Journal of Advances in Medical and Pharmaceutical Sciences
8(1): 1-12, 2016, Article no.JAMPS.25404
ISSN: 2394-1111

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Oxidant–Antioxidant Status and Renal Function in Wistar Rats after Administration of *Vernonia amygdalina* Fractions in Streptozotocin Induced Diabetes Mellitus

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AEO and MEA designed the study and wrote the protocol. Authors CUO and DMET managed the animals and the field study, authors IPN, EO and EOL conducted laboratory analysis and collected all data. Authors EAU and JU performed the statistical analysis and author AEO wrote the first draft of the manuscript. Authors AEO and IPN did the literature search and completed the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2016/25404
Editor(s):
(1) Hamdy A. Sliem, Internal Medicine, Suez Canal University, Egypt and College of Dentistry, Qassim University and EL-Jouf University, Saudi Arabia.
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(3) Triveni Krishnan, National Institute of Cholera and Enteric Diseases, Kolkata, India.
Complete Peer review History: http://sciencedomain.org/review-history/14539

Original Research Article

Received 29th February 2016
Accepted 26th April 2016
Published 9th May 2016

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ABSTRACT

Diabetes mellitus is a group of metabolic diseases characterized by sustained hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It accounts for about 3% of all deaths globally and affects all glucose utilizing tissue leading to severe complications. One third of diabetes patients eventually develops renal complications and is the major cause of end stage renal disease the pathogenesis of which has been associated to oxidative stress. An alternative approach in the management of diabetes has focused on the role of herbs. In this study, *Vernonia amygdalina* a tropical savanna herb was studied for its potent antioxidant property and its ameliorative effect on diabetic renal complication. 40 adult male wistar rats were divided into eight groups (n=5) A-H. Fresh *Vernonia amygdalina* leaves were extracted and fractionated using four solvents of varying polarity. The extracts were further concentrated and reconstituted and was administered to the animals. Group A were used as control, groups B-H were induced diabetes with a single intravenous injection of 60 mg/kg B.W. streptozotocin. Group B were induced with diabetes but left untreated, group C received metformin (50 mg/kg B.W.) while groups D-H received 300 mg/kg B.W of crude Vernonia and the respective fractions of the plants. Laboratory analysis at the end of the analysis showed that the plant crude and fraction reduced the level of fasting blood glucose between 30–80%, increased serum catalase and superoxide dismutase level while decreasing malondialdehyde level. The plant caused significant reduction in serum creatinine level compared to the untreated diabetic groups and the control groups. The study shows that *Vernonia amygdalina* reduces oxidative stress by causing a balance in the oxidant–antioxidant level as well restores deranged renal function seen in diabetes mellitus.

Keywords: Diabetes; oxidative stress; renal function; streptozotocin; Vernonia amygdalina; antioxidants.

1. INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by sustained hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The incidence of diabetes has become a global pandemic affecting people of all race, nations, gender, works of life and social class with a global burden of 247 million in 2013 and an estimated global incidence of 438 million in 2030 [1]. Diabetes accounts for 3.3% of all global deaths [2]. Tropical countries in Asia and Africa have witnessed increasing risk and incidence of diabetes in recent times. This rise has been attributed to the adoption of a western diet and sedentary lifestyle. Irrespective of type, diabetic patient presents with a variety of signs and symptoms ranging from polyuria to skin manifestations such as tags and vitiligo. However, classical symptoms of diabetes include; Polyuria, polydipsia, fatigue, blurring of vision, weight loss, glycosuria and ketonuria [3]. The chronic hyperglycemia causes many of the major complications of diabetes, with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels [4]. These latter effects of hyperglycaemia may result from oxidative stress.

1.1 Oxidative Stress and Tissue Damage in Diabetes

Oxidative stress results from increased content of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) above antioxidant levels. ROS include charged species such as superoxide and the hydroxyl radical and uncharged species such as hydrogen peroxide [5]. There is evidence that oxidative stress which is a persistent imbalance between the production of highly reactive molecular species (chiefly oxygen and nitrogen) and the body's antioxidant defenses, leads to tissue damage in diabetes [5]. Research shows that ROS formation is a direct consequence of hyperglycemia [6]; Because of their ability to directly oxidize and damage DNA, protein, and lipid, ROS are believed to play a key direct role in the pathogenesis of late diabetic complications [6,7]. In addition to their ability to directly inflict macromolecular damage, ROS can function as signaling molecules to activate a number of cellular stress-sensitive pathways that cause cellular damage, and are ultimately responsible for the late complications of diabetes.

