NEPHROPROTECTIVE EFFECTS OF LEAVES OF *Vitex doniana* AQUEOUS EXTRACT ON STREPTOZOTOCIN-INDUCED DIABETIC RATS

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O.E. Yakubu[1], O.F.C. Nwodo[2] and E.P.K. Imarenezor[3]
[1]Department of Biochemistry, Federal University Wukari, Nigeria
[2]Department of Biochemistry, University of Nigeria, Nsukka, Nigeria
[3]Department of Microbiology, Federal University Wukari, Nigeria
Corresponding author: oj4real_2007@yahoo.co.uk;

Abstract: Several herbal preparations are used to treat diabetes, but their reported hypoglycemic effects are complex. In this study, thenephroprotective effects of leaves of *Vitex doniana* aqueous extract onstreptozotocin-induced diabetes in rats was evaluated. Twenty five male rats were used in the study by randomly allocating them into five groups, each of five rats. Diabetes was induced intraperitoneally using 50 mg/kg streptozotocin, while diabetic rats were treated with 100 mg/kgaqueous extract and glibenclamide (2.5 mg/kg) for 28 days. Normal rats received distilled water. Serum creatinine and urea levels thiobarbituric acid reactive substances (TBARS) in the kidney as well as kidney catalase (CAT) and superoxide dismutase (SOD) activities were assayed. The results revealed that the TBARS, creatinine and urea levels were increased while SOD and CAT activities decreased significantly ($P<0.05$) in control rats. Treatment with extract was able to reverse these parameters to normal by increasing SOD and CAT and decreasing TBARS, urea and creatinine. Extract treatment demonstrated more effect compared to glibenclamidetreatment. However, the extract was able to manage hyperglycaemia and diabetes -induced oxidative changes in the kidney, thus suggesting its use for the management of diabetic complications affecting the kidney.

Keyword: Nephroprotective, streptozotocin-induced diabetic, *Vitex doniana*.

Introduction

Diabetes is a chronic disorder that arises from either, defects in peripheral insulin action and/or insulin secretion resulting in hyperglycemia (Ferrannini, 1998). Abnormally elevated blood glucose level causes oxidative stress and the formation of advanced glycation end products which result in diabetic complications (Baynes, 1991; Ahmed, 2005). Among the complications, nephropathy seems to be prevalent (Selby *et al.*, 1990; Held *et al.*, 1991). Clinical trials suggest that there is no effective treatment for diabetic nephropathy, thus efforts are focusing on traditional herbal medicine to find a novel therapeutic agents for treatment of diabetic nephropathy(Kang *et al.*, 2006).

Vitex doniana (family *Verbanaceae*) is a perennial shrub widely distributed in tropical West Africa, and some East African countries including Uganda, Kenya and Tanzania; and high rainfall areas. It is found in Nigeria particularly Kogi, Benue, and parts of the savannah regions of Kaduna, Sokoto and Kano states (Etta, 1984). It is variously called *vitex* (English), *dinya* (Hausa), *dinchi* (Gbagyi), *uchakoro* (Igbo), *oriri* (Yoruba) *ejiji* (Igala) and *olih* (Etsako) (Burkill, 2000). *V. doniana* is employed in the treatment of a variety of diseases. Hot aqueous extracts of the leaves are used in the treatment of stomach and rheumatic pains, inflammatory disorders, diarrhea, dysentery and diabetes (Irvine, 1961; Etta, 1984) indicating that the plant's leaves may possess antidiabetic properties among others. The roots and leaves are used for nausea, colic and epilepsy (Bouquet and Debray, 1971; Iwu, 1993). In North-Central and eastern parts of Nigeria, the young leaves are used as vegetables or sauces and porridge for meals, especially for diabetic patients.

Hence, this study is aimed at investigating the nephroprotective effects of the aqueuos extracts of the leaves of *Vitex doniana* in diabetic rats.

