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

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

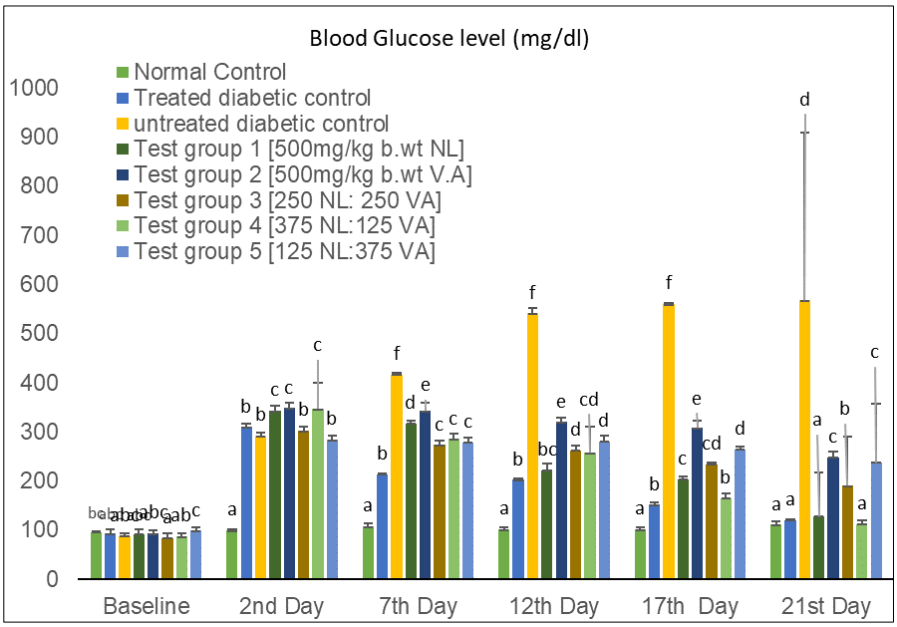

Figure 1 Effect of combined methanol extract of *N. latifolia* and *V. africana leaves* on blood glucose level of alloxan-induced diabetic rats.

3.1. Body Weight Changes in Rats

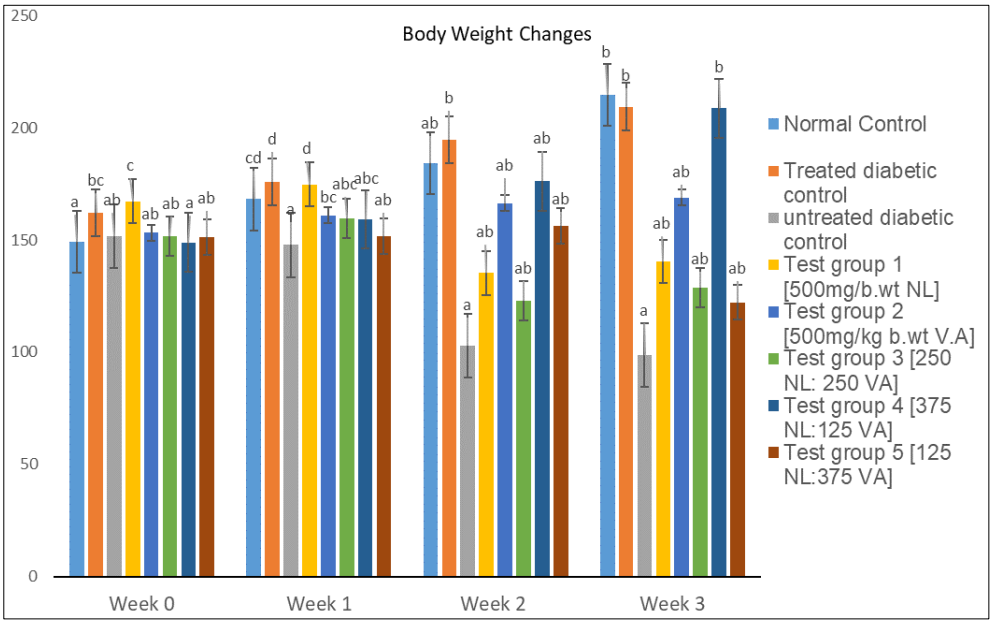

Figure 2 Effect of combined methanol extract of *N. latifolia* and *V. africana leaves* on body weight changes of alloxan-induced diabetic rats.

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.

References

[1] Adolfina, R.K.H., Rodolfo, J., Daniel, K., Hanson, A., Juliana, A. and James, E.S. (2009). *Voacanga africana*: Chemistry, Quality and Pharmacological activity. African Natural Plant Products: New Discoveries and Challenges in Chemistry and Quality: Chapter 20, Pp. 363-380.

[2] Agbafor, K.N. (2004). The Effects of Aqueous and Organic Extract of *Baphian nitida* on tissue acetylcholine Estrase in Guinea Pigs. *Journal of Science and Technology*, 10(7), 1-6

[3] Al- Akour, N.A., Khader, Y.S. and Alaoui, A.M. (2011). Glycemic Control and its Determinants among Patients with Type 2 Diabetes Mellitus Attending a Teaching Hospital. *Journal of Diabetes Metabolism.*2: 129

[4] Arbonnier, M. (2000). Arbres, Arbustes et Lianes des Zones Sechesd'Afrique de I'Ouest. IstEdn. CIRAD Publishers, 431-541.