The resultant effect of oxidative stress in tissues is progressive lipid peroxidation of lipids of cell

membranes. Lipid peroxidation or reaction of oxygen with unsaturated lipids produces a wide variety of oxidation products. The main primary products of lipid peroxidation are lipid hydroperoxides (LOOH). Among the various aldehydes which can be formed as secondary products during lipid peroxidation, malondialdehyde (MDA), propanal, hexanal, and 4-hydroxynonenal (4-HNE) have been extensively studied by Esterbauer et al. [8]. Malondialdehyde appears to be the most mutagenic product of lipid peroxidation. It is an end-product generated by decomposition of arachidonic acid and larger polyunsaturated fatty acids (PUFAs) [8], through enzymatic or non-enzymatic processes. Once formed MDA can be enzymatically metabolized or can react on cellular and tissular proteins or DNA to form adducts resulting in biomolecular damages. The body's antioxidant system checks and balances the activity of reactive oxygen species to prevent its harmful effect on tissues. Superoxide dismutase and catalase constitute part of the antioxidant defense mechanisms that maintain this balance.

Catalase an enzyme antioxidant found in nearly all living organisms exposed to oxygen catalyses the decomposition of hydrogen peroxide; (a harmful by-product of many normal metabolic processes) to water [9]. Catalase has the highest turnover number of enzymes, one catalase molecule can convert millions of molecules of hydrogen peroxide to water and oxygen each second [10]. All known animals use catalase in every organ with particularly high concentrations occurring in the liver [11]. Superoxide Dismutase are enzymes that catalyses the dismutation of superoxide ion into oxygen and hydrogen peroxide. Thus, they are an important antioxidant defense in nearly all cells exposed to oxygen [12]. There are three major families of superoxide dismutase, depending on the metal cofactor: Cu/Zn (which binds both copper and zinc), Fe and Mn (which binds iron or manganese) and Ni (which binds nickel) [13].

1.2 Diabetes and Renal Complication

The diabetic patient is at risk of serious large vessel disease in the kidneys, glomerular microvasculopathy which poses serious and predictable threat to the victim's longevity [14]. Diabetic nephropathy occurs in approximately one third of all type II diabetes [15] and diabetes has been identified as the major cause of end-stage renal disease (ESRD) around the world having enormous medical, social and economic consequences. Diabetes affects the kidney in stages; at the onset of diabetes, the kidney grows large and the glomerular filtration rate (GFR) becomes disturbed. Most recent basic and clinical research has pointed toward sclerosis and kidney failure. Several mechanisms have been attributed to the development of diabetic nephropathy. Of scientific importance are the roles of renal glucose transporter (GLUT1) expression [16], abnormalities of vasodilatation and generation of reactive oxygen species (ROS) mediated by endothelial derived nitric oxide (NO) [17], suggesting linkage between vascular and metabolic abnormalities. Angiotensin II and aldosterone, interacting with pulse pressure and increased systolic blood pressure, activate NADP oxidase, which acts as mediator of oxidative stress. Sowers et al. [18] discussed the relationship between dyslipidemia and Chronic Kidney disease, hypothesizing that the mechanism of action of statins is an increase in endothelial NO synthase transcription via the phosphatidylinositol 3-kinase (PI3K) pathway and statin-induced inhibition of the mobilization of small molecular weight G-proteins. Also, Sowers suggested that the direct renin inhibitor aliskiren has similar benefits in renal disease to those of angiotensin receptor binders.

The inadequate cellular glucose utilization in diabetes leads to increased gluconeogenesis where protein and amino acid provide the substrate for new energy production increasing deamination of proteins and production of uric wastes such as urea and creatinine to be cleared off by the kidneys. Since the kidneys are the sites of excretion of metabolic wastes, renal complications especially in disease conditions are diagnosed in the laboratory as the ability of the kidney to clear off these metabolic wastes. A diagnostic criterion for renal function is the measurement of serum urea and creatinine levels. At all stages of renal insufficiency as seen in diabetic nephropathy, the serum creatinine is a much more reliable indicator of renal function than the blood urea nitrogen because the blood urea nitrogen is far more likely to be affected by dietary and physiologic conditions not related to renal function. However, the blood urea nitrogen and creatinine, taken together, are valuable screening tests in evaluating renal disease.

1.3 Herbal Remediation for Diabetes and Its Complications

Several allotrophic medications have been used over the years to treat and manage diabetes, but

the costs of such medications are exorbitant and they also have some side effects which reduce patient's compliance. Hence, recent attempts for the development of new antidiabetic therapy have focused on harnessing the medicinal potency of herbal plants [19]. *Vernonia amygdalina* also known as bitter leaf, a tropical savanna shrub of the order *Asterace*s and family *Compositae* has attracted attention for its broad spectrum medicinal uses in the tropics. Several studies have evaluated the antidiabetic property [20-22] of *Vernonia amygdalina*; its hypoglycaemic potency [22,23] and its metabolic effect on various organ systems [24,25]. Also, *Vernonia amygdalina* ethanol extract was shown to possess antioxidant activity from free radical scavenging test by Ayoola et al. [26], its total flavonoid and phenolic contents was found to be correlated positively with total antioxidant activity of the plant. Phenolic compounds identified in *Vernonia amygdalina* can be grouped into flavonoides, tannins and caffeoyl qunic acid [27]. Flavonoids protect the cell as antioxidant against free radicals and reactive oxygen species (ROS).

