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Research Article

AMELIORATION OF LIPID PEROXIDATION AND OXIDATIVE STRESS IN HEPATOCYTES OF STREPTOZOTOCIN-INDUCED DIABETIC RATS TREATED WITH AQUEOUS EXTRACT OF *VITEX DONIANA* LEAVES

[*]O.E. Yakubu[1], O.F.C. Nwodo[2], V.O. Nwaneri-Chidozie[3] and E. Ojogbane[4]
[1]Department of Medical Biochemistry, Cross River University of Technology, Calabar, Nigeria
[2]Department of Biochemistry, University of Nigeria, Nsukka, Nigeria
[3]Department of Biochemistry, Salem University, Lokoja, Nigeria
[4]Department of Biochemistry, Kogi State University, Anyigba, Nigeria
**Author for Correspondence*

ABSTRACT
Several herbal preparations are used to treat diabetes, but their reported hypoglycemic effects are complex. This study therefore was designed to evaluate the effect of aqueous extract of *Vitex doniana* leaves on oxidative stress and lipid peroxidation in streptozotocin-induced diabetic and non diabetic rats. Diabetes was induced intraperitoneally using 50mg/kg streptozotocin, while diabetic rats were treated in 12-h cycles for 4 weeks with 100mg/kg of the extract and glibenclamide (2.5mg/kg). Nondiabetic control rats received distilled water. The levels of thiobarbituric acid reactive substance (TBARS), Aspartatate aminotransfrease (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), catalase (CAT) and superoxide dismutase (SOD) activities total, conjugated and unconjugated bilirubin concentration were assayed. The results indicated that the concentrations of TBARS, ALT, AST, ALP and bilirubin were significantly increased while the activities of SOD and CAT were reduced in the diabetic animals ($p<0.05$). The extract significantly increased CAT and SOD activity and reduced TBARS, ALT, AST, ALP and bilirubin concentrations ($p<0.05$). However, glibenclamide treatement showed slight modification in the changes observed compared to the extract. The study concluded that the extract reversed diabetes -induced oxidative changes in the hepatocytes, thus suggesting its use for the management of diabetic complications.

Key Word: *Lipid Peroxidation, Streptozotocin-Induced Diabetic, Vitex Doniana*

INTRODUCTION
Diabetes mellitus is a chronic metabolic disorder characterize by degeneration of carbohydrates, protein and fat metabolism (O'Brien and Granner, 1996). Such alterations result in increased blood glucose, which causes long-term complications in many organs.
Oxidative stress in cells and tissues results from the increased generation of reactive oxygen speces and/or from diseases in antioxidant defense potential (Gumieniczek *et al.,* 2002). Lipid peroxidation of cellular structures, a consequence of free radical activity in turn seemed to play an important role in aging and late complications of diabetes (Ugochukwu and Cobourne, 2003, Hunkar *et al.,* 2002) disrrupting natural antoixidant defence systems and altering antioxidant enzyme activities in various tissues like the liver (Rauscher *et al.,* 2000, Rauscher *et al.,* 2001). On the other hand, an increase in circulating lipids may be a reason for increased lipid peroxidation in diabetes. Currently, there is a renewed and growing interest in the use of plant-based products as drugs or as 'leads' in the manufacture of more potent drugs (Ogbonnia *et al.,* 2008). Several secondary plant metabolites have been shown to modify biological processes, which may reduce the risk of chronic diseases in humans (Ugochukwu *et al.,* 2003).
Globally the prevalence of diabetes mellitus is increasing. The increase in prevalence has accelerated due to the aging population structure in the developed countries and due to the globally increasing obesity, as well as stressing life style. Diabetes mellitus is the sixth leading cause of death globally (Nash *et al.,* 2001).

Vitex doniana sweet, (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 the middle belt of 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), *ucha koro* (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, diarrhoea dysentery and diabetes (Irvine, 1961; Etta, 1984) indicating that the plant's leaves may possess antidaibetic properties among others. The roots and leaves are used for nausea, colic and epilepsy ((Bouquet *et al.*, 1971 and 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.

