

Modification of Lipid Peroxidation and Oxidative Stress In Hepatocytes of Diabetic Rats Treated with Root Extract of *Sarcocephalus latifolius* and *Daniella oliveri*.

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Abstract: Increased oxidative stress and lipid disorders may exacerbate complications of diabetes mellitus. This study, therefore was designed to evaluate the effect of aqueous root extract of *Sarcocephalus latifolius* and *Daniella oliveri* (SDAE) on oxidative stress and lipid peroxidation in non-diabetic and alloxan-induced diabetic rats. The alloxan (65mg/kg) induced diabetic rats were treated in 12-h cycles for 3 weeks with 250mg/kg of the extract and glibenclamide (5mg/kg); while the non-diabetic control rats received distilled water. The results indicated that hepatic catalase (CAT) and superoxide dismutase (SOD) activities were significantly increased in the diabetic animals ($p < 0.05$) for both activities. The extract significantly reduced catalase activity ($p < 0.05$) but non-significantly reversed the superoxide dismutase (SOD) activity observed in diabetic liver tissues. However, glibenclamide treatment showed slight modification in the changes observed in diabetic liver tissues. A significant increase in MDA content and decrease in reduced glutathione (GSH) was observed in the hepatocytes of the diabetic untreated rats. The diabetic induced changes in both MDA and GSH were distinctly reversed by the extract. The study concluded that the combined root extract reversed diabetes - induced oxidative changes in the hepatocytes, thus suggesting its use for the management of diabetic complications.

Key word: *Sarcocephalus latifolius*, *Daniella oliveri*, alloxan, hepatocytes, antioxidant.

INTRODUCTION

Diabetes mellitus is a metabolic disorder that precipitates disturbances in glucose, lipid and protein homeostasis (Van den Berghe *et al.*, 2006). In unmanaged diabetes mellitus, chronic hyperglycaemia give rise to a variety of biochemical events like oxidative stress. These contributes to the progression of the disease and its complications (Guigliano *et al.*, 1996, Hayashi *et al.*, 2001). Oxidative stress in cells and tissues results from the increased generation of reactive oxygen species 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) disrupting natural antioxidant 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).

A decoction of the roots of two tropical plants: *Sarcocephalus latifolius* (Rubiaceae) and *Daniella oliveri* (Caesalpiniaceae) (Rolfe) Hutch and Dalz in equal proportion is used in South Eastern Nigeria in the management of diabetes. Parts of both plants have been used in isolation for various ethropharmacological purposes. (Madubunyi, 1995; Boye *et al.*, 1990; Basile *et al.*, 1988; Raffauf 1992).

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Scientific studies also indicated that leaf extract of *Sarcocephalus latifolius* possesses hypoglycaemic effect (Gidado *et al* 2005 & 2008). Recently, we have reported the hypolipidemic, (Iwueke and Nwodo 2009), antihyperglycaemic (Iwueke and Nwodo 2008) and renal antioxidant (Iwueke *et al* in press) properties of the combined root extract of these plants.

This study sought to evaluate the potential antioxidant activity of the aqueous combined root extract of *S. latifolius* and *D. oliveri* in the hepatocytes of alloxan-induced diabetic rats.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials:

Fresh samples of *S. latifolius* and *D. oliveri* roots were collected from Nsukka, Enugu State, in January 2007. It was identified and authenticated by Mr. A. Ozioko of the International Centre for Ethnomedicine and Drug Development, Nsukka; Nigeria. Their voucher specimen numbers are Inter CEDD: 76AO and Inter CEDD: 158AO respectively. The cleaned roots were dried to a constant weight at room temperature under continuous ventilation and reduced to coarse powder with a mechanical grinder. Ground root powder of the plants in equal proportion was macerated in 4 volumes (w/v) of distilled water and decocted exhaustively. The filtrate was concentrated under reduced pressure to obtain an average yield of 7.46% w/w which was refrigerated. The extract was reconstituted freshly in distilled water at appropriate concentrations for the various experimental doses using the equation of Tedong *et al* (2007):

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

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

Experimental Animals:

Adult male Sprague Dawley rats weighing between 200 – 250g were used for the experiments. The animals were allowed to acclimatize for 7 days and maintained under normal laboratory condition of humidity, temperature (25±2°C) and light (12-h light/dark cycle) with free access to food and water *ad libitum*. All experimental protocols were in compliance with the National Institute of Health Guide for Care and Use of laboratory Animal (Pub. No.85-23, revised 1985).

