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Effects of combined methanol extracts of *Nauclea latifolia* and *Voacanga africana* leaves on some biochemical indices of alloxan-induced diabetes in albino rats

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Abstract

Diabetes mellitus is a rampant global epidemy of multifactorial aetiology and risk factors. This present study was aimed at investigating the effects of combined methanol extracts of the leaves of *N. latifolia* and *V. africana* on some biochemical indices of alloxan-induced diabetes in albino rats. The rats were divided into eight groups of five rats each with group 1 as normal control, group 2 to 8 as treated diabetic control (2.0 mg/kg b.w. of glibenclamide), untreated diabetic control, 500mg/kg body weight. N.L., 500 mg/kg body weight V.A., 250 N.L.: 250 V.A., 375 N.L.:125 V.A and 125 N.L.:375 V.A respectively. The administration of the extracts was by oral (gavage) and the treatment lasted for 21 days. After 21 days, the rats were fasted overnight and sacrificed by cervical dislocation to get blood serum. All the analyses were done using standard analytical methods. The combined extracts exhibited antihyperglycaemic effect especially the 500 mg/kg b.w.N.L. and 375 NL and 125 V.A. The results disclosed that the rats of the normal control, treated diabetic control and the group administered with the 375 N.L.:125 V.A. significantly ($p<0.05$) increased the body weight of the rats. There was significant decrease ($p <0.05$) in the concentration of MDA in the rats of the treated diabetic control group and test groups when compared with rats of the untreated diabetic control. There was increase in catalase and GPx concentrations in the rats of the test groups when compared to rats of untreated diabetic control. The study also disclosed significant ($p < 0.05$) reduction in LDL-c, TC, TG and VDL-c, with an increase HDL-c. This study unveiled that the dose ratios of 375 N.L.:125 V.A, 500 mg/kg b.w. N.L and 250 N.L.:250 V.A efficiently reduced the blood glucose concentration and had better effect on the biochemical indices and therefore may be useful in the treatment of diabetes and diabetes-related complications.

Keywords: *Nauclea latifolia; Vocanga africana*; Methanol Extract; Biochemical Indices; Alloxan-induced diabetes; Albino rats.

1. Introduction

The use of traditional remedies in the amelioration of diabetes is widespread at urban as well as rural areas in Africa (Hu, 2011). Many patients opt for traditional remedies due to some factors including ease of accessibility of traditional medicine as well as financial constraint (WHO, 2013). Diabetes mellitus is characterised by hyperglycaemia due to

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defects in secretion and action of insulin (Kangralkar *et al*., 2010). This manifests as a result of failure of the pancreatic cells to produce enough insulin or when the body lacks the ability to utilise the insulin it produced effectively (Krishnasamy and Abell, 2018). Insufficient secretion caused by β-cells diruption results to insulin-dependent diabetes (Type 1) triggering. The gradual development of pancreatic β-cell dysfunction (abnormal insulin cellular action) has resulted to type-2 diabetes initiation. Diabetes mellitus has been documented as one of the chronic diseases of man and its frontiers are expanding by day. Some diabetic conditions can be managed only with diet while most require oral hypoglycaemic medications and/or insulin. There are shortcomings and side effects in the use of insulin and/or oral hypoglycaemic drugs (Rang and Dale, 2001).

Nauclea latifolia is used in sub-Saharan traditional system of medicine to treat different ailments indicating that they may represent a natural source of pharmacologically bioactive substances (Karou *et al*., 2011). *N. latifolia* root bark is used as antipyretic, tonic, analgesic and antidepressant. The leaf has the potential to relieve dysentery and diarrhoea (Etukudoh, 2013). The natives of Abia State in South-East, Nigeria use it exhaustively for the management of gastrointestinal ulcers (Alaribe *et al.,* 2014). Virtually all the plant parts are useful in the treatment of diseases (Arbonnier,2000).

Voacanga africana belongs to the family of Apocynaceae. It is an abundant, deciduous, mesophytic, sapwood, perennial shrub of the primary and secondary rain forest, within the tropical rain forest especially in Nigeria and the Guinea savannah wood belt. All the parts of *V. africana* plant has been used extensively for the treatment of many diseases. Its bark has been employed in wide range of disease reversal (Aldophina *et al.,* 2009). The roots and leaves decoction are used for antinociceptive effects (Igbe and Edike, 2015), anti-inflammatory and antioxidant properties (Ayoola *et al.,* 2008), antiulcer property (Tan *et al.,* 2000), effective in the treatment of anaemia and other blood diseases (Omodamiro and Nwankwo, 2013), also in the treatment of malaria, diarrhoea, heart aches and infant convulsion (Tan *et al.,* 2000).

