

## BLOOD GLUCOSE LOWERING ACTIVITY OF FIVE NIGERIAN MEDICINAL PLANTS IN ALLOXAN-INDUCED DIABETIC WISTAR ALBINO RATS

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### ABSTRACT

*The blood glucose lowering effects of the aqueous leaf extracts of Cassia alata, Acalypha torta and Breynia nivos, and aqueous root extracts of Daniellia oliveri and Nauclea latifolia traditionally employed in Nigeria in the management of diabetes mellitus were compared in alloxan-induced diabetic Wistar rats. The same dose, 100.0 mg/kg body wt. of each extract was used. Alloxan (150.0 mg/kg body wt.), extracts, and the reference drug, glibenclamide (20.0 mg/70kg body wt.) were solubilized in normal saline and administered intraperitoneally. Investigation into the mechanisms of action of the most potent extract was carried out by determining its effects on lipid-peroxidation, superoxide dismutase (SOD), catalase and glucokinase. The extracts caused significant ( $p < 0.01$ ) reductions in the blood glucose concentrations of the diabetic animals, thereby validating their antidiabetic properties. The order of potency was Nauclea latifolia (79.9%) > Acalypha torta (66.5%) > Breynia nivos (50.4%) > Cassia alata (39.2%) > Daniellia oliveri (31.9%). The most active extract, Nauclea latifolia, was more potent than glibenclamide. N. latifolia extract decreased malonylaldehyde concentration and superoxide dismutase activity, although these effects were not statistically significant, whereas significant reduction in catalase activity was noted. Alteration of body's oxidant-antioxidant balance may be enhancing the antidiabetic activity of Nauclea latifolia root. Glucokinase activity was also significantly ( $p < 0.05$ ) elevated.*

**Keywords:** Antidiabetic activity, *Cassia alata*, *Breynia nivos*, *Acalypha torta*, *Nauclea latifolia*, *Daniellia oliveri*, Lipid-peroxidation, Superoxide dismutase, Catalase, Glucokinase

### INTRODUCTION

The successful use of plant extracts in the prevention, management and treatment of communicable and non-communicable diseases has over the years attracted the attention of scientists worldwide (Falodun *et al.*, 2006; Palombo, 2011). At least 80% of Africans depend on herbal medicine for their health care (Iwu *et al.*, 1999; Kilani, 2006; Ajose, 2007; Okwu and Uchegbu, 2009). Presently, in Nigeria, many people in the rural communities depend on herbal remedies due to its easy accessibility and low cost of treatment (Okigbo and Mmeka, 2006, Oreagba *et al.*, 2011); and

this practice has been recommended and encouraged by the World Health Organization in countries where the conventional treatment is inadequate and expensive (WHO, 1980).

Diabetes mellitus which is a disorder of carbohydrate metabolism characterized by high concentration of glucose in the blood (hyperglycemia), frequent urination and excretion of glucose in the urine (glucosuria) has been recognized as one of the silent killer diseases. Precisely, diabetes mellitus is now considered one of the leading causes of death all over the world, affecting over 100 million people worldwide (Abdullahi *et al.*, 2001). The World Health Organization has estimated that

by the year 2025 the number of diabetic patients worldwide would rise to 300 million people (Aravind *et al.*, 2002; WHO, 2014). Chronic uncontrolled hyperglycemia seen in diabetes mellitus causes glycation of body proteins, a factor responsible for many of the secondary complications affecting the eyes, kidneys, nerves and vascular tissues. The microvascular and macrovascular complications, in addition to hyperglycemia and hyperlipidaemia, are the leading causes of morbidity and mortality in diabetic subjects (Nagappa *et al.*, 2003; Mathers and Loncar, 2006).