Diabetes was induced by intravenous injection of freshly prepared alloxan monohydrate (65mg/kg body weight) (Sigma-Aldrich, U.S.A) in sterile normal saline. Control rats received only normal saline. Three days later, diabetes was confirmed in the alloxan treated rats by the determination of fasting blood glucose level (FBGL) of 200mg/dl and above. The experimental animals were then divided into four (4) groups (table 1). Treatment were administered using an intubator twice daily for 21 days. After 21-day-treatment, the rats were euthanised by anaesthesia using chloroform.

Table 1: Experimental design

Normal Control (NC)	5	Distilled water (5ml/kg)
Diabetic Control (DC)	5	Distilled water (5ml/kg)
Diabetic SDA (DSDAE)	5	SDAE (250mg/kg)
Diabetic Glibenclamide	5	Glibenclamide (5mg/kg)

250mg/kg was the effective dose of the SDAE extract from a previous dose-response study.

Livers were surgically removed, immediately washed with ice-cold 1.15% KCl and stored at – 10°C.

Tissue Preparation/Biochemical Assays:

Weighed liver samples (0.5g) were homogenised separately in 10 parts (w/v) of ice-cold 50mm Tris-HCl, 1.157 KCl using a homogeniser (Janke and Kunkel, Germany). The homogenised liver tissues were used for measurement of scavenging enzyme activities, non-enzymic antioxidant (GSH) and lipid peroxides (TBARS). In the presence of the homogenising buffer and low temperature, adequate care was taken to avoid unnecessary exposure to oxygen by following each step quickly.

Assay of Enzymatic Antioxidant:

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 glutathione (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. The quantity of GSH in tissue sample was calculated using standard GSH and values were represented as $\mu g/mg$ protein.

Thiobarbituric Acid Reactive Substances (TBARS):

Hepatic lipid peroxide formation was detected spectrophotometrically by assessing the level of thiobarbituric acid substances and expressed as malondiadehyde (MDA) equivalent. This was measured according to the method of Varshney and Kale (1990). The pink coloured adduct formed at $80^{\circ}c$ was read at $532nm$. Calculation of MDA is based on $E_{MDA} = 1.56 \times 10^{-5} M^{-1} Cm^{-1}$. Values of MDA were expressed as $n mol/mg$ protein.

Protein Estimate:

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

RESULTS AND DISCUSSION

Effects of the extract (SDAE) on enzymatic antioxidants:

Catalase:

A significant ($p < 0.05$) increase in catalase activity was observed in the diabetic untreated rats compared to the normal rats (Figure 1)

Treatment with the extract (SDAE) and glibenclamide showed a significant ($p < 0.05$) and non-significant ($p > 0.05$) decreases in activity respectively.

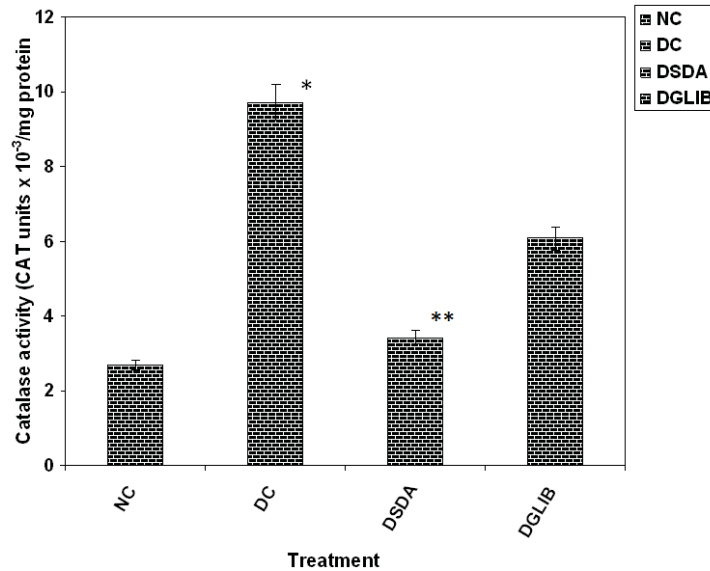


Fig. 1: CAT activities in normal and diabetic rats treated with SDA extract and glibenclamide. * $P < 0.05$ compared with NC; ** $P > 0.05$ compared with DC.