2. Materials and Method

2.1. Collection of Plant Material and Identification

Leaves of *N. latifolia* and *V. africana* were collected from a local farm in Ukwa East Local Government Area of Abia State and were identified by the botanist, Dr. Duru C.M. of Department of Biological Sciences, Federal University of Technology Owerri, Imo State.

2.2. Preparation of Plant Material

The preparation of the leaves was done using the method as described by Agbafor (2004). The leaves of *N. latifolia* and *V. africana* were washed in running tap water to remove adhering debris and were chopped into small sizes and dried for 14 days by air-drying under shade. The dried leaves were pulverised into coarse powder using a pulverising machine.

2.3. Extraction of Plant Material

The extraction of the leaves was done using the method as described by Agbafor (2004). A 500g of each of the ground plant samples was weighed and dispensed into 1500 ml of 80% methanol. The mixture was allowed to stand for 72 hours with intermittent shaking. Filtration of the samples were carried out using muslin cloth and then Whatman No. 3 filter paper and each of the filtrate was concentrated using rotary evaporator (at 40^0c). The obtained semi-solid crude extracts were stored in a refrigerator at 4^0C until usage.

2.4. Experimental Animals

Adult albino rats (140 -180 g) of either sex were purchased from Nano Farms Ihiagwa, Owerri, and was housed in the animal house of Department of Biochemistry, Federal Polytechnic Nekede, Owerri. All the animals were fed with a regular diet and water ad libitum. The rats were protected and cared for according to the principle of laboratory animal care. The rats were allowed to acclimatize for the period of 7 days under standard environmental conditions (temperature 25 ± 2 °C; 12hrs light/dark cycle).

2.5. Induction and Assessment of Diabetes

The method of Osinubi *et al*. (2006) and Battu *et al.* (2007) were employed. Diabetes was induced by injecting freshly prepared 120mg/kg alloxan monohydrate intraperitoneally to overnight fasted albino rats. Hyperglycemia was confirmed three days after induction by drawing blood from the tail vein of the experimental animals. The animals

showing Random Blood Glucose (RBG) level ≥ 250 mg/dl were selected for further study. The animals were maintained in a diabetic state for 21 days.

2.6. Experimental Design

Group 1 served as normal control and was given 10ml/kg distilled water, group 2 served as treated diabetic control and was administered 2mg/kg glibenclamide, group 3 served as untreated diabetic control, while group 4, 5, 6, 7 and 8 served as test groups and were administered 500 mg/kg b.w. of *Nauclea laifolia*, 500 mg/kg b.w. of *Voacanga africana*, 250 mg/kg: 250 mg/kg *Nauclea latifolia* and *Voacanga africana,* 375mg/kg : 125mg/kg *Nauclea latifolia* and *V. africana* and125 mg/kg : 375 mg/kg *Nauclea latifolia* and *V. africana* respectively. The extracts and standard drug were administered orally and daily using gavage and administration lasted for 21 days.

2.7. Measurement of Body Weight

This was done following the method of Nagappa *et al.* (2003). Rats were individually weighed by means of a sensitive digital weighing balance (model B-218). Whole body weights were recorded to determine weekly changes. Initial body weight before starting the experiment as well as at the end was recorded for all animals.

2.8. Measurement of Blood Glucose/ Anti-diabetic Evaluation

The method of Twari and Singh (2013) was used for the glucose measurement. Blood glucose concentration was measured on the initial 0 (baseline), 5th, 10th, 15th and 21st days of the study, to ascertain the hypoglycaemic effect of the extract on the rats being treated. This was done by slightly cutting the tail vein with a sharp scissors. Collection of blood samples was done by nipping and smearing the tail on the indicated portion of glucometer strip until it was fully soaked by blood after it was inserted into the Accu-check glucometer and blood glucose concentration was read within few seconds.

2.9. Collection of Blood Sample for Analysis

At the end of the experimental period, the animals were fasted overnight and sacrificed by cervical dislocation. The blood sample collection was through the ocular into a set of heparin bottles, then centrifuged at 2500rpm for 10 minutes to obtain the blood serum for analysis.

2.10. Biochemical Parameter Estimation

- Glycosylated haemoglobin (HbA1c) determination:
- Glycosylated haemoglobin kit (Spectrum, Egypt) was used.