The importance of natural products (their derivatives and analogs) has been documented in literature. These products represent more than 50% of all drugs in clinical use, in which plant derived natural products represent about 25% of the total (Lawal *et al.*, 2010). Many plants with anti-diabetic potentials are currently in use in various parts of the world. For instance, in South-Eastern Nigerian ethnomedicine, aqueous leaf extracts of *Cassia alata*, *Breynia nivosa*, *Acalypha torta* and the roots of *Daniellia oliveri* and *Nauclea latifolia* are useful herbal remedies for diabetes mellitus. They are commonly found in Nigeria and other tropical African countries and are endowed with many therapeutic potential. The leaf extracts of *Cassia alata* exhibit analgesic, antifungal and anti-inflammatory activities (Villasenor *et al.*, 2002). *Acalypha torta* leaves possess antibacterial, antifungal, anti-malarial and antihypertensive effects (Irobi *et al.*, 1994; Ezekwesili *et al.*, 2008). *Daniellia oliveri* root has analgesic and stimulant properties (Lawal *et al.*, 2010), whereas *Nauclea latifolia* root extract is well-known for its anti-bacterial, anti-malarial and antihypertensive activities (Lawal *et al.*, 2010).

The search for a more effective and safer hypoglycemic plant extract that will render a protective and, or curative effects from diabetes and its complications would therefore continue to be a research work of considerable interest. This research study was therefore, designed to investigate and compare the anti-diabetic values of these selected Nigerian plants

in experimental rats with a view to determining the most effective of them.

Oxidative stress, caused by imbalance between the production of reactive oxygen species (ROS) and a biological system's ability to readily detoxify or scavenge released reactive intermediates, has been implicated by many researchers in the pathogenesis of some chronic conditions such as Alzheimer's disease, Parkinson's disease, diabetes mellitus (David *et al.*, 2005; Brooks *et al.*, 2011), rheumatoid arthritis and neurodegeneration (Valko *et al.*, 2007; Shaw *et al.*, 2014). Precisely, increased oxidative stress has been emphasized as the key factor in the development and progression of diabetes mellitus and its associated complications (Dallak and Bin-Jaliah, 2010), as well as the red blood cells damage clinically manifest in diabetic patients.

In view of this, the effects of the most potent extract on lipid-peroxidation (which generates free radicals in the system) and the body's endogenous anti-oxidant enzymes (superoxide dismutase and catalase) were also examined. Its effect on the glycolytic enzyme, glucokinase, was also examined.

## MATERIALS AND METHODS

**Animal:** Fifty-five male Wistar albino rats weighing between 120 – 160g purchased from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, were used. They were housed and maintained in animal cages in the Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka. The animals were kept on commercial animal pellets (Guinea Feeds Ltd, Delta State, Nigeria) and were allowed seven days for acclimatization within the laboratory environment. All the animals had access to both water and feed *ad libitum*.

**Antidiabetic Plants:** The leaves of *Cassia alata*, *Breynia nivosa* and *Acalypha torta*, and the roots of *Nauclea latifolia* and *Daniellia oliveri* were collected from Awka, Anambra State, Nigeria, on the 27<sup>th</sup> day of January, 2010. The samples were identified and authenticated by a taxonomist, Prof. J. C. Okafor, of the Agro-forest Research Institute, Enugu, where voucher

specimens were kept. The plant samples were properly washed with water, air dried at laboratory temperature and pulverized.

**Extraction:** Hot extraction method was used for the extraction procedure. A portion (500g) of each sample was boiled in 2000 ml of distilled water for 1 hour. The boiled plant leaves and roots were left in the containers for 24 hours for complete extraction. The infusions were then filtered using cheese cloth and Whatman number 1 filter paper. The filtrates were concentrated using rotary evaporator and the crude extracts obtained were refrigerated pending use for the experiments.

**Phytochemicals:** Phytochemical screening of the five extracts was carried out according to established procedures of Sofowora (1993) for the presence of alkaloids, flavonoids, saponins, tannins, cardiac glycosides, cyanogenic glycosides and anthracene glycosides.

**Antidiabetic Activity:** After acclimatization for seven days, animals used were weighed and their baseline blood glucose concentrations measured using One Touch Basic Glucometer (Code – 12). The control group 1 (seven rats) was separated and diabetes mellitus was induced in the remaining animals (48) with single intraperitoneal (i.p) injection of alloxan monohydrate at a dose of 150.0 mg/kg body weight.

Three hours after alloxan administration, the test animals were kept on 50% oral glucose solution to prevent hypoglycaemia usually caused by hyperactivity of the pancreas induced by alloxan two to three hours after its administration.

