THE EFFECT OF *SARCOCEPHALUS LATIFOLIUS* AND *DANIELLA OLIVERI* ROOTS EXTRACT ON RENAL OXIDATIVE STRESS IN DIABETIC RATS.

Iwueke, A.V[1*]. Nwodo O.F.C. [2], Shoyinka S.V. [3], Joshua P.E[2]., Iheanacho K.M.E[1] Ojiako O.A. [1]

1. *Department of Biochemistry, School of Science Federal University of Technology, Owerri, Nigeria.*
2. *Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu Nigeria.*
3. *Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu Nigeria*

ABSTRACT

Sarcocephalus latifolius and *Daniella oliveri* are tropical plants whose roots are used in combination for the management of diabetes mellitus among other medicinal uses. This study investigated the effect of the aqueous extract of these roots (SDAE) on renal lipid peroxidation products (MDAs) and oxidative stress in non-diabetic control and alloxan-induced diabetic rats. 20 male wistar rats were induced in this study. Moderate diabetes mellitus was induced with alloxan (65mg/kg body weight) in 15 rats; these animals were further grouped into three (B,C & D). Group B was left untreated, Group C and D were treated intragastrically with SDAE (250mg/kg) and glibenclamide (5mg/kg) respectively. Treatments were administered twice daily for three weeks. MDA significantly ($p<0.05$) increased, while the reduced glutathione (GSH) concentration decreased in the kidney of untreated diabetic rats. This was accompanied by a significant increase ($p<0.05$) in both catalase and superoxide dismutase activities. The diabetes-induced increase in MDA was non-significantly ($p>0.05$) reduced by treatment with the extract. The result also indicated that the aqueous extract normalized the GSH concentration in the diabetic kidney. The extract caused a non-significant decreased in the SOD activity and almost normalized the catalase activity in the diabetic kidney. Renal histopathological changes induced by diabetes were improved by the extract to an extent. The current study suggests that the extract is effective in preventing oxidative/peroxidative stress and also ameliorates endogenous antioxidant enzyme activities in the diabetic kidneys; confirming its ethnopharmacological use for the management of diabetes and diabetes-induced complications.

INTRODUCTION

Hyperglycaemia in diabetes mellitus generates free radicals by mechanisms that are thought to involve metal-catalysed oxidation of glucose, oxidative degeneration and protein glycation (Hunt *et al.,* 1988; Hunkar *et al.,* 2002). Increased production of reactive oxygen species seems to be a vital biochemical occurrence in some pathological events that cause diabetic complications such as atherosclerosis, and diabetic nephropathy. (Gupta *et al.,* 2002). Diabetes also disturbs natural antioxidant defense systems, altering antioxidant enzymes activities in various tissues including the kidneys (Rauscher *et al.,* 2000, 2001; Mak *et al.,* 1996; Kedziora-Kornatowska *et al.,* 1998; Genet *et al.,* 2002).

In recent times, there is a renewed and growing interest in the use of plant-derived biologically active compounds as drugs or as leads in the manufacture of more potent medicaments (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).

A herbal formulation prepared with *Sarcocephalus latifolius* [Rubiaceae] and *Daniella oliveri* [Caesalpiniaceae] roots in equal proportion is employed in the management of diabetes in the south eastern part of Nigeria. Different parts of these plants are also known to be used independently and variously for health management in the tropics (Boye *et al.,* 1990; Madubunyi, 1995; Gidado *et al.,* 2005; Basile *et al.,* 1988; Raffauf, 1992). The results from our previous studies indicated that the combined extract of these plants possesses antihyperglycaemic (Iwueke and Nwodo, 2008) and hypolipidaemic (Iwueke and Nwodo,

2009) activities in the serum of alloxan-induced diabetic rats.

In view of the above consideration, the present study was designed to investigate the effect of the combined root extract on the diabetes-induced changes in renal oxidative stress and histopathology.

EXPERIMENTALS

Plant Material

Roots of *S. latifolius* and *D. oliveri* were collected in December, 2007 and were authenticated at the International Centre for Ethnomedicine and Drug Development, Nsukka; Voucher specimens were deposited as InterCEDD:76 AO and InterCEDD:158 AO respectively. The plant roots were cleaned, dried at room temperature under continuous ventilation and reduced to coarse powder with a grinder. The root powder (500g) containing an equal amount of each plant material was macerated in 2L of distilled water and extracted exhaustively by decoction. After cooling and filtering, the filtrate was concentrated under reduced pressure at 40^{o}C to obtain a residue of 38.499g (7.46% yield) SDAE. For the dose used, the volume administered was calculated using the equation of Tedong et al (2007): V (ml) = (D x P) /C
where D = dose used (g/kg body weight). P = body weight (g), C = concentration (g/ml); V = Volume.