Materials and Methods

Collection and preparation of plant materials

Fresh leaves of *V. doniana* were collected from its natural habitat in Ankpa, Kogi State, and it was identified and authenticated by the Ethnobotanist in the Department of Medicinal Plant Research and Traditional Medicine of the National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria. A voucher specimen number NIPRD/H/6415 was deposited at the herbarium of the department. The plant material was dried in the laboratory at room temperature and pulverized using laboratory mortar and pestle.

The pulverized sample was soaked in 1:4 volumes (w/v) of distilled water and decocted exhaustively overnight. The filtrate was concentrated under reduced pressure using rotary evaporator, the extract was reconstituted freshly in distilled water at appropriate concentrations for the various experimental doses using the equation of Tedong *et al.* (2007);

$$V(ml) = (D \times P)/C$$

Where: D = dose used (g/kg body weight); P = body weight (g); C = concentration (g/ml) and V = volume.

Animal management

Male albino rats weighing 150-180 g (7-8 weeks old) were purchased from the animal house of the Department of Biosciences, Salem University, Lokoja, Nigeria. They were acclimatized for two weeks prior to commencement of experiment. They were kept at room temperature and maintained *ad libitum* on growers mash (feed) and weighed prior to experiment.

Induction of diabetes

Rats were fasted overnight and experimental diabetes was induced by intraperitoneal injection of streptozotocin (STZ) with a single dose of 50 mg/kg body weight. STZ was dissolved in a freshly prepared 0.1M cold citrate buffer pH 4.5 (RakietenRadkarni, 1963). Control rats were similarly injected with citrate buffer. Because STZ is capable of inducing fatal hypoglycemia as a result of massive pancreatic insulin release, STZ treated rats were provided with 10% glucose solution after 6 h for the next

24 h to prevent severe hypoglycemia. After 3 days for development and aggravation of diabetes, rats with moderate diabetes (i.e. blood glucose concentration 250 mg/dl) that exhibited hyperglycemia were selected for experiment (Canepa *et al.*, 1990).

Experimental design

In the experiment, the rats were divided into 5 groups of 5 rats each. Treatment was carried out orally for four weeks.
GROUP 1: Normal Control (N. control)
GROUP 2: Diabetic Control (D.Control)
GROUP 3: Diabetic Glibenclamide (D.STD) (2.5 mg/kg)
GROUP 4: Diabetic treated with Extract (D. Aqueous) Aqueous extract (100 mg/kg)
GROUP 5: Non diabetic treated with Extract (N. Aqueous) Aqueous extract (100 mg/kg)
On the 28th day of post-treatment, the animals were fasted overnight, anesthetized with chloroform and sacrificed by humane decapitation. The blood was collected in test tubes and serum collected and stored in deep-freezer prior to analysis. Fasting blood glucose and packed cell volume were monitored weekly. Liver and kidneys were surgically removed, immediately washed with ice-cold normal saline and stored in deep freezer.

Tissue preparation

Weighed liver and kidney samples were homogenized separately in 10 parts (w/v) of ice-cold 50mM Tris-HCl, (pH 7.4) using a homogenizer (Janke and Kunkel, Germany). The homogenates were centrifuged at 3,000 rpm for 15 min and the supernatant collected. The supernatants were used for measurement of scavenging enzyme activities and lipid peroxides (TBARS).

Determination of thiobarbituric acid reactive substances (TBARS)

Hepatic Lipid peroxidation was determined as thiobarbituric acid reactive substances as described by Torres *et al.* (2004). Lipid peroxidation generates peroxide intermediates which upon cleavage release malondialdehyde, a product which react with thiobarbituric acid. The product of the reaction is a coloured complex which absorbs light at 535 nm. The extinction coefficient, 1.56 x 10^{-5} m^{-1} cm^{-1} was used in the calculation of TBARS and values were expressed as nmol/mg protein.