2.10.1. Assay principle

A haemolysed preparation of whole blood was mixed continuously for 5 min with a weakly binding cation-exchange resin. The labile fraction was eliminated during the hemolysate preparation and during the binding. During this mixing, non-glycosylated haemoglobin binds to the ion-exchange resin leaving glycosylated haemoglobin free in the supernatant. After the mixing period, a filter separator was used to remove the resin from the supernatant. The percent glycosylated haemoglobin was determined by measuring absorbance of the ratio of the absorbance of the glycosylated haemoglobin (GHb) and the total haemoglobin fraction (THb). The ratios of the absorbance of GHb and THb of the control and test were used to calculate the percent HbA1c of the sample.

2.10.2. Haemolysate preparation

Into a test tube was dispensed 0.5 ml of lysing reagent and labelled as test and control. Thereafter, 0.1 ml of the reconstituted control or well-mixed sample was added into the appropriately labelled tubes and mixed until complete lysis was evident.

2.10.3. Glycosylated haemoglobin separation

The cap from the ion exchange resin was removed and labelled as control and test and 0.1 ml of hemolysate from hemolysate preparation above was added into appropriately labelled ion exchange resin tubes. Thereafter, a resin separator was inserted into each tube so that the rubber sleeve was approximately 1 cm above the liquid level of the resin suspension and the tube was vortexed continuously for 5 min. The resin was allowed to settle and the resin separator was pushed until the resin was firmly packed and the supernatant decanted directly into a cuvette and absorbance measured at 415 nm against distilled water.

2.10.4. Total haemoglobin fraction

Into test tubes labelled as 'test' and 'control' were dispensed 5 ml of distilled water and 0.02 ml of hemolysate from hemolysate preparation above and added to appropriately labelled tubes. The mixture was mixed well and the absorbance read against distilled water.

Calculations

$$Ratio\ of\ control(Rc) = \frac{Absorbance\ of\ control\ GHb}{Absorbance\ of\ control\ THb}$$

$$HbA1c(\%) = \frac{Ratio\ of\ test\ (Rt)}{Ratio\ of\ control\ (Rc)} \times 10$$

Where 10 = value of control

Estimation of Antioxidant Parameters

Estimation of Lipid Peroxidation

Lipid peroxidation was assessed by measuring malondialdehyde (MDA) formation, using the method of Okhawa *et al*., (1979). Malondialdehyde (MDA), the end product of lipid peroxidation, is a good marker of free radical–mediated damage and oxidative stress. The principle of this method consists in the reaction of MDA with thiobarbituric acid (TBA) in acidic conditions and at a higher temperature (90-100°C) to form a pink MDA-(TBA)2 complex, which can be quantified spectrophotometrically at 530 nm. In the procedure, 0.5mL of 20% TCA was added to 0.5mL of the tissue homogenate, then there was an addition of 1 mL of 0.67% TBA. The mixture was incubated at 100°C for 15 min in a water bath, cooled and then added with 4 mL of n-butanol and centrifuged at 3000rpm for 15min. The absorbance of the clear pink supernatant was then read against a blank at 532nm spectrophotometrically. The concentration of MDA is expressed in nmol / g of the tissue.

2.10.5. Assay of Glutathione Peroxidase Activity

The method of Paglia and Valentine (2001) of glutathione peroxidase assay was used.

Principle: This method uses the principle of oxidation of NADPH to NADP+ which is accompanied by a decrease in absorbance at 340nm. This assay is an indirect measure of the activity of glutathione peroxidase.

Method: In background or Non-enzymatic well, 120 ul of Assay Buffer and 50 ul of Co-substrate mixture were added to the three Wells. In positive control well (borine erythrocyte Gpx), 100 ul of Assay Buffer, 50ul of Co-substrate mixture and 20ul of dilute Gpx (control) were added to the three wells.