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 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) = (DxP)/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 (7-8weeks old) will be purchased from the animal house of the Department of Biosciences, Salem University, Lokoja, Nigeria. They will be 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 will be induced by intraperitoneal injection of streptozotocin (STZ) with a single dose of 50mg/kg body weight. STZ was dissolved in a freshly prepared 0.1M cold citrate buffer pH4.5 (Rakieten *et al.*, 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 hr for the next 24 hr to prevent severe hypoglycemia. After 3 days for development and aggravation of diabetes, rats with moderate diabetes (i.e. blood glucose concentration 250mg/dl) that exhibited hyperglycemia were selected for experiment (Canepa *et al.*, 1990).

Experimental Design

In the experiment, the rats will be divided into 5 groups of 5 rats each. Treatment was carried out orally.

Normal Control (N. control) Distilled water (5ml/kg)
Diabetic Control (D.Control) Distilled water (5ml/kg)
Diabetic Glibenclamide (D.STD) (2.5mg/kg)
Diabetic Extract (D. Aqueous) Aqueous extract (100mg/kg)
Non diabetic Extract (N. Aqueous) Aqueous extract (100mg/kg)

On the 28th day of post-treatment, the animals were fasted overnight, anesthetized with chloroform and sacrificed by humane decapitation. Blood was collected in centrifuge tubes, and serum collected after

centrifugation at 2,000rpm for 10 minutes and stored in deep-freezer prior to analysis. Fasting blood glucose was and packed cell volume was monitored weekly. Kidneys were surgically removed, immediately washed with ice-cold normal saline and stored in deep freezer.

Tissue Preparation

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

Determination of Biochemical Parameters

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 535nm. The extinction coefficient, 1.56x10-5 M-1 Cm-1 was used in the calculation of TBARS and values were expressed as nmol/mg protein.

Aspartate aminotransferase (AST)

Aspartate aminotransferase (AST) catalyzes the transamination of aspartate to alpha-keto glutarate to form glutamate and oxaloacetate, which then reacts with 2,4-dinitro-phenylhydrazine to form hydrazone derivative of oxaloacetate, a coloured complex which can be measured at 546nm. Aspartate aminotransferase was determined as described by Reitman and Frankel, (1957) using assay kits (Agape Laboratories Ltd, UK).

Alanine aminotransferase (ALT)

Alanine aminotransferase (ALT) catalyzes the transamination 0f alanine to alpha-keto glutarate to form glutamate and pyruvic acid, which then reacts with 2,4-dinitro-phenylhydrazine to form hydrazone derivative of pyruvate, a coloured complex which can be measured at 546nm. Alanine aminotransferase was determined as described by Reitman and Frankel, (1957) using assay kits (Agape Laboratories Ltd, UK).

Alkaline Phosphatase (ALP)

Serum alkaline phosphatise was determined as described by Klein *et al.,* (1960). Serum alkaline phosphatase catalyses the hydrolysis of a colourless substrate of phenolphthalein monophosphate giving rise to phosphoric acid and phenolphthalein which, at alkaline pH values turns into a pink colour that can be determined photometrically at 550nm.

Serum bilirubin

This was determined colorimetrically according to the method described by Jendrassic and Grof, (1938) using assay kits (Agape Laboratories Ltd, UK). Conjugated bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

Assay of Enzymatic Antioxidant

Superoxide Dismutase (SOD)

The activity of superoxide dismutase was measured at 560nm 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.

Catalase (CAT)

Catalase activity was measured using the method of Abei (1974). The decomposition rate of H_2O_2 was measured at 240nm for 5 minutes 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 ANOVA and post hoc Duncan's -test using 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 liver 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 (Figure 1).