Assay of serum urea and creatinine

Serum urea and creatinine were determined by Agape Diagnostics (Switzerland), according to the manufacturer's instructions.

Assay of superoxide dismutase (SOD)

The activity of superoxide dismutase was measured at 560 nm according to the method described by Martin *et al.* (1987). Briefly, auto-oxidation of hematoxylin is inhibited by SOD at assay pH, the percentage of inhibition is linearly proportional to the amount of SOD present within a specific range, and was expressed as unit/mg protein.

Assay of catalase (CAT)

Catalase activity was measured using the method of Abei (1974). The decomposition rate of H_2O_2 was measured at 240 nm for 5 min using a spectrophotometer. A molar extinction coefficient of 0.041 mM^{-1}-$cm^{-1}$was used to calculate the catalase activity and was expressed in unit/mg protein.

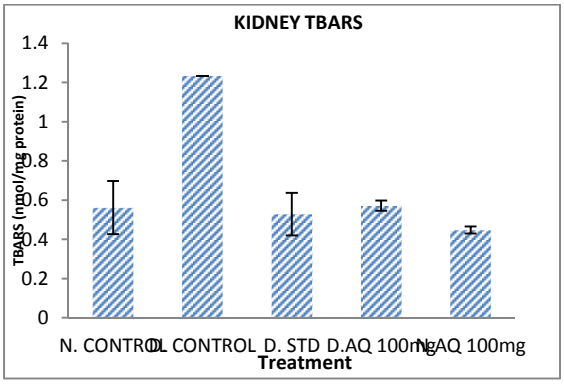
Statistical analysis

All the values estimations were expressed as mean ± standard deviation and analyzed for Duncan's post-hoc ANOVAand student's t-test using statistical package for social sciences (SPSS). Differences between groups were considered significant at P <0.05 levels.

Results and Discussion

Thiobarbituric acid reactive substances (TBARS) levels

TBARS level was significantly (P<0.05) elevated in the kidney of diabetic control rats, when compared with the normal control rats. This increase was reduced significantly (P<0.05) in the extract treated rats as well as in the rats treated with glibenclamide (Fig. 1).

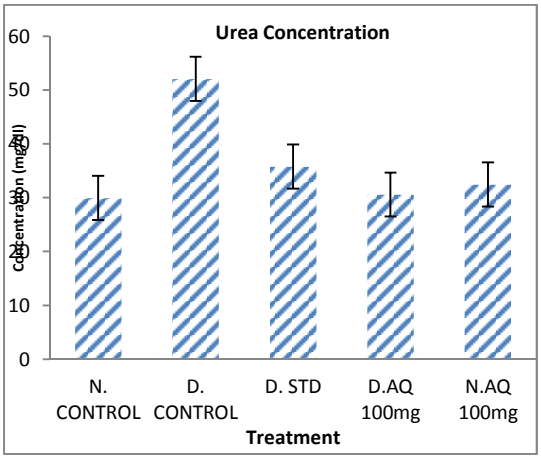


N. CONTROL= Normal Control; D. CONTROL = diabetic Control; D. STD = Diabetic Standard Drug (Glibenclamide); D.AQ = Diabetic Aqueous extract; N. AQ = Non Diabetic Aqueous extract.

Fig. 1: TBARS levels in normal and diabetic rats treated with *V. doniana* aqueous extract and glibendamide.

Effects of the extract on urea and creatinine

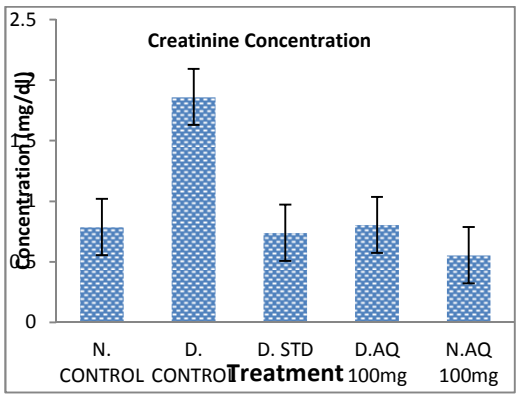
Urea and creatinine are shown in Figs. 2 and 3, respectively. Glibenclamide caused significant elevation (P<0.05) in the activities of these markers in the serum. Treatment with *V. doniana* aqueous extract at the dose of 100 mg/kg significantly reduced the activity of the urea and creatinine compared to the normal. However, treatment of non-diabetic rats caused no significant decreases in both urea and creatinine levels.