It is against this backdrop that the present study aims at evaluating the hypoglycaemic potency of *Vernonia amygdalina*, investigate the antioxidant activity of *Vernonia amygdalina* and its fractions and the consequent effect on the renal complication in diabetes mellitus.

2. MATERIALS AND METHODS

2.1 Chemicals

Chemicals (analytical grade) used for this study were purchased from Rovet Scientific Ltd. 1, Wire Road, Benin city, Edo State. All the reagents for the assays were commercial kits and products of Randox Laboratories Ltd, Antrim, United Kingdom. Metformin (Glucophage) was purchased from a local Pharmacy in Abraka. The tablets were dissolved in distilled water to get the appropriate concentrations (0.5 gm/100 g) that were administered to the rats, using the formula: Rat dose = (human dose/average BW) x 7. Streptozotocin (STZ) (Batch 1378), Address: Sigma. Aldrich Co.3050 Spruce Street St. Louis Mo 63103 U.S.A 314-771-5765, Acetic Acid (Batch – 70419322), H_2SO_4 SL 704l419322, E .Merk, Darmstadt 2.5l, Weigh: 1.05lg, Ethyl Acetate (1502 batch 1356o517) Gato Perez, 33-P.1. Masden Gsa 08181 sentmenat Spam. Shelf Life 5/2017, N-Butanol, Guangdong Guanghua Sci-Tech Co., Ltd. Shatou, Guangdong, China, 515000, Benzene (Batch No. 704 L419322). Gato Perez, 33-P.1. Masden Gsa 08181sentmenat Spam. Shelf Life 5/2017,

2.2 Methods

2.2.1 Source of *Vernonia amygdalina*

Fresh *Vernonia amygdalina* leaves were collected at the staff quarter, located at Site III, Delta State University Abraka, Delta State, Nigeria and was authenticated in Forestry Research Institute of Nigeria, Ibadan, with herbarium number; FHI 110336. The plants were transported to Emma-Maria Scientific and Research Laboratory Abraka for extraction.

2.2.2 Extraction procedure

Vernonia amygdalina leaves were air-dried, crushed and soaked in ethanol for 48 hours after which the ethanolic extract was sieved out and allowed to dry. The resultant ethanol-free juice was subjected to a serial liquid – liquid fractionation method using solvents of varying polarity from non-polar to highly polar following Ekam et al. [28]. Solvents used include; Benzene, Chloroform, Ethyl acetate and butanol. The fractions gotten were: Benzene Fraction, Chloroform fraction, Ethyl acetate fraction and butanol fraction. Fractionation procedure was as follow: 200 ml of the crude extract of bitter leave was measured using a measuring cylinder into a 500ml separating funnel, held using a retort stand and clamp. 200 ml of benzene was added to it, shook properly and allowed to stand 30 minutes. The mixture separated into two layers; one layer containing benzene soluble constituents of *Vernonia amygdalina* which was collected into a beaker, and the other layer containing non benzene soluble residue. The resultant residue was allowed to air dry to remove any trace of benzene in the residue. After drying, 200 ml of the residue was measured into a separating funnel and 200 ml of chloroform was added to it, shook properly and allowed to stand for separation. The mixture separated in two layers as above (Chloroform soluble constituents and non-chloroform soluble residue). The non-chloroform soluble phase was collected, allowed to air dry and then put into another separating funnel and further fractioned using Ethyl acetate; the mixture separated into two phases and collected as above. 200 ml of the residue from ethyl acetate fractionation was allowed to air-dry and further fractioned using 200 ml of butanol and allowed to stand, separate and the fraction collected. The fractions gotten

were allowed to dry to powder and identified by the solvent used for the extraction. The extracts were then reconstituted using water for water soluble extracts and Tween-80 (a placebo) for lipid soluble extracts.

2.2.3 Handling of animals

After receiving approval from the Faculty bioethics committee for the use of laboratory animals for research, forty (40) adult male wistar rats, weighing between 100-200 g were used in this research. The animals were bred and purchased from the Emma-Maria Laboratory Animal unit, Abraka, Delta State and transported in plastic basket to the College of Health Sciences Laboratory Animal Facility, Delta State University, Abraka and housed under standard laboratory conditions in stainless steel cages, supplied with clean drinking water and fed *ad libitum* with standard commercial pelleted feed (Vital feed, UAC, Lagos).

