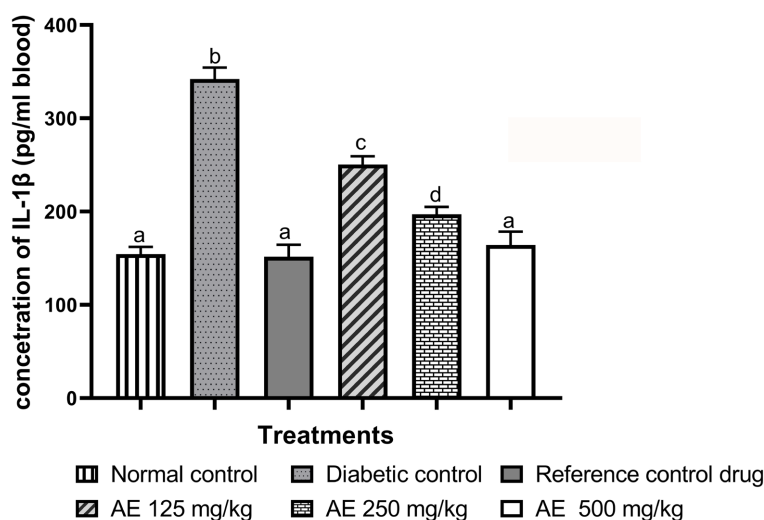


Figure 4. Effect of the aqueous extract of *V. ampla* leaves on the concentration of interleukin 1 beta (IL-1 β) in the serum of high fat diet and STZ-induced diabetic rats. The histograms represent means $\pm$ SEM of 5 rats per group. The small letters represent changes across the groups. Same letters indicate non-significant difference and different letters indicate significant difference across groups. STZ = Streptozotocin; AE = Aqueous extract.

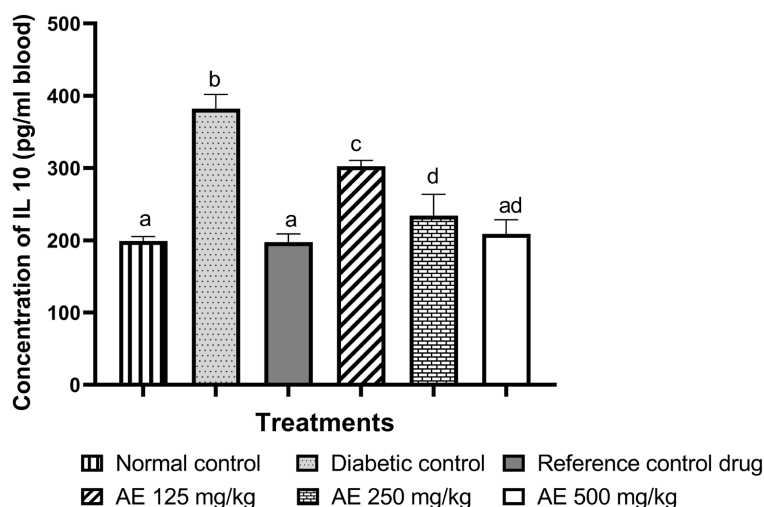


Figure 5. Effect of the aqueous extract of *V. ampla* leaves on the concentration of interleukin 10 (IL-10) in the serum of high fat diet and STZ-induced diabetic rats. The histograms represent means $\pm$ SEM of 5 rats per group. The small letters represent changes across the groups. Same letters indicate non-significant difference and different letters indicate significant difference across groups. STZ = Streptozotocin; AE = Aqueous extract.