In sample Wells 100ul of Assay buffer, 50ul of Co-subatrate mixture and 20ul of blood sample were added to three Wells. The reaction was initiated by adding 20 ul cumene hydro-peroxide to Wells been used. The plate was shaken for few seconds and the absorbance was read once every minute at 340nm using a plate reader to obtain at least 5 times points.

$$Gpx\ activity\ = \frac{\Delta 340}{min} \times \frac{volume\ of\ sample \times sample\ dilution}{0.00373 NM^{-1}}$$

2.10.6. Determination of catalase activity

The enzymatic activity of catalase was determined by the method of Clairborne, (1985). The principle is based on the hydrogen peroxide H_2O_2 degradation in the presence of the enzyme. The reaction mixture contained 50 mM potassium phosphate buffer pH 7.4, 19mM H_2O_2 and 16.5 uL tissue homogenate. The consumption of H_2O_2 was monitored spectrophotometrically at 240 nm for 1 min. and the enzymatic activity is calculated according to the formula:

$$K = \frac{2.303}{T} \times log\ \left(\frac{A1}{A2}\right) (4)$$

Where: K: Rate of reaction

T: Time interval (minutes)
A2: Absorbance at 60 seconds interval
A1: Absorbance at time zero

Determination of Lipid Profile Test

All the lipid profile tests were determined according to method described by Ochei and Kolhatkar (2000).

2.10.7. Estimation of Total cholesterol (TC)

Principle:

In the presence of excess acid such as phosphoric acid and ferric (Fe^{+++}) ions, cholesterol is oxidized to disulphoric acid which is reddish in colour (salkowski reaction). It is read colorimetrically at 560 nm (green yellow filter).

Method: The serum 1:20 was diluted with water (0.1 ml serum + 1.9 ml distilled water). The cholesterol standard 1: 20 was diluted with glacial acetic acid. Then three glass stoppered test tubes were set up, 5.0 ml of ferric chloride reagent and 0.5 ml of diluted serum were added to the first test tube (labelled as test), 5.0 ml of ferric chloride and 0.5 ml of diluted cholesterol standard (250 mg/dl) were added to the test tubes labelled as standard, while 5.0 ml of ferric chloride and 0.5 ml of distilled water were added to the test tube labelled as blank. The tubes were shaken for 10 seconds to mix the content of each tube and were placed immediately in a boiling water bath for 90 seconds. They were cooled in a running tap water for 5 minutes and the absorbance read against the blank at 560 nm.

Calculation:

$$Serum\ cholesterol\left(\frac{mg}{dl}\right) = \frac{Absorbance\ of\ test}{Absorbance\ of\ standard} \times 250$$

2.10.8. Estimation of High Density Lipoprotein (HDL-Cholesterol)

Principle:

Chylomicrons, VLDL and LDL are precipitated by phosphotungstic acid in the presence of magnesium ions, leaving HDL in the solution.

Method:

A volume, 1 ml of serum was pipetted into a centrifuge tube and 0.1 ml of phosphotungstic acid reagent (PTA) was added and mixed well. 0.05 ml of magnesium chloride was added and mixed well. It was centrifuged at 2500rpm for 30minutes, and the supernatant was carefully removed with a Pasteur pipette. 2.5 ml of colour reagent and 0.1 ml of supernatant were added to the test tube labelled as test. 2.5 ml of colour reagent and 0.1 ml of cholesterol standard (100mg/dl) were added to the test tube labelled as standard while 2.5 ml of colour reagent and 0.1 ml of distilled water were added to test tube labelled as blank. All the tubes were mixed well and cooled in running tap water. The absorbance was read at 560nm against blank.

Calculation:

$$Serum\ HDL - cholesterol\left(\frac{mg}{dl}\right) = \frac{Absorbance\ of\ test}{Absorbance\ of\ standard} \times 115$$

2.10.9. Estimation of serum triglycerides (TG)

Principle:

The enzymatic method for serum triglycerides involves the following steps.

Triglycerides are hydrolysed by the enzyme lipase to produce glycerol and fatty acids.

$$Triglyceride + H_2O \xrightarrow[lipase]{} Glycerol + fatty\ acids$$

The enzyme glycerol kinase acts on glycerol in the presence of ATP to form glycerol- 3- phosphate and ADP.

$$Glycerol + ATP \xrightarrow[Glycerol\ kinase]{} Glycerol - 3 - Phosphate + ADP$$

Glycerol-3- phosphate is oxidized by glycerol phosphate oxidase to dihydroxyacetone and hydrogen peroxide.

$$Glycerol - 3 - phosphate + O_2 \xrightarrow[Glycerol-3-phosphate\ oxidase]{} dihydroxyacetone + H_2O_2$$

Oxygen is released from H_2O_2 in the presence of peroxidase, which oxidized p- chlorophenol chromogen to form a coloured compound.

$$H_2O_2 \xrightarrow[peroxidsae]{} H_2O + O_2 \rightarrow chlorophenol \rightarrow coloured\ product$$

Method:

Three clean test tubes were set up and labelled as test, standard and blank. 3.0 ml of colour reagent and 0.03 ml of serum were added to test tube labelled test. 3.0 ml of colour reagent and 0.03 ml of triglyceride standard (100 mg/dl) were added to tube labelled as standard while 3.0 ml of colour reagent and 0.03 ml of distilled water were added to the tube labelled as blank. They were mixed well and incubated at 37^0C for 15 minutes. The absorbance of the test and standard were read against the blank at 420nm.