2.2.4 Induction and treatment

Diabetes was induced using Streptozotocin dissolved in sodium citrate buffer. Fasting blood glucose levels of the animals were checked after an overnight fast prior to induction. Streptozotocin was prepared by dissolving 2 g of Sodium Citrate in 100 ml of water to yield 0.1 mole of citrate buffer; 0.6 g of Streptozotocin was dissolved in 10ml of citrate buffer to yield 60mg of Streptozotocin. The resultant solution was injected into the animals based on their body weight, through the lateral tail vein at 60mg/kg B.W. Fasting blood glucose level was assessed using ACCUCHEK active blood glucometer, 72 hours after induction. A 50% increase in pre-induction fasting Blood glucose level was considered to be diabetic.

2.2.5 Treatment group

The study comprised of 40 animals divided in eight groups (n=5) and were treated as follow;

- Group A: Non diabetic, untreated (Control) group.
- Group B: Untreated diabetic group (negative control).
- Group C: Diabetic group treated with 50 mg/kg/day of metformin
- Group D: Diabetic group treated with 300 mg/kg/day of Crude *Vernonia amygdalina*
- Group E: Diabetic group treated with 300 mg/kg/day of Benzene fraction
- Group F: Diabetic group treated with 300 mg/kg/day of chloroform fraction
- Group G: Diabetic group treated with 300 mg/kg/day of Ethyl acetate fraction
- Group H: Diabetic group treated with 300 mg/kg/day of Butanol fraction

2.3 Observation

The fasting blood glucose levels of the experimental animals were monitored weekly to determine the progression of the disease and the effect of the extract on the blood glucose level.

2.4 Sacrificing of animals and Sample Collection

At the end of the experiment, the animals were subjected to an overnight fast, and their final fasting blood level checked. The animals were sacrificed by cervical dislocation and a laparotomy was carried out on each animal to access the internal organs; blood was collected by cardiac puncture, using 5ml syringes and 21G needle into plain blood sample containers for biochemical analysis. Biochemical analysis was carried out on the samples collected to determine the level of antioxidant activities, lipid peroxidation and renal function as shown below;

2.5 Assessment of Kidney Function

2.5.1 Urea (Colorimetric method)

Urea content of the serum samples was estimated by means of an automated analyzer, Blood Urea Analyzer, Beckman Coulter Inc., USA. The analysis procedure required a set up of reagents, Hichem kit of reagents for blood urea nitrogen analyzer. The kit is supplied by Elan Diagnostics, USA [29].

2.5.2 Creatinine (Colorimetric method)

Creatinine Analyzer-2 (Beckman Coulter Inc., USA) in combination with a specific kit of reagents (Hichem Creatine Pak, Elan Diagnostics, USA) was employed to calculate creatinine content of the serum samples [29].

2.6 Assay of Enzymatic Antioxidants

2.6.1 Assay of catalase (CAT) activity

Catalase activity was measured according to the method of Aebi [30]. 0.1 ml of the homogenates (supernatant) was pipetted into cuvette containing 1.9 ml of 50 mM phosphate buffer, pH 7.0. Reaction was started by the addition of 1.0

ml of freshly prepared 30% (v/v) hydrogen peroxide (H_2O_2). The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of enzyme was expressed as units /mg protein.

2.6.2 Assay of superoxide dismutase (SOD) activity

Superoxide dismutase activity was measured according to the method of Misra and Fridovich [31] and adopted by Isamah, et al. [32]. The principle of the assay was based on the ability of SOD to inhibit the autooxidation of epinephrine by superoxide radical (O_2^-). Briefly, the reaction mixture contained 0.2 ml of the Stomach suspension; 2.5 ml of 0.05 ml carbonate buffer pH 10.2 was added. The reaction will start by adding 0.3 ml freshly prepared 0.3 mM adrenaline. This was mixed by inversion. A reference cuvette containing 2.5 ml of the buffer and 0.2 ml of water and 0.3 ml of the substrate (epinephrine). The increase in absorbance was monitored at 480 nm every 30 seconds for 150 seconds. Percentage inhibition of SOD activity will then be calculated: One unit of SOD was defined as the amount of enzyme required to inhibit the autooxidation of epinephrine by 50% under the specific conditions. It was expressed as u/mg protein.