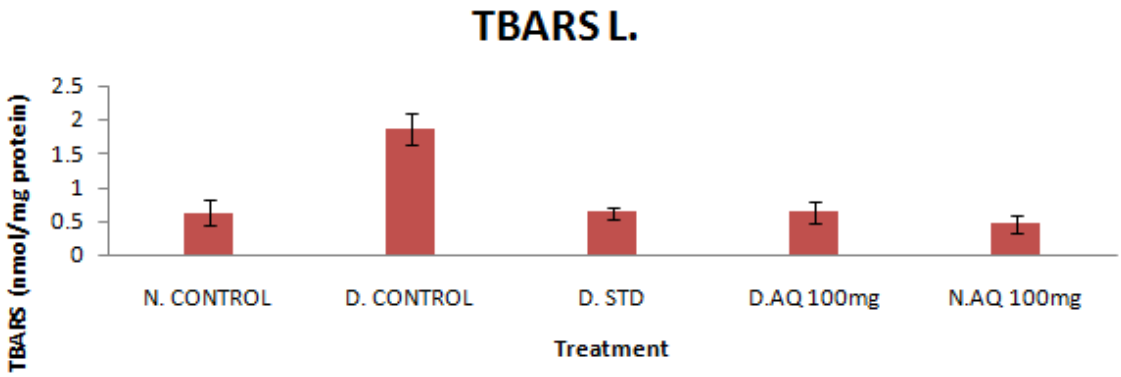

Figure 1: TBARS levels in normal and diabetic rats treated with V. doniana ethanol extract and glibendamide

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.

Effects of the Extract on Hepatic Enzymes

Hepatic enzymes; alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are shown in figure 2, 3 and 4 respectively. Glibenclamide caused significant elevation (P<0.05) in the activities of these enzymes in the serum. Treatment with *V. doniana* aqueous extract at the dose of 100mg/kg significantly reduced the activity of the enzymes compared to the normal. Similarly, treatment with glibenclamide was able to reduce ALP activity significantly but non-significant in ALT and AST. However, treatment of non-diabetic rats caused no significant decrease in the activity of the enzymes.

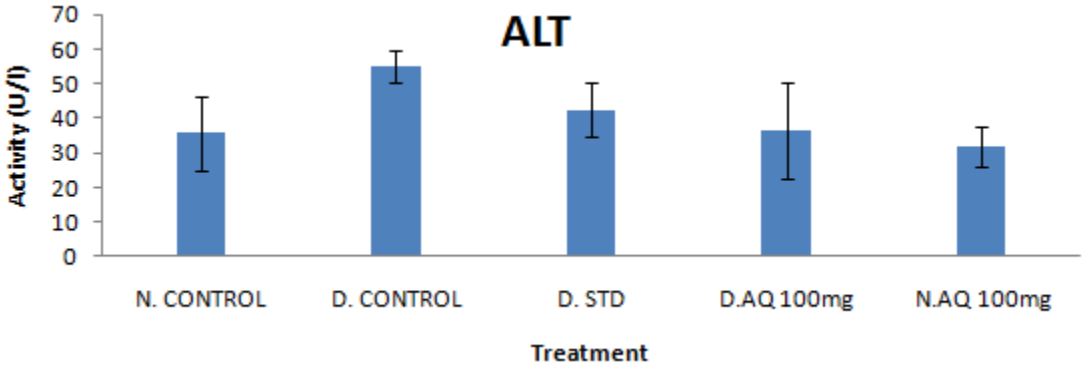

Figure 2: Alanine aminotransferase (ALT) activity in normal and diabetic rats treated with V. doniana ethanol extract and glibendamide.

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.