Calculation:

$$Serum\ triglyceride(mg/dl) = \frac{Absorbance\ of\ test}{Absorbane\ of\ standard} \times 100$$

Estimation of serum VLDL and LDL cholesterol

This was calculated by mathematical formulae (Friedewald formulae) as described by by Ochei and Kolhatkar (2000).

$$Serum\ VLDL - cholesterol\ (mg/dl) = \frac{Triglyceride}{5}$$

$$Serum\ LDL - cholesterol\left(\frac{mg}{dl}\right) = Total\ cholesterol\ (HDL - C + 0.46 \times TG)$$

2.11. Statistical analysis

Data generated from the study was presented as mean ± SEM. Statistical analysis was done by Oneway analysis of variance using the SPSS version 21.0. The mean difference at $P<0.05$ was considered statistically significant.

3. Results

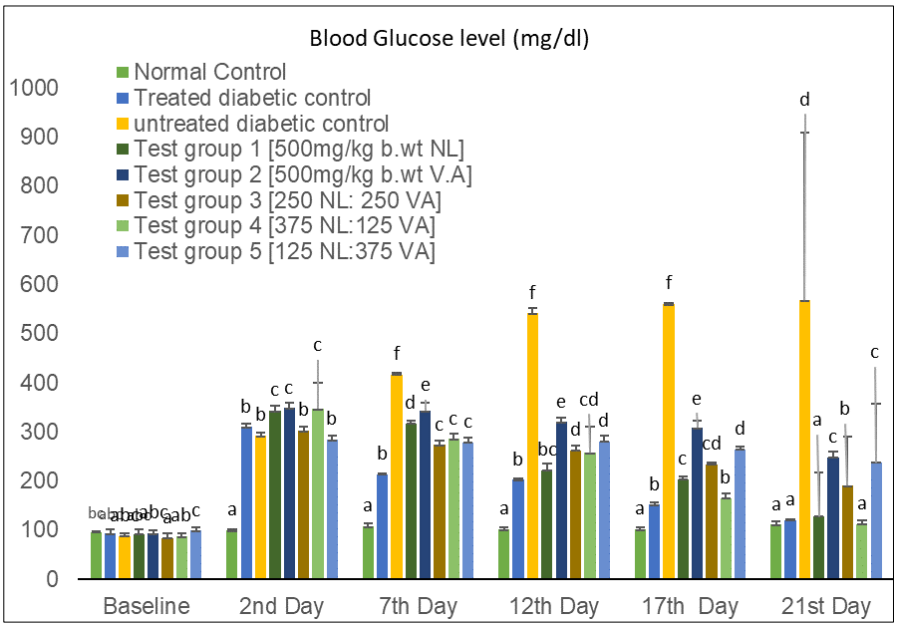

Figure 1 Effect of combined methanol extract of *N. latifolia* and *V. africana leaves* on blood glucose level of alloxan-induced diabetic rats.