Superoxide Dismutase:

There was a non-significant ($p > 0.05$) increase in activity of SOD in the diabetic untreated rats compared to the normal control rats (figure 2). The activity of SOD in the diabetic treated rats reduced non-significantly ($p > 0.05$) with extract administration.

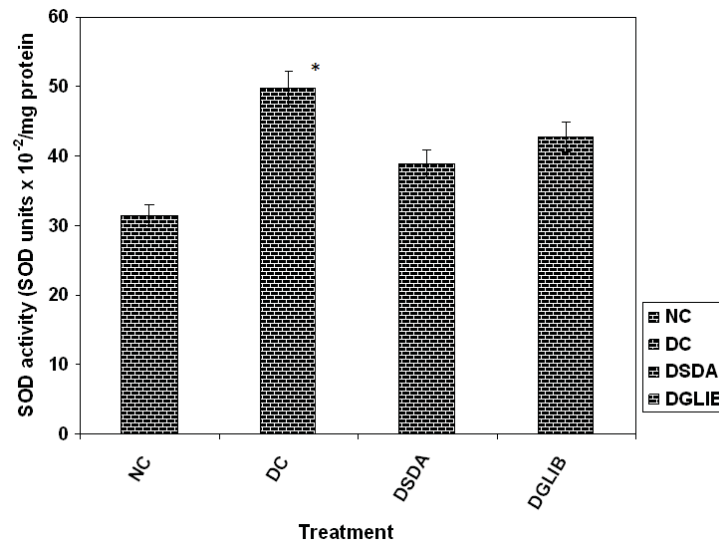


Fig. 2: SOD activities in normal and diabetic rats treated with SDA extract and glibendamide. *P<0.05 compared with NC; **P>0.05 compared with DC.

**Alterations in the Levels of Non-enzymatic Anti-oxidant:
Reduced Glutathione Gsh:**

GSH content was significantly (p<0.05) depleted in the hepatocytes of diabetic control rats. (Figure 3). Treatment with the extract (SDA) almost normalised the level of GSH.

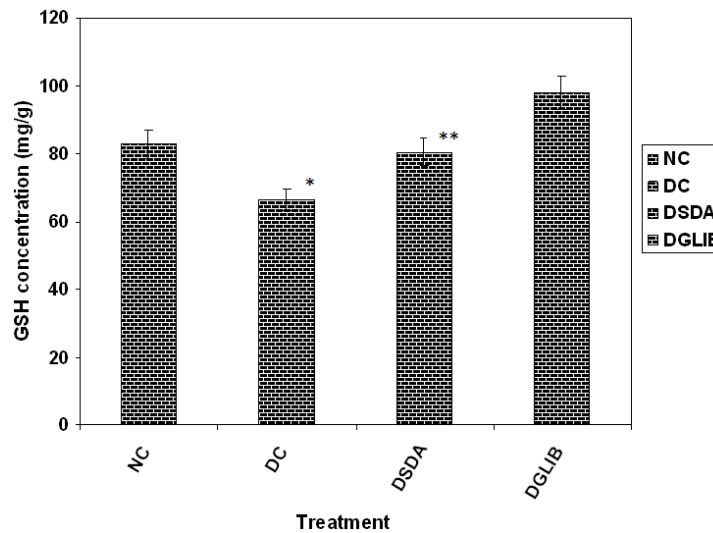


Fig. 3: Reduced glutathione concentrations in normal and diabetic rats treated with SDA extract and glibendamide. *P<0.05 compared with NC; **P>0.05 Compared with DC.

Malondialdehyde (MDA) levels:

MDA level was significantly (p<0.05) elevated (Figure 4) in the liver of diabetic control rats, when compared with the normal control rats. This increase was reduced significantly (p<0.05) in the SDA treated rats and non-significantly (p>0.05) in the rats treated with glibenclamide.