Animals

The experimental animals used in this study were male Sprague Dawley rats with an average weight of 230g. The animals were allowed to acclimatize to the laboratory conditions (room temperature, 12-h light/dark cycle) for seven days and were maintained on standard animal feed and water *ad libitum*.
All animal experiments were in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals (Pub. No.85-23, revised 1985).

Twenty animals used for the study were distributed into 4 groups, 3 groups were made diabetic by intravenous injection of freshly prepared alloxan monohydrate (65mg/kg body weight) (Sign-Aldrich, U.S.A.) in sterile normal saline. Diabetes was confirmed by the determination of fasting blood glucose level (FBGL) of 200mg/kg and above on the third day after administration of alloxan.

The animal groups were as follows:
Group A: Normal control, received only distilled water.
Group B: Diabetic control, received only distilled water.
Group C: Diabetic treated with the extract (SDAE 250mg/kg).
Group D: Diabetic treated with glibenclamide (5mg/kg)
All the treatments were given twice daily for 21 days via an intubator.

After 3 weeks of treatment, the rats were euthanized by anesthesia using chloroform. The kidneys were surgically excised immediately, washed separately in 1.15% KCl, dried on filter paper and stored at -10^{0}C.

Tissue Preparation/Analysis

Weighed amounts (0.5g) of kidney tissues were separately homogenized in 10 parts of ice-cold 50M Tris-HCL, 1.15% KCl using a homogenizer and centrifuged at 1000 x g for 15mins at 4^{0}C. The supernatant was collected after centrifugation and refrigerated at $\leq$ 4^{0}C until use.

The homogenates were used for measuring the antioxidant scavenging enzyme activities, non enzymic antioxidant (GSH) and lipid peroxides. The presence of the homogenizing buffer and low temperature protects tissue antioxidants during processing. Adequate care was taken to avoid unnecessary exposure to oxygen by following each step quickly.

BIOCHEMICAL ESTIMATIONS

Assays of enzymatic antioxidants

The activity of superoxide dismutase (SOD, E.C.1.15.1.1) was assayed using adrenaline as a substrate by the method of Fridovich (1989) and was expressed as unit/mg protein. One unit of enzyme is defined as the amount required for 50% inhibition of adrenaline auto oxidation.

Catalase activity (CAT, E.C. 1.11.1.1) was assayed by measuring spectrophotometrically the rate of decomposition of hydrogen peroxide (H_2O_2) at A_{570} over 3min (1min interval) according to the method of Sinha (1972). The enzyme activity for tissues was expressed in terms of 'Kat f' as kS^{-1} mg^{-1} protein, where k is the first order rate constant.

Assay of Non-enzymatic Antioxidant.
Reduced glutathone (GSH) was determined by the method of Jollow et al (1974). The method is based on the formation of a relatively stable chromophoric product (A_{412nm}) on reacting a sulfhydryl compound (GSH) with Ellman's reagent. Quantity of GSH in tissue sample was calculated using standard GSH and values were represented as µg/mg protein.

Thiobarbituric acid reactive substances (TBARS). Renal lipid peroxide formation was detected spectrophotometrically by assesing the level of thiobarbituric acid reactive substances and expressed as malondialdehyde (MDA) equivalent. This was measured according to the method of Varshney and Kale (1990). The pink coloured adduct formed at 80^0c was read at 532mm. Calculation of MDA is based on $\varepsilon_{MDA}= 1.56x10^{-5}\ M^{-1}\ Cm^{-1}$. Values of MDA were expressed as n mol/mg protein.

Protein Estimation
Protein in the kidney samples was determined by the Biuret method as described by Gornall *et al* (1949). Bovine serum albumin (BSA) was used as a standard.

Histopathological Study
Histopathological investigation of the kidneys were done according to the method of Lamb (1981) reported by Pieme (2006). Thin sections (1-2cm diameter) of the tissues were fixed in 10% formalin for 24 hr, washed for 24 hr, dehydrated and cleared in xylene to remove absolute alcohol. The cleared samples were infiltrated and embedded in paraffin at 50^0C. The embedded bodies were microtomed and mounted on slides which were stained with Haematoxylin-eosin and observed under the light microscope.