2.6.3 Estimation of lipid peroxidation (Malondialdehyde)

Lipid peroxidation was estimated spectrophotometrically by thiobarbituric acid reactive substances TBARS method of Buege and Aust [33]. A principal component of TBARS being malondialdehyde (MDA), a product of lipid peroxidation. 0.1 ml of the serum was treated with 2 ml of (1:1:1 ratio) TBA-TCA-HCl reagent (thiobarbituric acid 0.37%, 0.25 N HCl and 15% TCA) and placed in water bath for 15 min, cooled. The absorbance of clear supernatant was measured against reference blank at 535 nm. Concentration was calculated using the molar absorptivity of malondialdehyde which is 1.56 x105 M-1 cm-1 and expressed as nmol/mg protein.

2.7 Statistical Analysis

All the data are expressed as mean±standard error of mean SEM. Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD). The SPSS software (version 20) was used in the statistical analysis using multiple comparison tests. A p-value of less than 0.05 ($p < 0.05$) was considered significant.

3. RESULTS

3.1 Effect of *Vernonia amygdalina* on the Fasting Blood Glucose Level of Experimental Rats

The effect of administration of *Vernonia amygdalina* and its fractions on the fasting blood glucose level (FBGL) of normoglycaemic, diabetic rats and the percentage FBGL change is represented in the Fig. 1.

From the above Fig. 1, *Vernonia amygdalina* and its fraction caused a significant decreased in the final blood glucose level with the highest percentage decrease observed in groups treated with crude extract, ethyl acetate and chloroform fractions of the plant.

3.2 Effect of *Vernonia amygdalina* on the Level of Antioxidant Enzymes

The effect of administration of *Vernonia amygdalina* and its fractions on the level of catalase and superoxide dismutase in normoglycaemic, diabetic rats is represented in the Fig. 2.

From the Fig. 2, *Vernonia amygdalina* and its fraction caused a significant increase in the level of superoxide dismutase activity in groups treated with crude extract, ethyl acetate and benzene fractions of the plant. While only crude extract caused a slight increase in catalase activity.

3.3 Effect of *Vernonia amygdalina* on the Level of Lipid Peroxidation

The effect of administration of *Vernonia amygdalina* and its fractions on the level of malondialdehyde in normoglycaemic, diabetic rats is represented in the Fig. 3.

From the Fig. 3, *Vernonia amygdalina* and its fraction caused a significant decrease in the level of malondialdehyde in groups treated with crude extract, ethyl acetate and chloroform fractions of the plant.

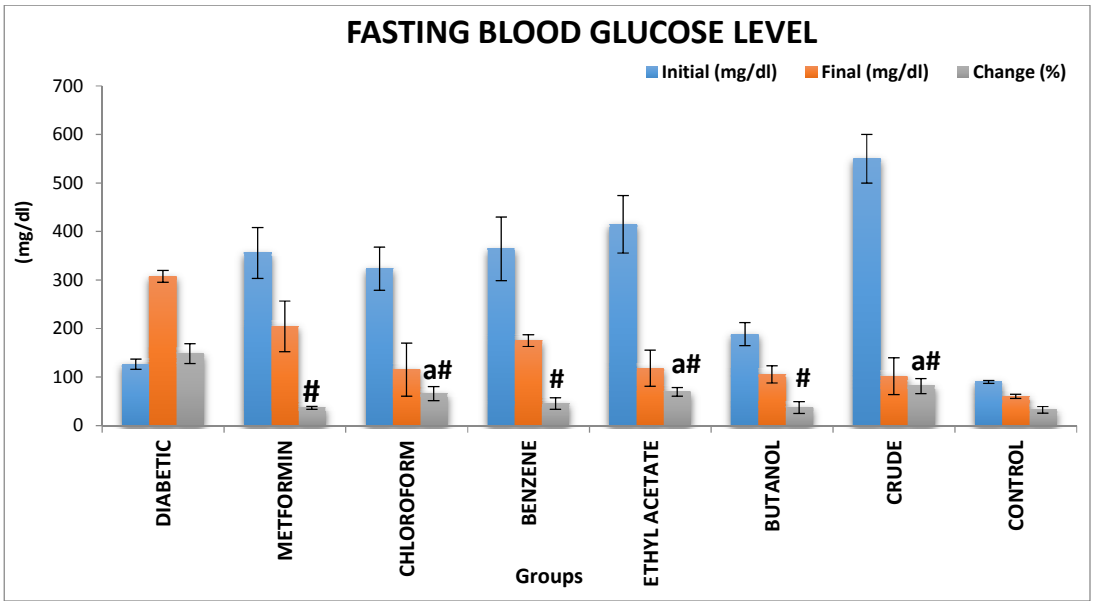

Fig. 1. Showing the effects of *Vernonia amygdalina* on the fasting blood glucose level in STZ-induced diabetic experimental rats expressed as mean±S.E.M

(a) Significant increase, (b) Significant decrease when compared with Control. (*) Significant increase (#), significant decrease when compared with diabetic control