N. CONTROL= Normal Control; D. CONTROL = diabetic Control; D. STD = Diabetic Standard Drug (Glibenclamide);

D.AQ = Diabetic Aqueous extract; N. AQ = Non Diabetic Aqueous extract.

Fig. 2: Effects of *V. doniana* aqueous extract on serum urea in streptozotocin-induced diabetes in rats.

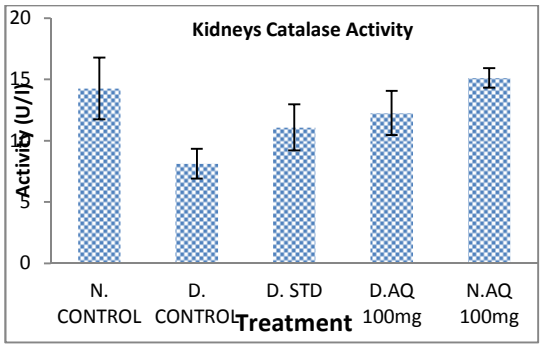


N. CONTROL= Normal Control; D. CONTROL = diabetic Control; D. STD = Diabetic Standard Drug (Glibenclamide); D.AQ = Diabetic Aqueous extract; N. AQ = Non Diabetic Aqueous extract.

Fig. 3: Effects of *V. doniana* aqueous extract on serum creatinine in streptozotocin-induced diabetes in rats.

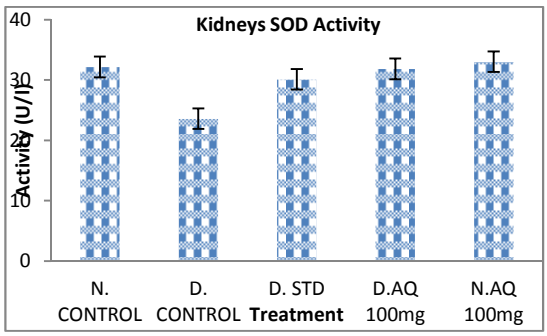
Effects of the extract on enzymatic antioxidants

A significant (P<0.05) decrease in catalase (CAT) and superoxide dismutase (SOD) activities were observed in the kidney of diabetic untreated rats compared to the normal rats (Figs. 4 and 5). Treatment with the extract and glibenclamide showed a significant (P<0.05) increases in catalase activity, glibenclamide treatment also significantly increased SOD activity but non-significant with extract treatment compared to the normal group. There was no significant increase in the activity of the enzymes in non-diabetic rats treated with the extract compared to the normal.



N. CONTROL= Normal Control; D. CONTROL = diabetic Control; D. STD = Diabetic Standard Drug (Glibenclamide); D.AQ = Diabetic Aqueous extract; N. AQ = Non Diabetic Aqueous extract.

Fig. 4: Kidney catalase (CAT) activity in normal and diabetic rats treated with *V. doniana* aqueous extract and glibeclamide.



N. CONTROL= Normal Control; D. CONTROL = diabetic Control; D. STD = Diabetic Standard Drug (Glibenclamide); D.AQ = Diabetic Aqueous extract; N. AQ = Non Diabetic Aqueous extract.

Fig. 5: Kidney superoxide dismutase (SOD) activity in normal and diabetic rats treated with *V. doniana* aqueous extract and glibenclamide.