3.9. Effect of the Aqueous Extract of *Vernonia ampla* on Histology of High Fat Diet and STZ-Induced Diabetic Rats

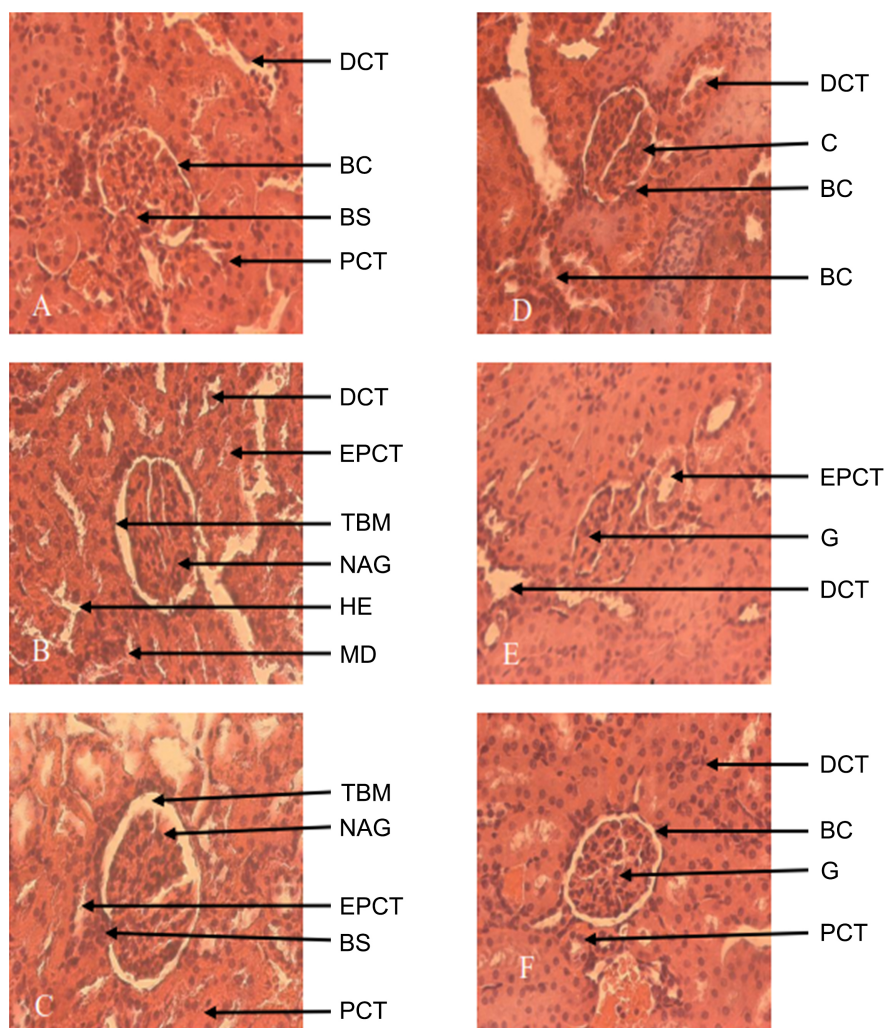


Figure 6. Histological sections of rat's kidneys using (H&E $\times$ 400) following diabetic treatment with *Vernonia ampla* extract. Normal control (**Figure 6(A)**), Diabetic control (**Figure 6(B)**), Reference control drug (**Figure 6(C)**), AE 125 mg/kg (**Figure 6(D)**), AE 250 mg/kg (**Figure 6(E)**), AE 500 mg/kg (**Figure 6(F)**). DCT = Distal convoluted tubule; BC = Bowman's capsule; BS = Bowman's space; G = Glomerulus; PCT = Proximal convoluted tubule; EPCT = Edematous proximal convoluted tubule; NAG = Necrotic area of the glomerulus; TBM = Thickening in the basement membrane. HE = Hypercellularity with edema; MD = Mucopolysaccharide deposits.

The kidney sections of normal control (**Figure 6(A)**) and control showed normal Bowman's space, Bowman's capsule and glomerulus. They showed normal proximal tubule and distal tubule. Whereas, diabetic control (**Figure 6(B)**) showed kidney alterations characterized by the thickening in the basement membrane, necrotic area of the glomerulus. They showed proximal convoluted tubule characterized by hypercellularity with edema, proximal convoluted tubule with Mucopolysaccharide deposits and edematous proximal convoluted tubule. The refer-

ence control drug (**Figure 6(C)**) still presented few abnormalities like necrotic area of the glomerulus and thickening in the basement membrane. The rats treated group with the dose of 250 mg/kg aqueous extract **Figure 6(D)** still presented abnormalities like edematous proximal convoluted tubule. Treatment with aqueous extract at the dose of 500 mg/kg normalized the kidney architectures, and showed an improvement in kidney structures similar to the normal control (**Figure 6(F)**).

4. Discussion

Diabetes mellitus, more simply called diabetes, is a chronic and serious disease that occurs when the level of glucose in the blood is far above the normal value because the pancreas does not produce enough insulin or the body does not use it effectively [28]. Some medicinal plants have been tested and confirmed to have some antidiabetic effects; for example *Vernonia amygdalina* [4] a plant of the same family with *Vernonia ampla*.