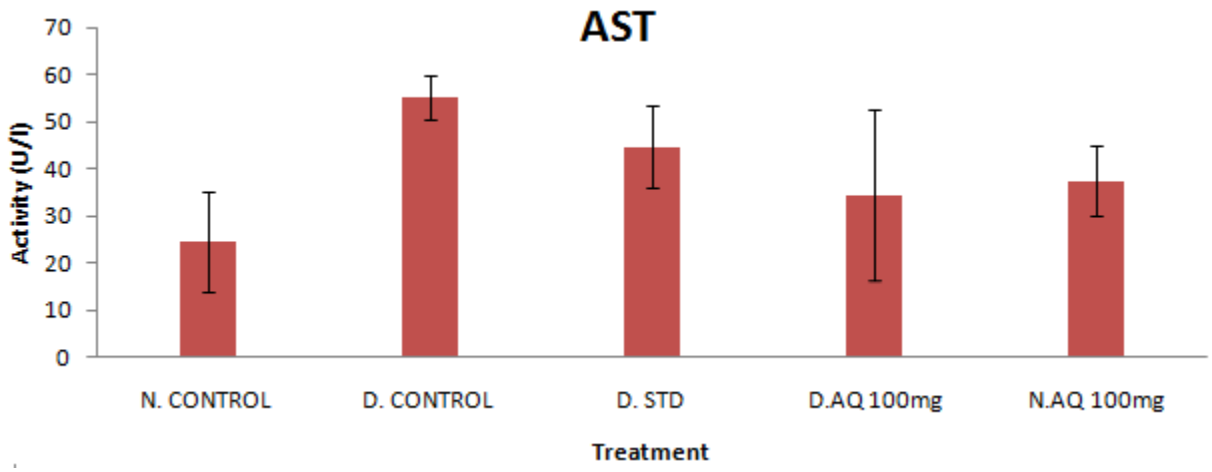

Figure 3: Aspartate aminotransferase activity in normal and diabetic rats treated with *V. doniana* ethanol extract and glibendamide.
N. CONTROL= Normal Control; D. CONTROL = diabetic Control; D. STD = Diabetic Standard Drug (Glibenclamide); D.AQ = Diabetic Aqueous extract; N. AQ = Non Diabetic Aqueous extract.

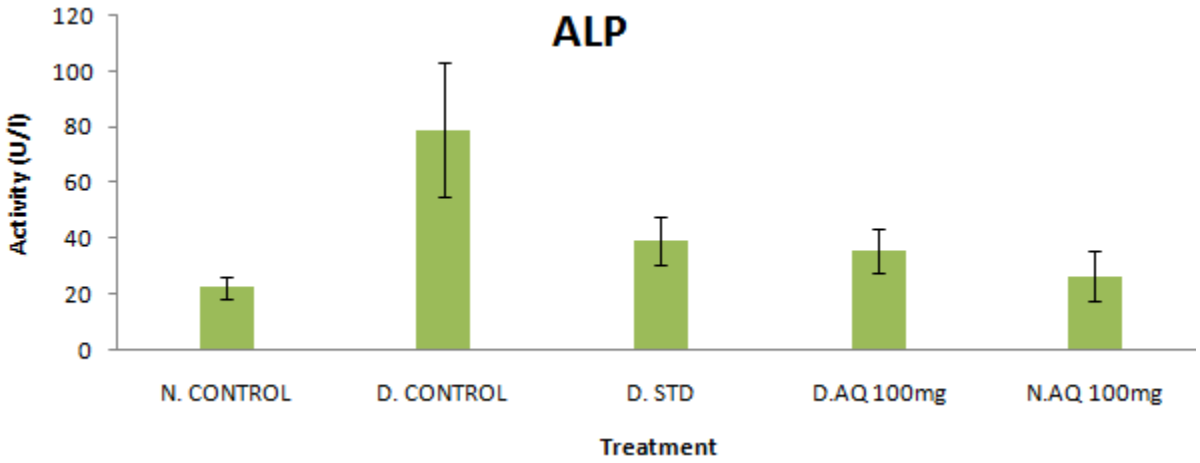

Figure 4: Alkaline phosphatase (ALP) activity in normal and diabetic rats treated with *V. doniana* ethanol extract and glibendamide.
N. CONTROL= Normal Control; D. CONTROL = diabetic Control; D. STD = Diabetic Standard Drug (Glibenclamide); D.AQ = Diabetic Aqueous extract; N. AQ = Non Diabetic Aqueous extract.

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 diabetic untreated rats compared to the normal rats (Figure 1). 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.