The current investigation revealed that induction of diabetes resulted in elevation of serum urea andcreatinine concentrations. These parameters are considered as significant markers of renal dysfunction (Prakasam *et al.* 2004; Fekete *et al.*, 2008; Yakubu *et al.*, 2016). *Vitex doniana* extract administration resulted indecrease of these parameters, a finding that was in agreement with that of Badr El-Din, (1997) and Kang *et al.*(2008) who reported ameliorated renal dysfunction of diabetic rats by the ginseng extract or 20(S)-ginsenosideRg3 administrations.

Concerning the renal antioxidant status, the current study revealed increased oxidative stress due to diabeteswhich was evidenced by increased tissue concentration of malondialdehyde and depletion of antioxidant enzymes concentration. This could be accompanied by significant glomerular pathology, namely; glomerularsclerosis orglomerular atrophy and accumulation of proteinous material in Bowman's space together with thickening of Bowman's capsule membrane. The reported oxidative stress resulted from hyperglycemia–induced increases inglucose autoxidation, protein glycation and the subsequent oxidative degradation of glycated protein leading toenhanced production of reactive oxygen species (Kakkar *et al.*, 1997). Oxidative stress may be both, the cause and the result of tissue damage, a primary and a secondary source ofdiabetic pathology (Baynes and Thorpe, 1996). The recorded rise in tissue concentration of malondialdehyde, anindex of endogenous lipid peroxidation, has been also reported by Turk *et al.* (2002) in diabetic patients and in diabetic rats reflecting increased state of oxidative stress. Both oxidative stress and advancedglycation end products result in Nuclear factor-kappa (NF-k) activation which is normally present in thecytoplasm of eukaryotic cells as an inactive complex with the inhibitor binding protein kB (Kang *et al.*, 2006).

When cells are exposed to various external stimuli, such as reactive oxygen species or advanced glycation endproducts, inhibitor binding protein kB undergoes rapid phosphorylation with subsequent ubiquitination, leadingto the proteosome mediated degradation of this inhibitor (Ahmed, 2005). Nuclear factor-kappa translocates to thenucleus, where it binds to enhancer regions of target genes, specially cyclooxygenase-2 (COX-2), induciblenitric oxide synthase (iNOS) genes, thereby altering their expression (Surh *et al.*, 2001). Cyclooxygenase-2 andinducible nitric oxide synthase expression was found to be increased in kidney of STZ-induced diabetic ratswhere they were involved in pathogenesis of nephropathy. Cyclooxygenase-2 increased the conversion of arachidonate to prostaglandin E_2, prostaglandin$F_{2\alpha}$, prostaglandin D_2, and thromboxane B_2 in glomeruli of diabetic rats thereby implicated in the alterations inrenal hemodynamics in diabetes (Komer*set al*. 2001). Although nitric oxide is a simple inorganic radicalexhibiting diverse physiological functions, including the regulation of neurotransmission and vascular tone yet itcould react with superoxide yielding peroxynitrite which is a potent nitrating and oxidizing agent that can nitrateand oxidize various biomolecules,

such as thiols, lipids, carbohydrates, and nucleic acids (Kasina *et al.,* 2005; Marcondes, 2006).

Moreover, ginsenoside fractions have been shown to induce the cytosolic antioxidant enzyme superoxide dismutase via enhanced nuclear protein binding to its gene regulatory sequences (Chang *et al.,* 1999). The reported results concerning the decrease in superoxide dismutase during diabetes by *V. doniana* aqueous extract agreed with those of Godin *et al*. (1988) who showed a decrease in Cu-Zn SOD activity in renal tissues during diabetes, however they contradicted those of Limaye *et al.* (2003) who demonstrated either a no change or an increase in SOD activity in renal tissues of diabetic rats.

In conclusion, *V. doniana* aqueous extract appears to be of benefit for dealing with diabetes-induced nephropathy, an effect thatseems to be rather dependent on antioxidant property of *Vitex doniana.*

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