Two days after alloxan injection, the animals were weighed again and their blood glucose concentrations recorded using the same glucometer. Forty-two diabetic rats (with blood glucose concentrations of 250.0 mg/dl and above) were selected and divided into six groups 2, 3, 4, 5, 6 and 7 of seven rats each according to their body weights.

The diabetic animals in groups 2, 3, 4, 5 and 6 were treated with daily doses of 100.0 mg/kg body weight of aqueous leaf extracts of

*Cassia alata*, *Breynia nivosa* and *Acalypha torta* and aqueous root extract of *Nauclea latifolia* and *Daniellia oliveri*, respectively. Animals in group 7 were treated with the reference drug glibenclamide, at 20.0 mg/70kg body weight dose, whereas the control group 1 rats received normal saline, 1.0 ml/kg body weight. All the extracts and drug were dissolved in normal saline and injected intraperitoneally (i.p). The animals blood glucose concentrations were measured every morning for five days using the same glucometer. Animals in the test group 5 treated with the most potent extract were then anaesthetized with chloroform and blood samples collected by cardiac puncture for antioxidant enzymes and glucokinase assays.

### Antidiabetic Mechanisms

**Lipid peroxidation activity:** The method of Biye and Aust as described by Usoh *et al.* (2005) was employed. Coagulated blood sample was spun in a centrifuge for ten minutes at 3,000 rpm to obtain the serum which was taken up with a Pasteur pipette into labeled specimen tubes that were refrigerated until used. Serum aliquots (0.4 ml) were pipetted into the test tubes and were mixed with 1.6 ml of 0.25N HCL, 0.5 ml of 15.0% trichloroacetic acid (TCA) and 0.5ml of 0.375% of thiobarbituric acid (TBA). The reaction mixtures were then placed in 100°C boiling water for fifteen minutes, cooled and centrifuged at 3,000 rpm for ten minutes. The optical densities of the supernatants were recorded at 532 nm against a reagent blank which contained only distilled water.

**Superoxide dismutase activity:** Superoxide dismutase activity was assayed using the method of Okpuzor *et al.* (2009). Whole blood (1.0 ml) was diluted in 9.0 ml of distilled water to make a one in ten dilution of the blood. An aliquot of 0.2 ml of diluted blood was added to 2.5 ml of 0.05 M sodium carbonate buffer (pH 10.2) and left to equilibrate in the spectrophotometer and the reaction was started by addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was done quickly by inversion.

The reference cuvette contained 2.5 ml buffer, 0.2 ml of distilled water and 0.3 ml of substrate (adrenaline). The increase in absorbance at 480 nm was monitored at thirty seconds interval for one hundred and fifty seconds.

**Catalase activity:** Serum catalase activity was determined according to Beer and Sizer as reported by Usoh *et al.* (2005), by measuring the decrease in absorbance at 240 nm in a UV recording spectrophotometer by monitoring the decomposition of H<sub>2</sub>O<sub>2</sub> as described by Aebi (1984). The reaction mixture (3.0 ml) contained 0.1ml of suitably diluted serum in phosphate buffer (50.0 mM, pH 7.0) and 2.9 ml of 30.0 mM H<sub>2</sub>O<sub>2</sub> in phosphate buffer. An extinction coefficient for H<sub>2</sub>O<sub>2</sub> at 240 nm of 40 MM<sup>-1</sup> was used for the calculation. The specific activity of catalase was expressed as moles of H<sub>2</sub>O<sub>2</sub> reduced /min/mg. protein.

**Glucokinase activity:** A modified method of Newguard *et al.* (1983) as reported by Ugochukwu and Babady (2003) was adopted for the determination of hepatic glucokinase activity. The production of glucose-6-phosphate (total glucose phosphorylating capacity) by glucokinase in the presence of ATP was linked to the reduction of NAD<sup>+</sup> by glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides*. Liver tissues (0.5g) were homogenized in nine volumes of the homogenizing Tris-HCL buffer. After centrifugation at 10,000 rpm for twenty minutes at 4°C, the supernatants were used to measure the enzyme activity.