Statistical Analysis
Data was analysed using analysis of variance (ANOVA) and reported as mean± S.D, n=5. The difference of the means was calculated using Fisher LSD post HOC test. P values less than 5% was considered statistically significant ($P<0.05$).

RESULTS
EFFECTS OF THE EXTRACT (SDAE) ON ENZYMATIC ANTIOXIDANTS

Superoxide dismatase (SOD)
A significant ($p<0.05$) increase in SOD activity was observed in the diabetic untreated animals as compared to the normal rats (Fig.1a). Treatment with the extract (SDAE) showed a non-signifcant ($p>0.05$) decrease in the activity while glibenclamide elicited no difference in the SOD activity compared to the diabetic control rats.

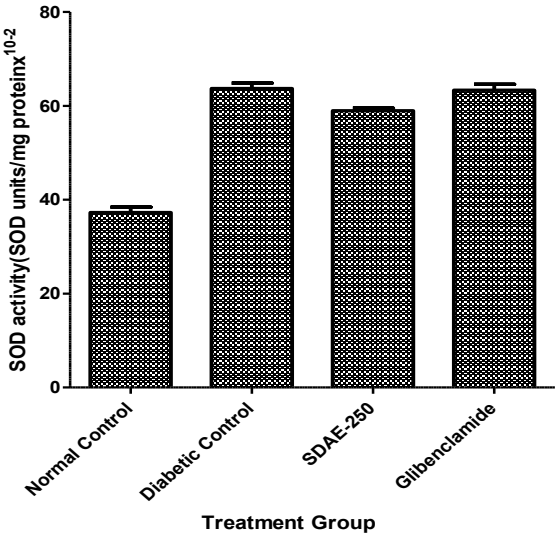

***Fig. 1a**: Effect of repeated administration (21 days) of the extract (SDAE) and glibenclaimide on SOD activity*

Catalase
It was observed that the catalase activity in the diabetic control animals was significantly higher ($p<0.05$) than those of the normal control group of rats (Fig.1b). The activity of catalase in the diabetic treated animals significantly ($p<0.05$) reduced and compared well with that of the normal control animals.

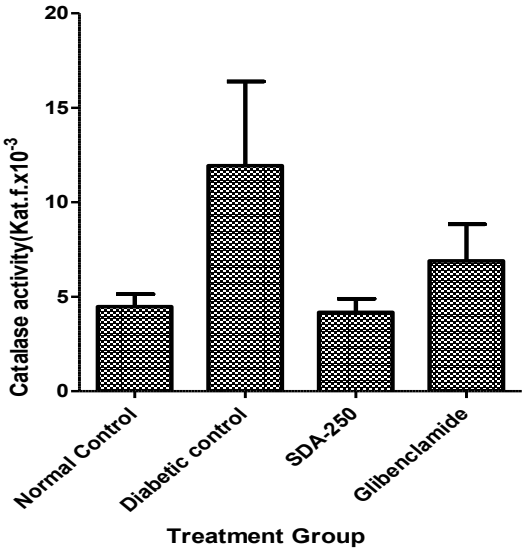

Fig. 1b: *Effect of repeated administration (21 days) of the extract (SDAE) and glibenclamide on catalase activity in the kidney.*

ALTERATIONS IN THE LEVELS OF NON-ENZYMATIC ANTIOXIDANT

Glutathione (GSH)

GSH content was significantly depleted in the kidneys ($p<0.05$) of diabetic control animals (Fig.2). Treatment with the extract improved the level of GSH. Glibenclamide treatment also caused a significant ($p<0.05$) increase in the GSH level when compared with the diabetic control animals.

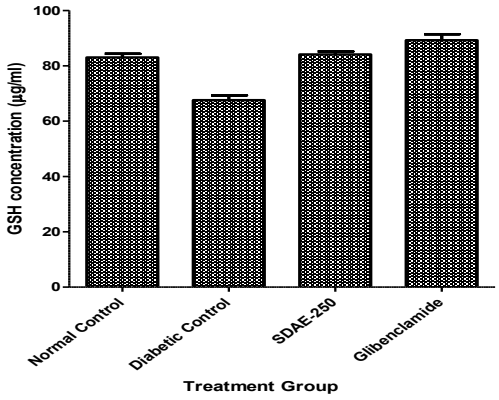

Fig. 2: *Effect of repeated administration (21 days) of the extract (SDAE) and glibenclamide on GSH concentration in the kidney*

Alteration in malondialdehyde Content

MDA content (Fig.3) was non-significantly elevated ($p<0.05$) in the kidneys of diabetic untreated rats as compared to normal animals. This was reduced in both the extract and glibenclamide treated animals.