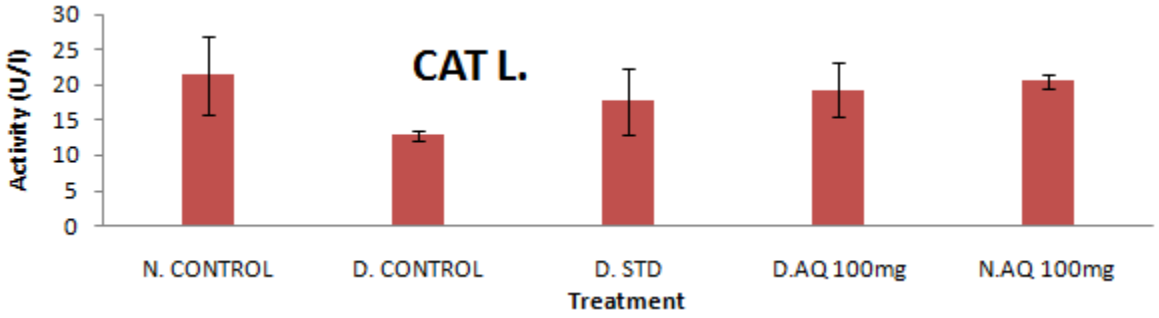

Figure 5: Catalase (CAT) activity in normal and diabetic rats treated with *V. doniana* ethanol extract and glibendamide
N. CONTROL= Normal Control; D. CONTROL = diabetic Control; D. STD = Diabetic Standard Drug (Glibenclamide); D.AQ = Diabetic Aqueous extract; N. AQ = Non Diabetic Aqueous extract.

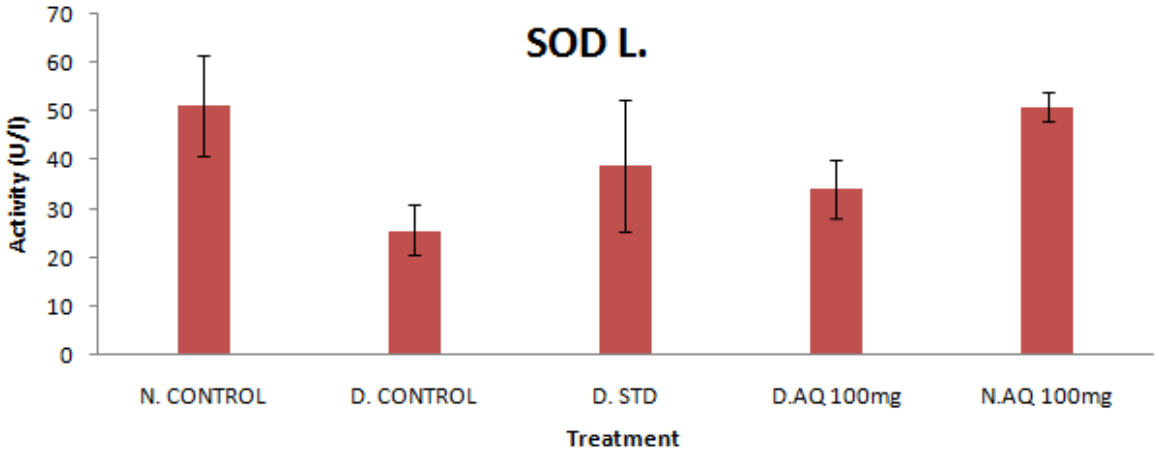

Figure 6: Superoxide dismutase SOD activity in normal and diabetic rats treated with *V. doniana* ethanol extract and glibendamide
N. CONTROL= Normal Control; D. CONTROL = diabetic Control; D. STD = Diabetic Standard Drug (Glibenclamide); D.AQ = Diabetic Aqueous extract; N. AQ = Non Diabetic Aqueous extract.

Effects of the extract on serum bilirubin
Administration of streptozotocin caused significant ($P<0.05$) elevation in serum total, direct and indirect bilirubin concentration in the control animals compared to normal (Figure 7, 8 and 9).