The protein content of the liver homogenate was determined using the Lowry method. The reaction mixture contained in a final volume of 1.0 ml, 0.3 ml of glucose-6-phosphate dehydrogenase, 10.0 µl of the diluted (1:10) liver homogenate and 0.1 ml of 10.0 mM D-glucose. The blank cuvette was devoid of glucose and glucose-6-phosphate dehydrogenase. Production of reduced nicotinamide adenine dinucleotide (NADH) was monitored at 340 nm.

**Statistical Analysis:** Arithmetic mean and standard error of mean were calculated and all

the data obtained analyzed statistically using Analysis of Variance (ANOVA). Statistical analyses were made by a SPSS for Windows version 13.0 packaged statistics program. All results represented were mean  $\pm$  standard error of mean (SEM) of six determinations.

## RESULTS AND DISCUSSION

The detected phytochemicals in the five plant extracts include alkaloids, anthracene glycosides, cardiac glycosides, cyanogenic glycosides, saponins and tannins (Table 1). Several reports have attributed the hypoglycaemic activity of many plants used in ethnomedicine for the management of diabetes to the presence of saponins and cyanogenic glycosides (Oliver and Zahnd, 1979; Omale and Haruna, 2011). Therefore these phytochemicals may be responsible for the observed biological activity of these plant extracts.

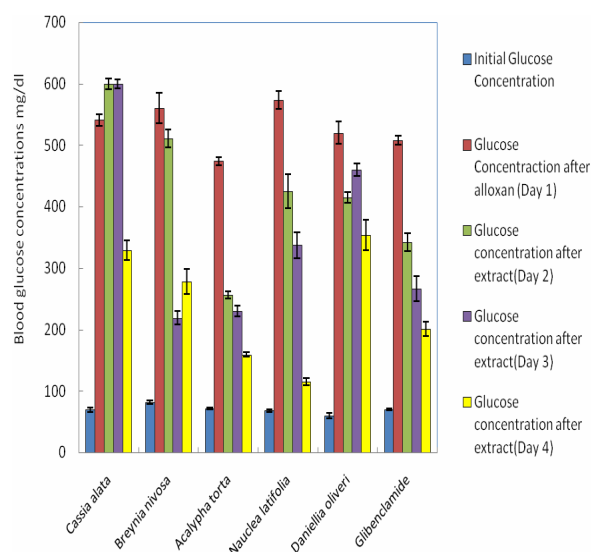
Intraperitoneal administration of alloxan elevated the blood glucose concentrations of all the rats (by up to 396.0%). Three - days treatment of the diabetic rats with the same dose (100.0 mg/kg) of aqueous leaf extracts of *Cassia alata*, *Breynia nivosa*, *Acalypha torta*, and aqueous root extracts of *Nauclea latifolia* and *Daniellia oliveri* caused decreases in the mean blood glucose concentrations of the diabetic rats (Figure 1). Their anti-diabetic potentials increased in the order, *Daniellia oliveri* < *Cassia alata* < *Breynia nivosa* < *Acalypha torta* < *Nauclea latifolia* (31.9%, 39.2%, 50.4%, 66.5% and 79.9%, respectively). This indicates that the most active extract is the aqueous root extract of *Nauclea latifolia* and it is more potent than the standard oral hypoglycaemic agent, glibenclamide, which reduced the blood glucose concentration by 59.0% (i.e. from 508.0  $\pm$  7.01 to 201  $\pm$  1.16). The blood glucose lowering effects of the five extracts were statistically significant at p<0.01.

These findings support the acclaimed use of these plant extracts in South Eastern Nigeria ethno medical practices for the management of diabetes mellitus.

**Table 1: Phytochemical composition of the five plant extracts**

Phytochemical	<i>Nauclea latifolia</i>	<i>Daniellia oliveri</i>	<i>Breynia nivosa</i>	<i>Cassia alata</i>	<i>Acalypha torta</i>
Alkaloids	++	++	-	+	++
Flavonoids	-	-	+	+	++
Saponins	+	++	+	-	+
Tannins	+	+	+	-	+
Cardiac glycosides	+	+	++	+++	+++
Cyanogenic glycosides	++	++	++	++	++
Anthracine glycosides	+	+++	-	+	-