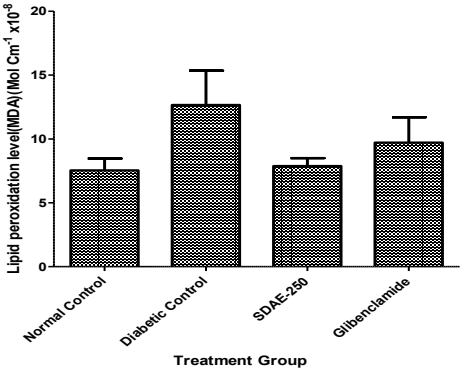

Fig. 3: Effect of repeated administration (21 days) of the extract (SDAE) and glibenclamide on lipid peroxidation level (MDA)

The light micrographs showing the histological appearance of the kidney of normal, diabetic untreated and treated rats are presented in Figs. 4a-4c. Histopathological examination of kidney sections of non-diabetic rats showed the normal histologic features for the mammalian kidney (Fig.4a). In comparison to the diabetic untreated rats (Fig.4b), there were severe degeneration and necrosis of tubular epithelial cells and mononuclear leucocytes. Glomerular and interstitial spaces were congested. Treatment with the extract moderately reduced the tubular degeneration and necrosis associated with diabetes (Fig.4c). The cells also showed mild mononuclear cells infiltration of the interstices and the peri-glomerular spaces.

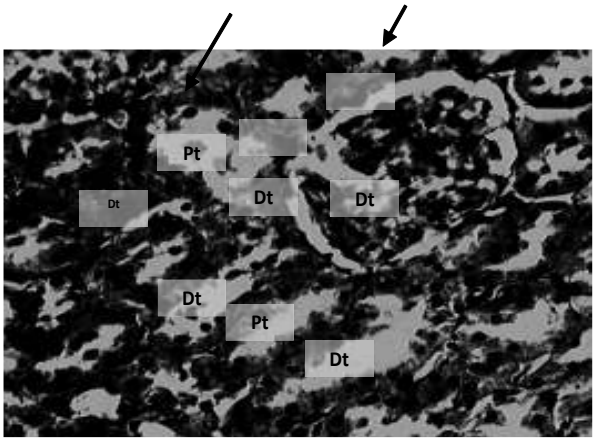

Fig. 4b: *Kidney section of diabetic untreated rat showing widespread degeneration of endothelial cells of the renal corpusules (arrow), Proximal tubule epithelia (Pt) cells and Distal tubule epithelial (Dt) cells. H & E Stain x 200*

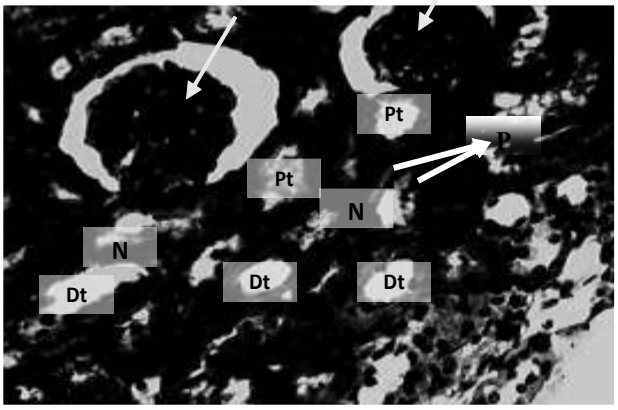

Fig.4a: *Kidney section of normal rat showing endothelial cells of the renal corpusules (arrow), Proximal tubule epithelia (Pt) cells and Distal tubule epithelial (Dt) cells. H&E Stain: × 200*