3.1. Body Weight Changes in Rats

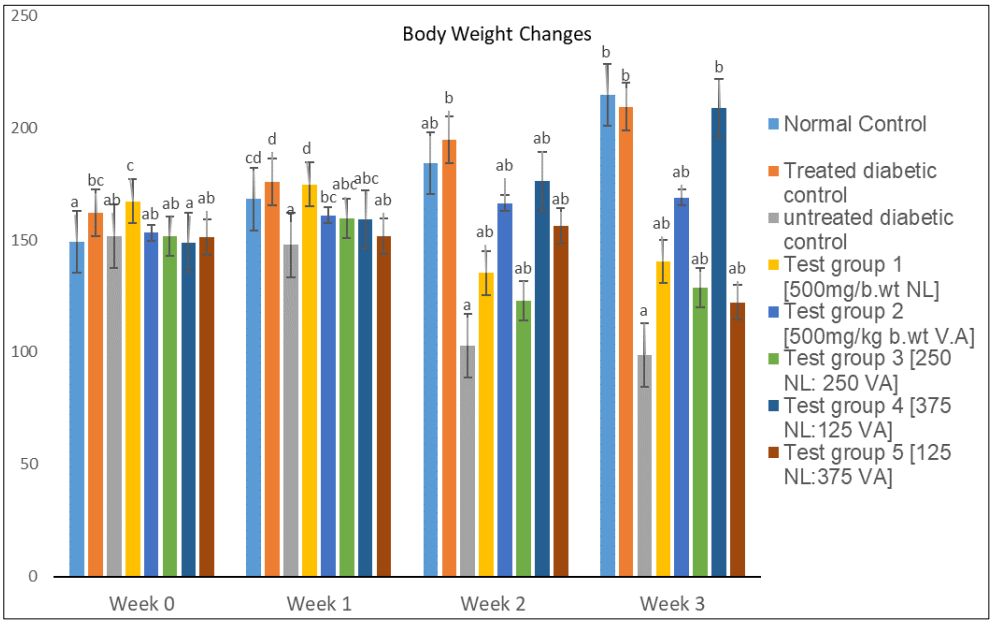

Figure 2 Effect of combined methanol extract of *N. latifolia* and *V. africana leaves* on body weight changes of alloxan-induced diabetic rats.

Table 1 Effect of combined methanol extract of *N. latifolia* and *V. africana leaves* on in –vivo antioxidant parameters of alloxan-induced diabetic rats.

GROUP	MDA(nmol/ml)	Glutathione peroxidase(µ/l)	Catalase(µ/l)
Normal Control	3.49±0.048^{a}	110.37±1.23^{d}	98.07±0.61^{bc}
Treated diabetic control	4.14±0.09^{a}	109.69±4.25^{d}	94.24±5.09^{bc}
Untreated diabetic control	7.27±2.44^{b}	62.92±35.20^{abcd}	67.41±37.82^{abc}
Test group 1 [500 mg/b.w. N.L.]	4.93±0.11^{a}	100.32±1.56^{bcd}	98.07±1.32^{bc}
Test group 2 [500 mg/kg b.w. V.A.]	3.14±2.87^{a}	60.71±55.42^{abc}	54.75±49.99^{ab}
Test group 3 [250 N.L.: 250 V.A.]	5.16±0.09^{a}	107.66±0.51^{cd}	99.72±1.42^{c}
Test group 4 [375 N.L.:125 V.A.]	3.142±2.86^{a}	57.05±52.08^{ab}	48.50±44.27^{a}
Test group 5 [125 N.L.:375 V.A.]	2.84±2.72^{a}	50.46±46.07^{a}	45.29±41.35^{a}

Table 2 Effect of combined methanol extract of *N. latifolia* and *V. africana leaves* on glycosylated haemoglobin of alloxan-induced diabetic rats.

GROUP	Glycosylated Haemoglobin (%)
Normal Control	4.96 ±0.082^{a}
Treated diabetic control	5.12± 0.05^{a}
Untreated diabetic control	6.27±3.50^{a}
Test group 1 [500 mg/b.w. N.L.]	6.53±0.30^{a}
Test group 2 [500 mg/kg b.w. V.A.]	4.19±3.83^{a}
Test group 3 [250 N.L.: 250 V.A.]	5.99±0.15^{a}
Test group 4 [375 N.L.:125 V.A.]	4.27±3.89^{a}
Test group 5 [125 N.L.:375 V.A.]	4.41±4.03^{a}

Table 3 Effect of combined methanol extract of *N. latifolia* and *V. africana leaves* on lipid profile parameters of alloxan-induced diabetic rats.

GROUP	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Normal Control	94.75±0.48^{a}	110.5±0.65^{a}	34.72±0.52^{e}	9.31±0.59^{a}	22.1±0.13^{a}
Treated diabetic control	105.75±0.48^{c}	120.0±0.41^{c}	37.88±0.28^{f}	12.68±0.16^{a}	24.0±0.08^{c}
Untreated diabetic control	242.25±1.03^{g}	144.25±1.11^{f}	15.75±0.48^{a}	160.15±1.69^{e}	28.85±0.22^{f}
Test group 1 [500 mg/b.w. N.L.]	99.0±0.91^{b}	113.5±0.65^{b}	35.83±0.31^{e}	10.97±1.00^{a}	22.70±0.22^{e}
Test group 2 [500 mg/kg b.w. V.A.]	162.75±1.56^{f}	135.0±1.08^{e}	21.89±0.22^{b}	80.0±2.99^{d}	27.00±0.22^{e}
Test group 3 [250 N.L.: 250 V.A.]	117.0±0.41^{d}	123.0±0.71^{d}	32.13±0.68^{d}	28.29±0.24^{c}	24.50±0.15^{d}
Test group 4 [375 N.L.:125 V.A.]	103.38±0.55^{c}	111.75±0.63^{ab}	30.70±0.59^{c}	21.24±0.54^{b}	22.35±0.13^{ab}
Test group 5 [125 N.L.:375 V.A.]	158.0±0.71^{e}	124.25±0.48^{d}	20.69±0.11^{b}	80.16±0.73^{d}	24.85±0.96^{d}