[5] Asanga, E.E., Ebong, P.E., Eseyin, O.A., Udohm I.E. and Eyo, R.A. (2013). Effect of ethanol extract and fractions of *Nauclea latifolia* leaves on some biochemical parameters of alloxan-induced diabetic rats. *International. Journal of Resource and Revolution. in Pharmaceutical Application Science.* 3(1), 147-154.

[6] Ayoola, G.A., Folawewo, A.D., Adesegun, S.A., Abioro, O.O., Adepoju-Bello, A.A. and Coker, H.A.B. (2008). Phytochemical and antioxidant screening of some plants of Apocynaceae from SouthWest Nigeria. *African Journal of Plant Science.*2(10), 124–128.

[7] Battu, G.R., Mamidipalli, S.N., Parimi, R., Viriyala, R.K., Patchula, R.P. and Mood L.R. (2007). Hypoglycaemic and antihyperglycemic effect of alcoholic extract of *Benincasa hispida* in normal and in alloxan induced diabetic rats. Pharmacognosy Magazine. 3: 101 – 105.

[8] Bopanna, K.N., Bhagy, A. N., Kanna, J., Balaraman, R. and Rathod, S.P. (1997). Antidiabetic and Antihyperlipidemic effects of neem seed and kernel powder on Alloxan-induced Diabetic rats. *Phamacoal. Res*. 46(3), 251-255.

[9] Clairborne, A. Catalase activity. In: Handbook of Methods for Oxygen Radical Research. Greenwald, R.A. ed Boca Raton, Fla: CRC Press 283-284.

[10] Delamater, A.M. (2006). Use of Hemoglobin A1c to Improve Diabetes Management. *Clinical Diabetes.* 24(1). 6-8

[11] Etukudoh, I. (2003). Ethnobotany: Conventional and Traditional uses of plants. Verdict Press, Uyo. Pp. 116 - 117.

[12] Folake, L.O., Olatunde, A.O., Olapade, S.A. and Daniel, U.M. (2020). Antidiabetic, Antilipidemic and Antioxidant Properties of Aqueous extracts of *Morinda lucida* and *Nauclea latifolia* leaves in alloxan-induced rats. *Platinum Access Open Journal*. 11(4), 11602-11615.

[13] Gidado, A., Ameh, D.A. and Atawodi, S.E. (2005). Effect of *Nauclea latifolia* leaves aqueous extract on blood glucose levels of normal and alloxan-induced diabetic rats. *African Journal of Biotechnology*. 4: 91–3.

[14] Gidado, A., Ameh, D.A., Atawodi, S.E. and Ibrahim, S. (2010). Hypoglycemic activity of *Nauclea latifolia* (Rubiceae) in experimental animals. *Afr J Tradit Complement Alternernative Medicine*. 5: 201-208

[15] Hu, F.B. (2011). Globalization of diabetes: the role of diet, life style, and genes. Diabetes Care; 34,1249ă57.

[16] Igbe, I. and Edike, T. (2015). In vivo antinociceptive activity of the aqueous leaf extract of *Voacanga africana* Stapf (Apocynaceae)in mice. *Journal of Science and Practice of Pharmacy.*2(1), 51–54.

[17] Igbe, I. and Edike, T. (2015). In vivo antinociceptive activity of the aqueous leaf extract of *Voacanga africana* Stapf (Apocynaceae)in mice. *Journal of Science and Practice of Pharmacy.*2(1), 51–54.

[18] Kangralkar, A.S., Fred, W.K., Griell, K.C. and Willey, W.C. (2010). Risk associated with Diabetes mellitus, *The Journal of Human Pharmacology and Drug Therapy*, 28(9),18-78

[19] Karou, S.D., Tchacondo, T., lboudo, D.P. and Simpore, J. (2011). SubSaharan Rubiaciaea: A review of their traditional uses, phytochemistry and biological activities. *Pakistan Journal of Biological Science*. 14, 149 -169.

[20] Katsumata, K.Y., Katsumata, T.O. and Katsumata, K. (1999). Potentiating Effects of combined usage of three sulphonylurea Drugs on the occurrence of Alloxan-induced Diabetes in rats. *Journal of Hormonal Metabolic Resources*. 25, 125-126.

[21] Kaur, G., Alams, M.S., Jabbar, Z., Javed, K. and Athar, M. (2006). Evaluation of antioxidant activity of *Cassia siamea* flowers. *Journal of Ethnopharmacology*. 108, 340-348.

[22] Krishnasamy, H. O. and Abell, F.G. (2018). The role of pancreas in diabetic disorder, *Journal of Physiology and Pharmacology*. 18, 215-232.