The results of this research reveal that there was a steady increase in the body weight of the rats through the treatment period in all the groups except in the diabetic control. Streptozotocin (STZ) is selectively accumulated in pancreatic beta cells via the low-affinity GLUT2 a transmembrane carrier protein that enables passive glucose movement across cell membranes [29]. The importance of the GLUT2 glucose transporter in this process is also shown by the observation that, streptozotocin damages other organs expressing this transporter such as muscles, integumentary organ, digestive organ, liver and kidney and reduce animal body weights. STZ-induced diabetes is associated with significant reduction in the body weight due to hyperglycemia, increased muscle wasting and loss of tissue proteins [29]. Treatment with the plant extract significantly increases body weight. *Vernonia ampla* improved the body weight significantly, suggesting that in the present of STZ, the aqueous extract increased the activity of GLUT2 and/or indicating the prevention of muscle tissue damage caused due to hyperglycemia. Although these mechanisms may offer plausible explanations for the observed effects, photochemical and molecular studies of the aqueous extract of *Vernonia ampla* are underway in our research unit.

The present research indicated a very significant rise in the glucose in the diabetic control compared to the normal control. Destruction of pancreatic beta cells result in the little or no secretion of the hormone insulin [30]. Insulin facilitates the uptake of glucose by animal cells, therefore, a decrease in the level of insulin results in the high blood glucose levels hence, diabetes [31]. The inability of cells to respond to the action of insulin or insensitivity of cells to the actions of insulin is a condition known as insulin resistance [31]. It should be noted that calculating the glucose disappearance rate (KITT) for the insulin tolerance test (ITT) allowed us to understand that a more pronounced drop in glucose levels following insulin administration indicates greater insulin sensitivity; this is because the KITT (%/min) reflects the rate at which blood glucose decreases after insulin administration. The

higher the KITT, the faster the glucose disappearance and the greater the insulin sensitivity. The half-life reflects the time interval during which half of the blood glucose disappears following insulin administration: the shorter the half-life the faster the glucose disappearance and the greater the insulin sensitivity [26]. Administration of aqueous leaf extract of *Vernonia ampla* to STZ-induced diabetic rats showed a significant reduction of the blood glucose levels a significant reduction of the half life and a significant increasing of (KITT). These results suggest that, *Vernonia ampla* promotes antidiabetic effects. Indeed, the plant's phenols inhibit α -amylase, thus increasing insulin levels and leading to a decrease in blood glucose levels [32]. These phenols stimulate pancreatic β cells, through the action on peroxisome proliferator-activated receptor gamma (PPAR γ) receptors and 5' AMP-activated protein kinase (AMPK) activation, stimulate insulin production [32]. Furthermore, the drop in the glucose levels in these animals suggests that the plant promotes regeneration of pancreatic beta cells and/or inhibits STZ action and reduces insulin resistance. The effects of aqueous leaf extract of *Vernonia amygdalina* on blood glucose levels in alloxan-induced diabetic rats indicated a decrease in blood glucose levels [4]. It is known that the principal phytoconstituents of *Vernonia ampla* are oxalate, phytates, tannins, saponins, flavonoids, cyanogenic glycosides, alkaloids [33]. Plant molecules like flavonoids are therapeutic opportunities for diabetic nephropathy [34]. These findings align with the results of researchers who tested ethanolic extracts of *Ocimum sanctum* leaves and demonstrated that the plant stimulates insulin secretion by the pancreas [35]. Further qualitative and quantitative phytochemical studies, as well as molecular biochemistry analyses, will be conducted as part of our future research within our unit to identify the extract's active constituents and determine the mechanisms responsible for its effects.

The results of this research indicated high creatinine and urea levels in the blood of untreated diabetic rats (diabetic control). Untreated or poorly treated diabetes results in chronic kidney diseases such as diabetic nephropathy. These diseases are manifested by excess creatinine and urea concentrations in the blood [34]. The importance of the GLUT2 glucose transporter in the process of facilitation of glucose transport is also shown by the observation that, streptozotocin damages other organs expressing this transporter, particularly kidney thus creatinine and urea are released in high quantity in bloodstream blood [34]. Administration of 500 mg/kg of aqueous extract showed reduced creatinine levels. From these results, it suggests that higher doses of the aqueous extract actively inhibit the adverse effect of STZ and/or stimulate GLUT2 and regenerate kidney cells.