Key: + = Trace, ++ = Moderately present, +++ = Abundant, - = Absent

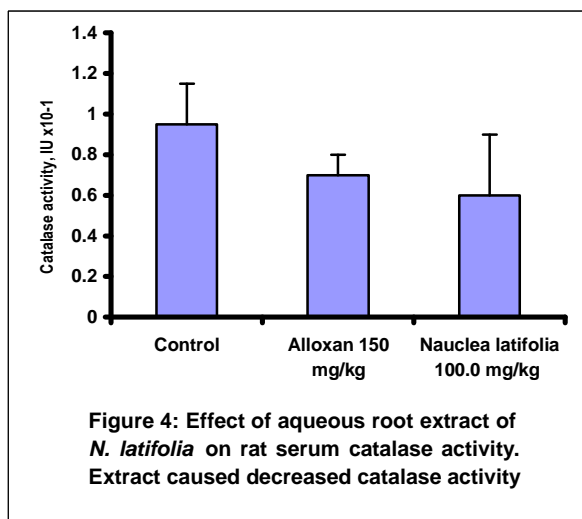
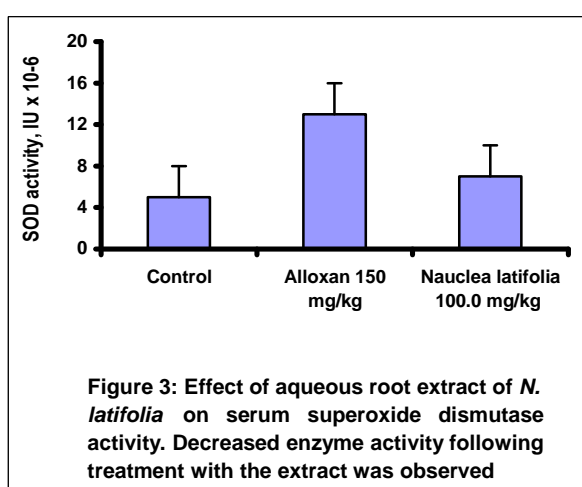
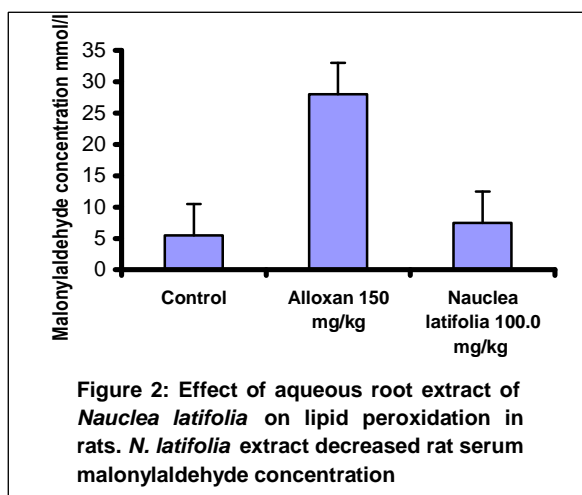


**Figure 1: The blood glucose levels of animals before and after treatments with extracts and glibenclamide. All the extracts produced decreases in blood sugar levels after 4 days**

Observations by many researchers revealed that many plants with hypoglycemic action may be exerting their effects through inhibition of endogenous glucose production (Eddouks *et al.*, 2003). Interference with gastrointestinal glucose absorption (Musabayane *et al.*, 2006) and, or presence of insulin-like substances may also account for the blood sugar lowering actions of some of these plants (Patel *et al.*, 2012). Inhibition of insulinase activity and, or increased insulin secretion from pancreatic  $\beta$  cells may also contribute to the antidiabetic property of some plants (Trivedi *et al.*, 2004; Yadai *et al.*, 2008; Hoda and Pierre, 2014), whereas some may increase  $\beta$  cells function in the pancreas by enhancing the regeneration of these cells (Jelodar *et al.*, 2007).