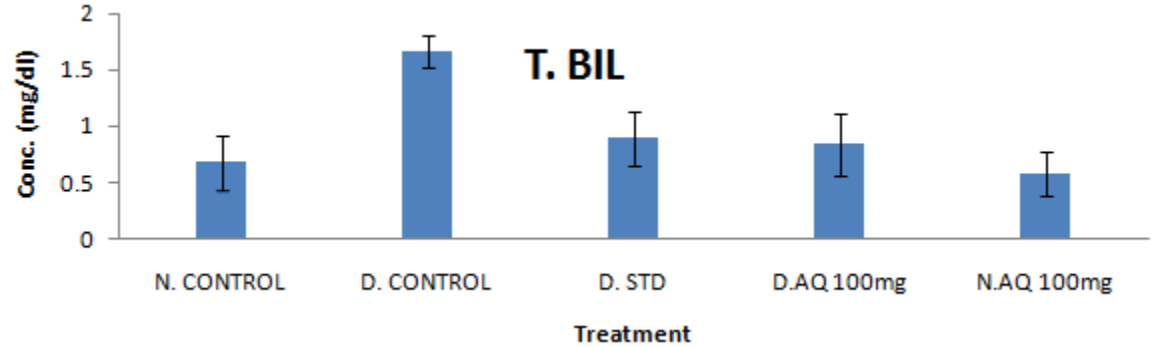

Figure 7: Serum total bilirubin concentration in normal and diabetic rats treated with *V. doniana* ethanol extract and glibendamide

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.

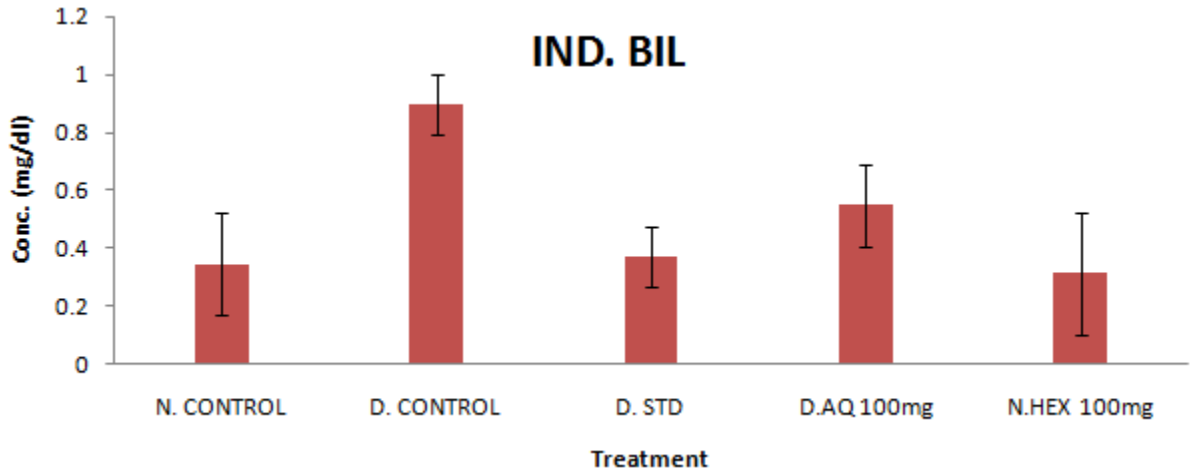

Figure 8: Serum indirect bilirubin concentration in normal and diabetic rats treated with *V. doniana* ethanol extract and glibendamide.

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.

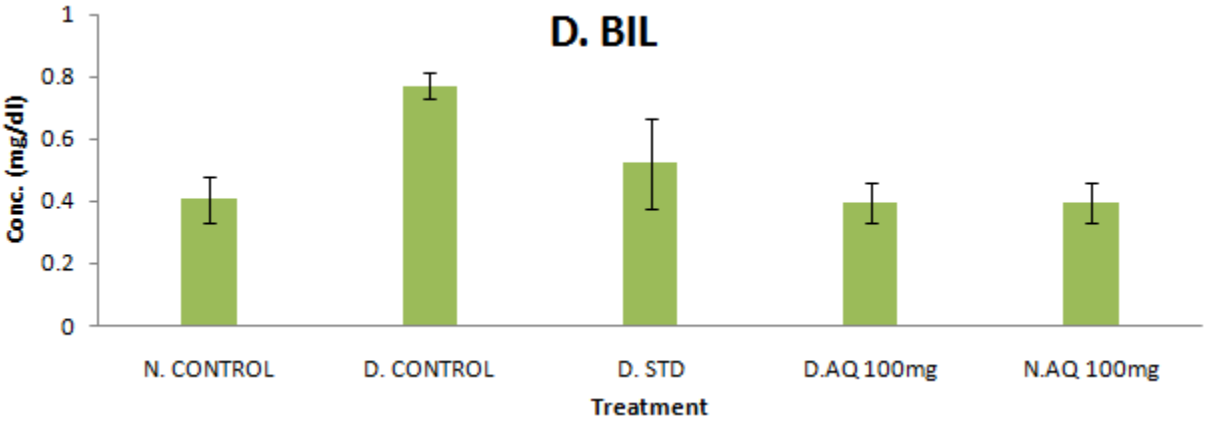

Figure 9: Serum direct bilirubin concentration in normal and diabetic rats treated with *V. doniana* ethanol extract and glibendamide

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.