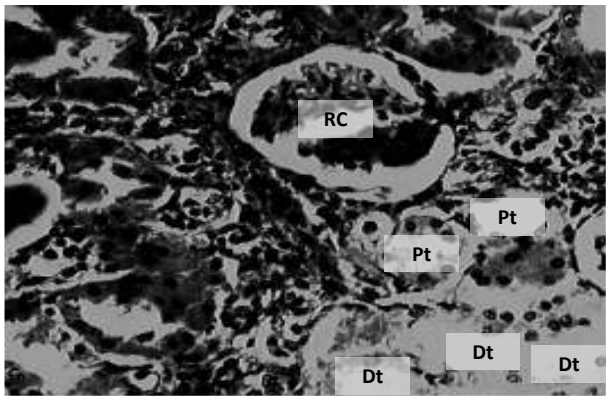

Fig4c: *Kidney section non-diabetic rat treated with 250mg/kg of SDA showing focal area of Proximal tubules (Pt) and Distal tubules (Dt) degeneration and necrosis. Note Renal Corpuscle (RC) with endothelial cells degeneration and mild mononuclear leukocytes aggregation around the Corpuscle (arrow). H&E Stain: × 200*

Discussion

Hyperglycemia in diabetes mellitus has been demonstrated to increase the generation of reactive oxygen species (ROS) (Ha and Kim, 1994). This invariably leads to increase in lipid peroxidation (Hunt *et al.,* 1988) and oxidative stress which is centrally implicated in the pathogenesis of diabetic complications like diabetic nephropathy. (Hayashi *et al.,* 2001; DCCT, 1995; Kedziora-Kormatowska *et al.,* 2002, Limaye *et al.,* 2003; Koya *et al.,* 2003). However, endogenous preventive antioxidants such as superoxide dismutase, catalase and glutathione peroxidase are the first line of defence against reactive oxygen species (ROSs) toxicity. (Ayidin *et al.,* 2001).

In the present study, superoxide dismutase (SOD) activity was increased in the diabetic situation, the observed increase may be due to enhanced level of superoxide radicals (O_2) in the diabetic rats in the bid to maintain homeostasis (Cho *et al.,* 2002). The diabetic rats treated with the extract (SDAE) showed reduced activity of SOD while treatment with glibenclamide elicited a very negligible reduction in SOD activity. This may suggest that there was an initial increase in SOD activity due to production of superoxide anions at the onset of diabetes, but treatment with the extract reduced further production of ROS and hence decreased the SOD activity.

A significant increase in catalase activity observed in the diabetic controls suggest an increase in the concentration of H_2O_2 since catalase scavenges and detoxifies H_2O_2. Catalase activity was also increased in the glibenclamide treated rats compared to the normal control rats. The catalase activity values obtained for the rats treated with the extract paralleled that of the normal control rats. Enhanced activities of catalase and glutathione peroxidase have been reported in rat models of diabetes (Ugochukwu and Courborne, 2003). This directly supports our findings.

The major endogenous thiol antioxidant in the biological system is reduced glutathione (GSH). Depletion of reduced GSH either by conjugation and removal from the cell or oxidation to GSSG could significantly affect the overall redox potential of the cell (Hansen *et al.,* 2001). From the results, the concentration of GSH was found to be reduced in the diabetic untreated rats compared to the normal control rats. This finding is in consonance with the observation of other workers (Obrosova *et al.,* 2003; Lee *et al.,* 2000; Ugochukwu and Courborne, 2003), suggesting that reduced GSH concentration may be instrumental to the development of diabetic complications. We observed significant ($p<0.05$) increases in the concentration of GSH in the diabetic rats treated with both the extract and glibenclamide relative to the diabetic control rats, suggesting that these treatment modulate oxidative stress in diabetes.

Renal lipid peroxidative status was assessed as MDA levels. In agreement with other re-

searchers' observation (Obrosova *et al*., 2003; Kakkar *et al.,* 1997b; Ugochukwu and Courborne, 2003), we observed an increase in MDA levels in the kidneys of diabetic rats when compared to normal control rats. The extract (SDAE) as well as glibenclamide non-significantly decreased MDA levels in the treated rats. These decreases in MDA levels in the treated animals, though not significant could lead to a decrease in oxidative stress and a concomittant decrease in the risk of diabetic complications over-time.

Histopathological examination of the kidneys of the normal, diabetic untreated and diabetic treated rats indicates that the extract showed slight degeneration of the tubular epithelium while those treated with the extract and glibenclamide showed moderate degeneration; indicating variable degrees of protection from diabetic-induced nepthrotoxicity.

In conclusion, the aqueous extract of *Sarcocephalus latifolius* and *Daniella oliveri* roots in combination may prevent the pathogenesis of diabetic complications resulting from oxidative damage, especially nephropathy when used in the management of diabetes mellitus. In addition, the extract when used over-time may also be useful for the protection of the renal architecture of diabetic subjects, lending credence to the use of these plants in Nigerian ethno medicine.

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