4. Discussion

The glycaemic change in blood of diabetic albino rats after administration of glibenclamide and extracts at different ratio are shown figure 1. The result revealed that there was no significant ($p > 0.05$) difference in blood glucose level of all the test groups when compared with control groups at baseline. On the 2nd and 7th day, there was significant ($p < 0.05$) increase in blood glucose level of the test groups and untreated diabetic control when compared to normal control while on the 12th and 17th day, there was significant ($p < 0.05$) reduction in blood glucose level in the group administered reference drug and 500 mg/kg b.w. of N.L. when compared to untreated diabetic control. On the 17th day group administered with 375 NL and 125 VA had significant ($p < 0.05$) reduction in blood glucose level while on 21st day, there was no significant ($p > 0.05$) difference between the group given 500 mg/kg b.w. of N.L and 375 NL and 125 VA when compared with normal control and treated diabetic control. However, 500 mg/kg b.w. of N.L and 375 NL and 125 VA were the most effective combination ratio that reduced the blood glucose of the diabetic rats to normal level with respect to time. It has been established that the two medicinal plants have antidiabetic property according to Okyar *et al.* (2001) and Rao *et al*. (2001) thus, the increased antidiabetic activity of dose ratio of 500 mg/kg b.w. of N.L and 375 N.L : 125 V.A. could be attributed to the increase in some phytochemicals associated with lowering of blood glucose such as alkaloid, terpenoids (Morah, 2005) and some inorganic elements of both plants. Flavonoids, alkaloid, terpenoids and some other secondary plant metabolites have been reported to possess hypoglycaemic effect (Ojewole, 2002). The hypoglycaemic and antihyperglycaemic activity of the extracts of *N. latifolia* and *Voacanga africana* leaves could arise through stimulation of insulin secretion from β-cells, increased availability of insulin. The exact mechanism of hypoglycaemic action of the plant extracts is similar to that of glibenclamide (Prince *et al*., 1999).

Consequently, diabetes is characterized by a severe loss in body weight due to the loss or degradation of structural proteins (Gidado *et al.,* 2008). The result in fig. 2 showed at week 1, the body weight of normal control group, treated diabetic control group and group administered 500mg/kg of NL significantly ($p < 0.05$) increased when compared to other groups. At week 2, there was no significant ($p > 0.05$) difference in body weight of normal control group when compared to test groups, but treated diabetic control had significant ($p< 0.05$) increase in body weight when compared to untreated diabetic control. At week 3, normal control group, treated diabetic control and group administered with 375 N.L.:125 V.A. significantly ($p< 0.05$) increased the body weight of the animals when compared to other groups. The increase in body weight implies that the well-being of the animals in these groups was maintained.

Long term hyperglycaemia increases the generation of free radicals via glucose auto-oxidation (Ugochukwu *et al*., 2003). Lipid peroxidation is one of the commonest symptoms of chronic diabetes and it is characterized by the high breakdown of intra- cellular macromolecules (Satheesh and Pari, 2004). Higher index of malondialdehyde is an indication of oxidative stress, thus, the level of antioxidant defense systems are mostly dwindling in alloxan- induced diabetic experimental rats (Prince and Menon, 2000). In this present research, there was a significant decrease ($p < 0.05$) in the concentration of malondialdeyde (a product of lipid peroxidation) in the treated diabetic control group and test groups when compared with untreated diabetic control as shown in table 1. The reduction in malondialdehyde as observed could be as a result of bioactive compounds present in the plant extracts. Glutathione peroxidase (GPx) is reported to have a high shielding antioxidant capacity than catalase, the over- expression of GPx could be a protective antioxidant mechanism against oxidative stress. Decreased activity of GPx may result from radical induced inactivation and enzyme glycation (Sozman *et al*., 2001). The study revealed that there was significant increase ($p< 0.05$) in the concentration of GPx in test group treated with 500mg/kg body weight of *N. latifolia* and more significant increase ($p< 0.05$) in test group treated with 250 + 250 mg/kg body weight of *N. latifolia* and *V. africana* respectively (107.66 ± 0.51) when compared with the untreated diabetic control (62.92 ± 35.20). This showed that combination of these plants in equal ratio could proffer a protective antioxidant mechanism. Catalase is one of the indices of antioxidant status of the body. Any reduction in the activity of this catalase may cause deleterious effects as a result of superoxide and hydrogen peroxide assimilation (Oyedemi *et al*., 2010). There was increased catalase activity in the treated diabetic control, the increased catalase concentration in test groups administered with 500 mg/kg body weight of *N. latifolia* and 250 + 250 mg/kg body weight of *N. latifolia* and *V. africana* respectively could be an indication of chemo- preventive potential of these plants in the pathogenesis of oxidative stress which may be linked to the presence of various bioactive principles such as flavonoids, alkaloids tannins, steroids and phenols (Oloyode *et al.,* 2010). The results of this work are in tandem with the report given by Mahrukh *et al.* (2015), who evaluated the antidiabetic effect of *Sida cordifolia* on diabetic rats.