Both extract and glibenclamide treatment caused significant reduction in bilirubin concentration in the experimental rats compared to normal. However administration of extract to normal rat showed no significant increase/decrease in total, direct and indirect bilirubin concentration.

Discussion

Diabetes is currently considered as a vascular disease (Ibrahim and Rizk, 2008). It has also been considered by researchers that hyperglycaemia-induced oxidative stress is a critical pathogenic mechanism that initiates a plethora of cascade metabolic and vascular perturbations (Ibrahim and Rizk, 2008; Housom *et al.*, 2001 and Hunt *et al.*, 1988). Studies have revealed the beneficial effects of some secondary plant metabolities that posses antioxidant activites in diabetes management.

Lipid peroxidation was investigated in our study by assessing the hepatic levels of TBARS, a significant increase in TBARS levels of diabetic rats was observed when compared to normal control rats. Numerous studies with human and animal models have also shown increased lipid peroxidative status in membranes of different tissues in diabetes (Feillet-Coudray *et al.,* 1999; Kakkar e*t al;* 1998; Aydin *et al*; 2001; Obresova *et al*; 2003 and Ugochukwu and Courbone, 2003). The extract produced significant decreases in TBARS levels in treated diabetic rats when compared to diabetic control rats. Treatment with glibenclamide also caused a slight decrease in TBARS levels of the treated rats. These reducctions could lead to a decrease in oxidative stress and hence a reductions in the rate of progression of diabetic complications in the liver.

Figure 2, 3 and 4 represent the changes in the activities of aspartate transaminase, alanine transaminase and alkaline phosphatase. In the assessment of liver damage by the determination of enzyme levels such as aspartate transaminase and alanine transaminase are largely used. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Watkins and Seef, 2006). Hepatocellular necrosis leads to high level of serum markers in the blood, among these, aspartate transminase, alanine transaminase represents 90% of total enzyme and high level of alanine transminase in the blood is a better index of liver injury, but the elevated levels of enzymes are decreased to normal levels after treatment with the extract. Alkaline phosphatase concentration is related to the functioning of hepatocytes, high level of alkaline phosphatase in the blood serum is related to the increased synthesis of it by cells lining bile canaliculi usually in response to cholestasis and increased biliary pressure (Handa and Sharma, 1990). Increased level was obtained owing to steptozotocin administration and it was brought to normal level by the extract treatment. Treatment with *V. doniana* aqueous leaf extract decreased the serum levels of aspartate transaminase, alanine transaminase and alkaline phosphatse towards the respective normal value that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by STZ. The above changes can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchymal cells.

Hyperbilirubinemia was observed due to excessive heme destruction and blockage of biliary tract. As a result of blockage of the biliary tract there was mass inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged and dead hepatocytes, this is in line with the report given by Gaw *et al.,* (1999). Administration of extract decreased the level of bilirubin, suggesting that it offered protection.

Catalase which has been known to scavenge and detoxify H_2O_2 showed a decreased activity in the diabetic control rats probably due to decreased concentration by H_2O_2 in the system (Esra *et al*., 2004). Treatment with the extract significantly ($p>0.05$) increased the activity in the treated rats indicating a possible attenuation of oxidant stress. SOD activity was observed to decrease in the diabetic rats compared to the normal control rats probably acting in a compensatory mechanism to maintain homeostasis. The increase in SOD activity may also be due to decreased mutation of superoxide anions due to their decreased production at the onset of diabetes. The diabetic rats treated with the extract showed increased SOD activity while treatment with glibenclamide demonstrated less increase in SOD activity. This suggests that the extract may have reduced the production of ROS with a concomitant increase in SOD activity.

Our results therefore indicate that the aqueous extract when used for diabetes management may control and or prevent the development of diabetic complications arising from increased oxidative stress and lipid peroxidation.

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