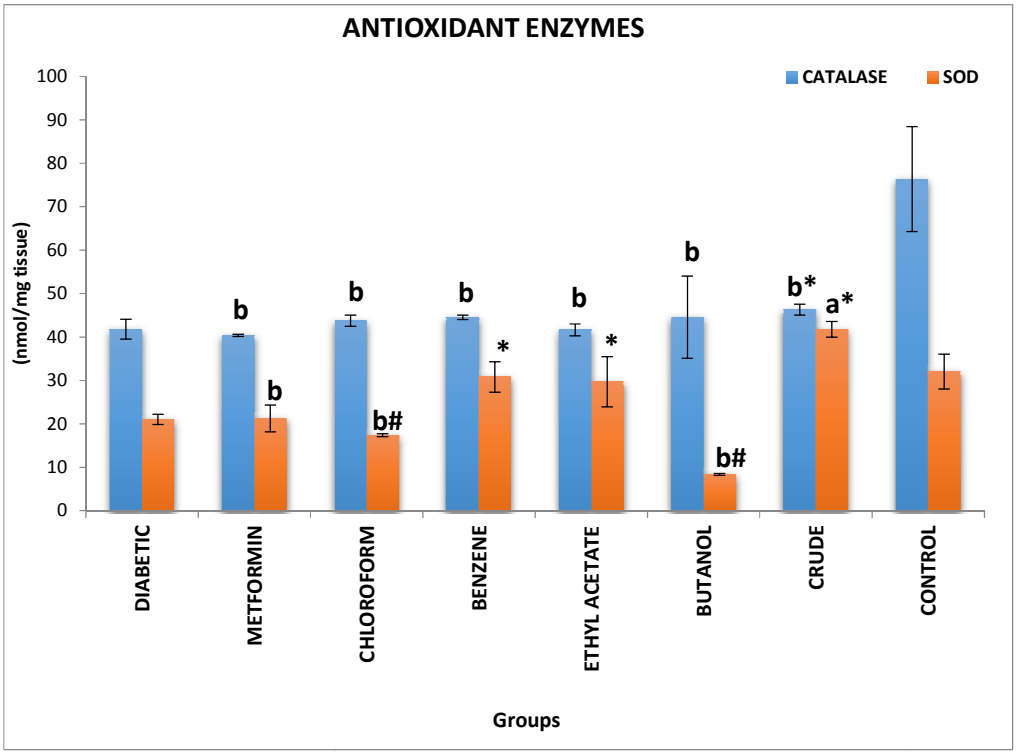

Fig. 2. Showing the effects of *Vernonia amygdalina* on the level of superoxide dismutase and catalase activity in STZ-induced diabetic experimental rats expressed as mean±S.E.M

(a) Significant increase, (b) Significant decrease when compared with control. (*) significant increase (#), significant decrease when compared with diabetic control

3.4 Effect of *Vernonia amygdalina* on Renal Function

The effect of administration of *Vernonia amygdalina* and its fractions on the level of blood urea nitrogen and serum creatinine in normoglycemic and diabetic rats is represented in the Fig. 4.

From the Fig. 4 the level of blood urea nitrogen was not improved by administration of *Vernonia amygdalina* and its fraction, while serum creatinine level was significantly decreased in rat groups treated with crude extract, butanol and chloroform fractions of the plant.

4. DISCUSSION

Diabetes affects principally the metabolism of glucose resulting in hyperglycaemia. All antidiabetic therapy is targeted at restoring euglycaemia, that is a plasma glucose level close or around physiological ranges. In this study, *Vernonia amygdalina* and its fractions caused a significant reduction in fasting blood glucose level at the end of the experiment, with the crude extract causing 81.5% reduction while administration of ethyl acetate and chloroform fractions caused 69.7%, and 65.9% reduction respectively, higher than that of metformin which showed 36.5% reduction in fasting blood glucose level of diabetic rats. This conforms to an earlier study by Nwanjo, [23] on the purported the hypoglycaemic property of *Vernonia amygdalina*. The result of this study shows that administration of *Vernonia amygdalina* and fractions caused a significant decrease in fasting blood glucose level similar to Ekam et al. [28] who observed that fractions from *Vernonia amygdalina* significantly reduced blood glucose levels of diabetic rats in similar percentages. This confirms the hypoglycaemic property of the plant and its fractions. The hypoglycaemic property of the plant found to be highest in the crude extracts may be attributed to the presence of known hypoglycaemic phytochemical constituents of the herb including but not restricted to sesquiterpene lactones and steroid glucosides [34,35]. It has also been suggested that bitter principle as seen in aloes may stimulate synthesis and/or release of insulin from the beta-cells of Langerhans [36]. *Vernonia amygdalina* contains both sesquiterpene lactones and bitter principle, may therefore act through stimulation of synthesis and/or release of insulin from the beta-cells of the pancreatic islets as suggested by Atangwho et al. [37].