Furthermore, in clinical management of diabetes, glycosylated haemoglobin (HbA1c) are commonly used as outcome measures of glycemic control (Delamater, 2006). Studies have clearly demonstrated that persons with higher level of adherence to their treatment regimens and lower level of HbA1c have better glycemic control and thus less likely to develop diabetic complications. In contrary, persistent higher levels of HbA1c indicate poorly controlled diabetes mellitus and hence increased risk of developing diabetic complications (Al-Akour *et al*., 2011). The results in table 8 shows the effect of combined methanol extract of *N. latifolia* and *V. africana leaves* on glycosylated haemoglobin of

alloxan- induced diabetic rats. The result disclosed that there was no significant ($p > 0.05$) difference in the percentage glycosylated haemoglobin of all the test groups when compared to the control groups. This indicated that all the groups exhibited considerable glycemic control and no diabetic complications.

Changes in the serum lipid profile is known to manifest in diabetes and this is likely to increase the risk of coronary heart diseases. A significant ($p < 0.05$) reduction in low density lipoprotein, total cholesterol, triglyceride and very low density lipoprotein cholesterol (VDL-C) concentrations was achieved through the administration of the extracts especially 500mg/kg b.w. N.L, 375 N.L. : 125 V.A. and 250 N.L. : 250 V.A. Flavonoids, phenols, saponins and sterols have been reported to be associated with hypolipidemia and hypocholesterolemia as reported by (Bopanna *et al.,* 1997; Katsumata, *et al.,* 1999), this may contribute immensely to the obtained result. Also the lipid lowering effect may be due to inhibition of hepatic cholesterol biosynthesis and increased fecal bile acid secretion as reported by Kaur *et al.* (2006). The reduction of the parameters after treatment with combined extracts was in consonance with the work reported by Okokon *et al*. (2011) and also compared well with the work by (Asanga *et al.,* 2013), thereby suggesting their ability in arresting some lipid related symptoms of diabetes mellitus and its recommendation for managing incidences of the disease. Moreover, high density lipoprotein cholesterol (HDL-C) showed a significant ($p < 0.05$) increase in all the test groups, more especially in groups administered with reference drug and 500 mg/kg b.w. N.L. when compared to untreated diabetic control. The lipid profile results were in accordance with the study done by Folake *et al.,* (2020), who studied the antilipidemic properties of aqueous extract of *Morinda lucida* and *N. latifolia* leaves in alloxan-induced diabetic rats.

5. Conclusion

The current study reveals that oral administration of combined methanol extracts of *N. latifolia* and *V. africana* leaves is relatively safe and did not have deleterious effect at the dosage investigated. The ability of the methanol leaf extracts of *N. latifolia* and *V. africana* in the reduction of hyperglycemic rats blood glucose towards normal confirms its antidiabetic activity. The leaf extracts of both plants especially 500mg/kg b.w. N.L., 375 N.L. : 125 V.A. and 250 N.L. : 250 V.A. inhibited diabetic complications by preventing alteration in serum level of antioxidant parameters, glycosylated haemoglobin and lipid profile parameters. The bioactive substances present in this plant leaves extract might be responsible for their pharmacological properties.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Animal were purchased from Nano Farms Ihiagwa, Owerri, and was housed in the animal house of Department of Biochemistry, Federal Polytechnic Nekede, Owerri. All the animals were fed with a regular diet and water ad libitum.

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