Diabetes is a primary cause of inflammation in the diabetic patients [36]. Cytokines play as great rule in immune response but having them in excess in the blood can lead to cytokine associated toxicity [37]. The results of this research showed that animals of the diabetic control show a higher concentration of cytokines (TNF- α , IL-1 β , and IL-10) in the blood compared to the normal control. It should be clarified here that IL-10 levels can vary over time. An increase may occur dur-

ing the initial phase of inflammation regulation, followed by a decrease once the inflammation has resolved. It is known that diabetes mellitus involves elevated blood glucose levels; this increases the production of reactive oxygen species (ROS) in mitochondria, which stimulates Nuclear Factor-kappa B (NF- κ B) an activator of macrophages and other immune cells leading to the production of TNF- α , and IL-1 β and IL-10 in certain cases. IL-10 is part of a complex immune regulatory network. This could reflect a global modulation of the immune response, involving a reduction in both the pro-inflammatory component and the compensatory anti-inflammatory response in some organisms [38]. The administration of the aqueous extract significantly reduces the concentration of TNF- α and IL-1 β in dependent doses when compared to the diabetic control and IL-10 also. The observed reduction in IL-10 does not in itself indicate an anti-inflammatory effect; rather, it may reflect a reduced need for compensatory anti-inflammatory signaling following the attenuation of the inflammatory response. This implies the aqueous extract has anti-inflammatory properties especially at higher doses. It could play an immunomodulatory role in case of IL-10. Many researchers reported that, plant molecules like alkaloids have anti-inflammatory activity [39]. These hypotheses could explain the reduction of TNF- α and IL-1 β by the aqueous extract of *Vernonia ampla* leaves. The similar results were obtained on Anti-diabetic effect of aqueous extracts of *Vernonia amygdalina* and *Dacryodes edulis* leaves and their combination in alloxan-induced diabetic rats in which the plant extracts significantly reduced the concentration of cytokines in the diabetic Wistar rats [40].

Furthermore, histological studies (Figure 3) have shown that, Intraperitoneal administration of 35 mg/kg of streptozotocin (STZ) was used to induce type 2 diabetes through the destruction of pancreatic beta cells. The diabetes caused nephropathy, resulting in renal alterations characterized by thickening of the basement membrane, necrotic areas within the glomeruli, and hypercellularity associated with edema of the proximal convoluted tubules, as well as edematous proximal convoluted tubules containing mucopolysaccharide deposits [29]. Treatment with the aqueous extract at a dose of 500 mg/kg normalized renal architecture and showed an improvement in renal structures comparable to that of the normal control. These results suggest that the extract inhibited the effects of streptozotocin (STZ) and regenerated kidney cells. It is clearly established that, quercetin and curcumin are antioxidants and anti-inflammatory agents that inhibit NF- κ B and possess nephroprotective and pro-regenerative properties [41].

5. Conclusions

In summary, the findings showed significant hypoglycemic effects of the aqueous extract leaves of *Vernonia ampla*, and thus can support hyperglycemic management. This effect can be related to the protective ability of the aqueous extract to reduce fasting blood glucose levels and improve glucose uptake in diabetes.

The group administered with 125 mg/kg increase creatinine levels and 250 mg/kg aqueous extract is remaining comparable to those of the diabetic control

with urea concentrations. Renal effects appear to be dose-dependent. In anti-inflammatory effects, the aqueous extract significantly reduces blood levels of cytokines in diabetic Wistar rats which consequence from the inhibition of TNF- α , IL-1 β and of IL-10. IL-10 levels can vary over time. An increase may occur during the early phase of inflammation regulation, followed by a decrease once the inflammation has resolved. Histological studies showed that the highest dose of 500 mg/kg of *Vernonia ampla* extract restored kidney architecture, with clearly defined glomeruli and Bowman's capsule.

Author Contributions

Oumar Mahamat, Kiyang Rane Muluh, and Youmbie Djanche Duplex Bonheur conceived and designed the study. Kada Sanda Antoine, Kiyang Rane Muluh, Oumar Mahamat and Youmbie Djanche Duplex Bonheur performed the experiments and acquisition of data. Kada Sanda Antoine, Kiyang Rane Muluh, and Youmbie Djanche Duplex Bonheur analysed and interpreted the data. Kada Sanda Antoine., and Youmbie Djanche Duplex Bonheur were involved in drafting the manuscript. All authors have contributed and approved the final manuscript.

Ethical Considerations

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Availability of Data and Materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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