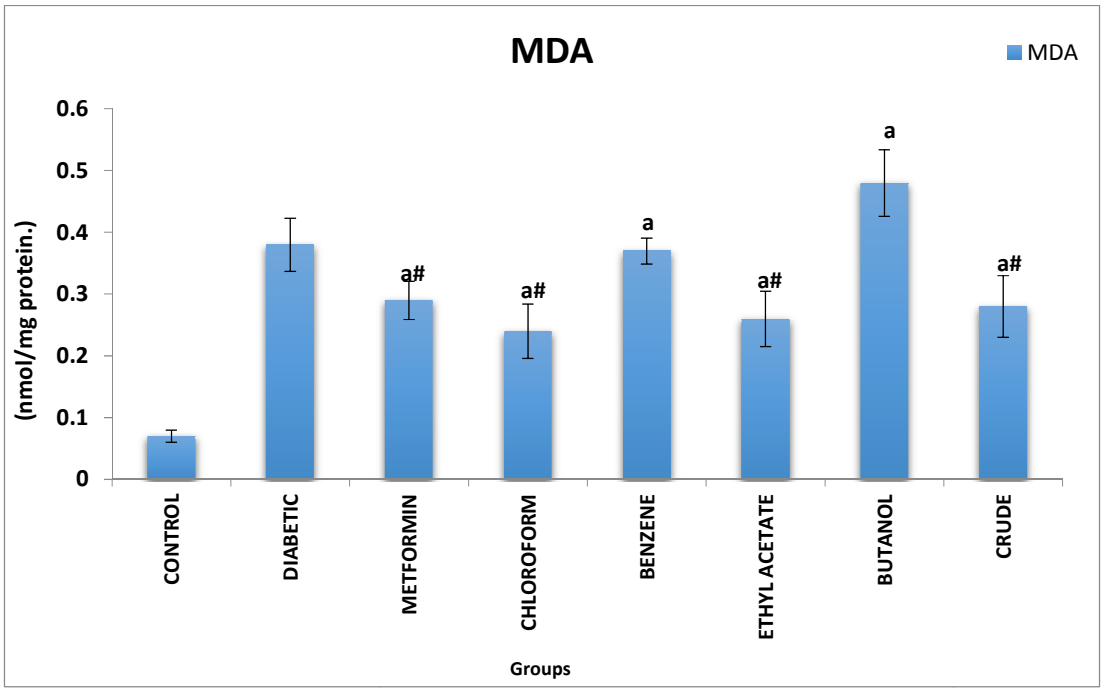

Fig. 3. Showing the effects of *Vernonia amygdalina* on the level of malondialdehyde in STZ-induced diabetic experimental rats expressed as mean±S.E.M

(a) Significant increase, (b) Significant decrease when compared with control. (*) significant increase (#), significant decrease when compared with diabetic control