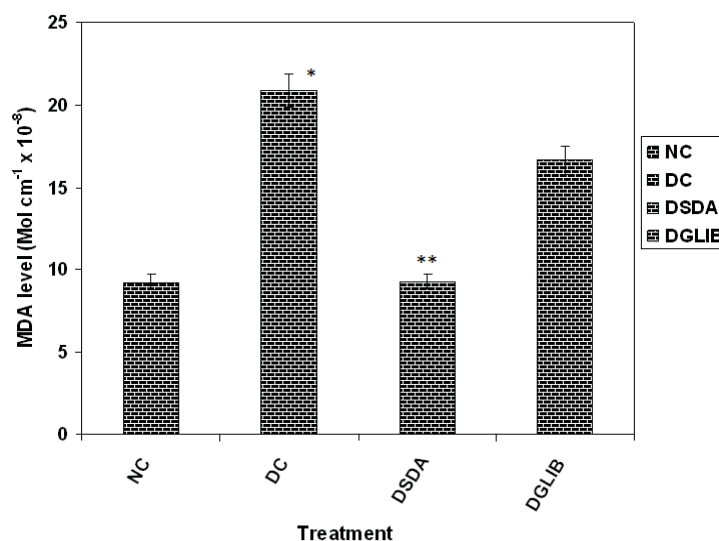


Fig. 4: MDA levels in normal and diabetic rats treated with SDA extract and glibendamide. *P<0.05 compared with NC; **P>0.05 compared with DC

Discussion:

There has been an explosive increase in the incidence of diabetes mellitus worldwide and so does the need for alternative and cost-effective therapies. 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, Hunt et al, 1988). Studies have revealed the beneficial effects of some secondary plant metabolites that possess antioxidant activities in diabetes management. Hence, we investigated the effect of the combined root extract of *S latifolium* and *D. Oliveri* on biomarkers of oxidative stress in hepatocytes of diabetic rats.

Catalase which has been known to scavenge and detoxify H₂O₂ showed an increased activity in the diabetic control rats probably due to increased concentration by H₂O₂ in the system. Our findings are in consonance with other studies with rat models of diabetes (Hunker et al; 2002, Ugochukwu et al, 2003). Some workers have found the reverse though. (Esra et al; 2004). Treatment with the extract significantly (p>0.05) reduced the activity in the treated rats indicating a possible attenuation of oxidant stress.

Studies have shown that oxidative stress induces the generation ROS scavenging enzymes (Nourooz-Zadeh et al, 1997). In this study, SOD activity was observed to increase 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 increased mutation of superoxide anions due to their increased production at the onset of diabetes. Our result is in tandem with other findings (Cho et al, 2002, Ugochukwu and Courbone 2003). The diabetic rats treated with the extract (SDA) showed reduced SOD activity while treatment with glibenclamide elicited a negligible reduction in SOD activity. This suggests that the extract may have reduced the production of ROS with a concomitant decrease in SOD activity.

Depletion of reduced glutathione (GSH) either by conjugation and removal from the cell or oxidation to oxidised glutathione could significantly affect the overall redox potential of the cell (Hansen et al, 2001). Our results indicate that the concentration of GSH in the diabetic rats was significantly depressed compared to the normal control rats. This is consistent with the reports of other researchers (Ibrahim and Rizk, 2008, Obrosora et al, 2003; Lee et al; 2000). Treatment of the diabetic rats with SDA and glibenclamide showed remarkable increases in the concentration of hepatic GSH. These increases may be contributory to the reduction of oxidative stress in the treated diabetic rats.

Lipid peroxidation was investigated in our study by assessing the hepatic levels of MDA, a significant increase in MDA 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 et al; 1998; Aydin et al; 2001; Obrosova et al; 2003, Ugochukwu and Courbone 2003).

The extract (SDA) produced significant decreases in MDA levels in treated diabetic rats when compared to diabetic control rats. Treatment with glibenclamide also caused a slight decrease in MDA levels of the treated rats. These reductions could lead to a decrease in oxidative stress and hence a reduction in the rate of progression of diabetic complications in the liver.

Our results therefore indicate that a decoction of the combined roots of *S. latifolius* and *D. oliveri* 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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