[23] Mahrukh, A., Shahid, P., Muasir, S., Rajinder, R., Pawan, K.V., Azad, A. A. and Nrip, K. P. (2015). Antidiabetic effect of *Sida cordifolia* (aqueous extract) on diabetes induced in wistar rats using streptozotocin and its phytochemistry. *Journal of Pharmaceutical Research and Innovation*, 8, 11-22.

[24] Morah, F.N.I. (2005). Naucleadal and Epinaucledal from an Antiviral preparation from *Nauclea latifolia.Global Journal of Pure and Applied Sciences.*1(2), 59-62

[25] Nagappa, A.N., Thakurdesai, P.A., Venkat, R. N. and Singh, J. (2003). Antidiabetic activity of Terminalia catappalinn fruits. *Journal of Ethanopharmacology.* 88, 45-50.

[26] Ochei, J. and Kolhatkar, A. (2000). Medical Laboratory Sciences. Theory and Practice. Tata McGraw – Hill Publishing Company Limited, New Delhi. Pp 191- 198.

[27] Ojowole, J. A. (2002). Hyoglycemic effect of *Clausenaanisata* hook methanolic rot extract in rats. *Journal of Ethnopharmacology*, 81, 231.

[28] Okhawa, H. Ohishi, N. and Yagi, K. (1979). Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. *Journal of Analytical Chemistry.*95, 351–358.

[29] Okokon, J.L., Paul, A., Nwafor, E. E., Umoh, P. J., Okokon, John, A. and Udobang, R.T. (2011). Antidiabetic and Hypolipidemic Activities of *Croton zambesicus* on Alloxan- induced Diabetic Rats. *Asian Journal of Pharmacology and Biological Research.* 5(8), 34-45

[30] Okya, A., Can, A., Akev, N., Baktor, G. and Sutlupinar, N. (2001). Effect of *Aloe vera* leaves on blood Glucose levels in Type 1 and Type 11 Diabetic Rats Models. *Phytother. Resources*. 15, 157-161.

[31] Oloyede, G.K., Onocha, P.A. and Soyinka, J. (2010). Phytochemical screening, antimicrobial and antioxidant activities of four Nigerian medicinal plants. *Ann Biol Res*1(2), 114-120

[32] Omodamiro, O.D. and Nwankwo, C.I. (2013). *European Journal of Experimental Biology*. 3(3),140–148.

[33] Osinubi, A.A., Ajayi, O.G., Adesiyun, A.E. (2006). Evaluation of the anti-diabetic effect of aqueous leaf extracts of *Tripinanthus butungil* in male spragne Dawley rats.

[34] Oyedemi, S.O., Bradley, G. and Afolayan, A.L. (2010). "In-vitro and in-vivo antioxidant activities of aqueous extract of *Strychnos henningsii Gilg" AJPP.* 4(2), 70-78

[35] Paglia, M. and Valentine, A. (2001). Glutathione peroxidase Activity, Oxford University Press, London, England, pp: 56-78.

[36] Prince, S.M. and Menon, V.P. (2000). Hypoglycaemic and other related actions of *Tinospera cardfolia* roots in alloxan induced diabetic rats.

[37] Rang, H. P., Dale, M. M., Ritter, J. M., Flower, R. and Henderson, G. (2007). Cannabinoids. *Rang & Dale's Pharmacology, 6th edition. Churchill Livingstone, London*, 248-255.

[38] Rang, H.P. and Dale, M.M. (2001). The Endocrine System Pharmacology, Longman Harlows. 504.

[39] Rao, B.K. and Rao, A. (2001). Hypoglycaemic and antihyperglycaemic Activity of Syzgiumal ternifolium (wt) Walp. Seed Extracts in Normal and Diabetic Rats. *Phytomed*. 8(2), 88-93.

[40] Satheesh, M.A. and Pari, L. (2004). Role of selected Indian plants in the management of type 2 diabetes. *Journal of Alternative Medicine*. 10 (2), 369-378.

[41] Sozmen, E.Y., Sozmen, B., Delen, Y. and Onat, Y. (2001). "Protective effect of *Raphanus sativus* extract against copper sulphate toxicity in *chemopharyn godonidella* fish" Arah *Medical Research*. 32, 283-287.

[42] Tan, P.V., Penlap, V.B., Nyasse, B. and Nguemo, J.D. (2000). Anti-ulcer actions of the bark methanol extract of *Voacanga africana* indifferent experimental ulcer models in rats. *Journal of Ethnopharmacology*. 73(3), 423–428

[43] Tiwari, V. and Singh, A. (2013). Antinociceptive and antioxidant potential of *Bauhinia tomentosa* extracts in experimental animal models. *National Productivity Journal.*3, 309–316.

[44] Ugochukwu, N. H., Babady, N. E., Cobourine, M. and Gasset, S. R. (2003). The effect of *Gongronema latifolium* extracts on serum lipid profile and oxidative stress in hypatocytes of diabetic rats. *Journal of Biosciences*. 28, 1 –5

[45] WHO (2013). WHO traditional medicine strategy 2014ă2023. Geneva: WHO; [Online] Available from: http://www.who.int/medicines/publications/traditional/trm_strategy14_23/en