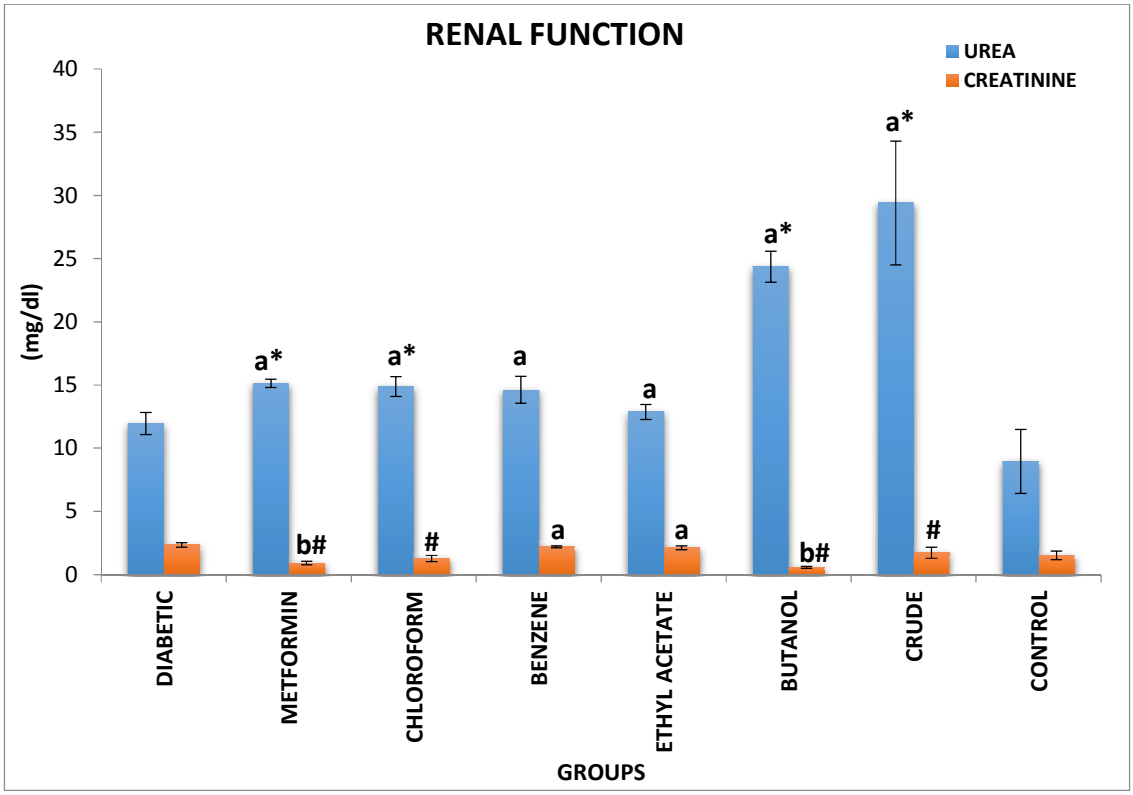

Fig. 4. Showing the effects of *Vernonia amygdalina* on the level of malondialdehyde in STZ-induced diabetic experimental rats expressed as Mean±S.E.M

(a) Significant increase, (b) Significant decrease when compared with Control. (*) significant increase (#), significant decrease when compared with diabetic control

Oxidant – antioxidant balance and lipid peroxidation level can be used to predict the severity of diabetes induced renal complications. Antioxidants enzymes such as Superoxide dismutase and Catalase dependently act in the metabolic pathways that involve free radicals scavenging. In diabetes mellitus, this balance is altered in favour of the oxidants resulting in organ complications as seen in the untreated diabetic group. The restoration of this balance, which is the increase in antioxidants (SOD and Catalase) and a consequent decrease in lipid peroxidation (MDA) was observed to be prominent after administration *Vernonia amygdalina* crude extract and its ethyl acetate fraction. This is in line with the finding of Ogunlade et al. [38] who observed a similar increase in the activity of antioxidant enzymes and decreased MDA level with administration of *Vernonia amygdalina* extract to alcohol induced toxicity in rats. This suggests the potent antioxidant capacity of *Vernonia amygdalina* and its fraction capable of restoring oxidant – antioxidant balance either by improving endogenous antioxidants or contributing its own antioxidant constituent to assist in scavenging free radicals in the body.

The kidneys maintain optimum chemical composition of body fluid by acidification of urine and removal of metabolic wastes such as urea, uric acid, and creatinine. The inadequate cellular glucose utilization in diabetes leads to increased gluconeogenesis. Proteolysis and hepatic amino acid deposition feed the liver for gluconeogenesis which constitutes deamination of proteins and production of uric wastes such as urea and creatinine to be cleared off by the kidneys. However, in diabetic nephropathy, the excretory efficiency of the kidney nephrons has been compromised causing the concentration of these metabolites to accumulate and increase in blood [39]. In this study, blood urea nitrogen levels of all diabetic rats were seen to be increased as compared to the non diabetic rats. Administration of *Vernonia amygdalina* extract and fractionates showed no significant reversal of this elevated values in the diabetic rats. Blood

creatinine levels on the other hand were reduced on administration of the plant extract and fractionate to diabetic rats. Several factors such as food type, physical activities and starvation may influence urea level while making it a non-specific diagnostic assay. Creatinine levels on the other hand are more specific and serve as better diagnostic tool for renal competence [40]. The relationship between urea and creatinine in determining the GFR is not a straight line but rather a parabolic curve; their values remain within the normal range until more than 50% of renal function is lost [41]. Therefore, in the early stages of renal disease, these tests could create a false sense of security. The blood urea nitrogen and creatinine, taken together, are valuable screening tests in evaluating renal disease. Though they may fall short as absolute indicators of renal function at any-given point in time, they are useful in following progression of disease. The result from this study suggests a restoration of renal function in diabetic rats. Similar renal restoration capacity was reported by Atangwho et al. [37].

5. CONCLUSION

This study has demonstrated the ameliorative property of *Vernonia amygdalina* on both the kidney as well as plasma glucose. The nephritic deraignment frequently seen in diabetes mellitus which has been attributed to the activity of free radicals was ameliorated by the restoration of oxidant-antioxidant balance with administration of *Vernonia amygdalina*.

Furthermore the study provides the basis for research into the possible of *Vernonia amygdalina* for the management of renal disorders not of diabetic origin but with etiology associated with free radical damage.

CONSENT

It is not applicable

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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