Increased generation of free radicals together with reduced level of antioxidant enzymes and vitamins in the body are considered to be the major contributors to oxidative stress (Dallak and Bin-Jaliah, 2010). Free radicals attack on membrane lipids, proteins and DNA have recently been implicated in many health disorders such as diabetes mellitus, cancers, neurodegenerative and inflammatory diseases (David *et al.*, 2005; Abheri *et al.*, 2010; Ochieng and Nandwa, 2010). Increased plasma lipid peroxidation and superoxide dismutase activity in type II diabetes mellitus have been well documented (Akalin *et al.*, 2008; Marjani, 2010). As a matter of fact, it has been deduced that alloxan monohydrate induces experimental diabetes mellitus in animals by enhancing the generation of hydrogen peroxide through the reaction of alloxan and reduced glutathione in vitro, as well as decreasing the catalase enzyme activity (Kazunori *et al.*, 2009). The effect of administration of 100.0 mg/kg body weight of aqueous root extract of *Nauclea latifolia* on lipid-peroxidation in the rats caused increased plasma level of malonylaldehyde (MDA) in the diabetic rats indicating that lipid-peroxidation is higher in all the diabetic rats compared to the control (Figure 2). Treatment of the diabetic rats, with *Nauclea latifolia* root extract produced a decrease in MDA concentration. This indicates inhibition of generation of reactive species by the extract.

The effect of *Nauclea latifolia* root extract on superoxide dismutase activity showed that the extract reduced the increase in SOD activity caused by alloxan injection due to elevated level of reactive peroxides.

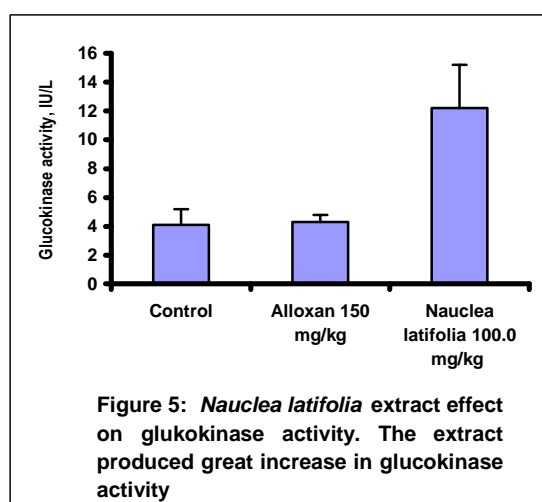


The extract's effects on lipid peroxidation and SOD activity were not significant ( $p > 0.05$ ) (Figure 3).

The effect of *Nauclea latifolia* root extract on the enzyme catalase revealed that the extract significantly diminished catalase activity ( $p < 0.01$ ) (Figure 4). The activity of

glucokinase enzyme in the liver of control rats, untreated diabetic rats, and diabetic rats treated with aqueous root extract of *Nauclea latifolia* indicated that alloxan injection did not have any remarkable effect on the activity of this enzyme (Figure 5). Rats treated with *Nauclea latifolia* extract showed a significant increase ( $p < 0.05$ ) in the activity of glucokinase when compared with control. This indicated that the extract activates this regulatory enzyme which is an integral part of glycolysis, a pathway responsible for the breakdown of glucose to pyruvate and consequent release of energy in form of adenosine triphosphate (ATP). The first step in the glycolytic sequence within the hepatocytes is the phosphorylation of glucose molecules to produce glucose-6-phosphate. This process is called the trapping process and it is catalysed by the enzyme glucokinase. Subsequently, the trapped glucose-6-phosphate can then be metabolized through the glycolytic pathway or the pentose phosphate pathway.

Activation of glucokinase may, therefore, be one of the mechanisms by which *Nauclea latifolia* produce anti-diabetic effect. Further investigations are required to elucidate the exact mechanism(s) of the hypoglycaemic action of *Nauclea latifolia* root.



**Conclusion:** Findings from our investigations validate the acclaimed antidiabetic potentials of *Cassia alata*, *Breynia nivosa*, *Acalypha torta*, *Nauclea latifolia* and *Daniellia oliveri*. *Nauclea latifolia* root extract is the most potent of the screened plant extracts. It exerted 79.9% reduction in the blood glucose concentration.

*Nauclea latifolia* extract may be acting by ameliorating the oxidative stress induced by alloxan, and or activating the key glycolytic enzymes such as glucokinase. Phytochemicals present in the extract such as saponins and cyanogenic glycosides could be contributory to the antidiabetic activity of the extracts.

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