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ANTI-HYPERGLYCEMIC AND HEPATOPROTECTIVE PROPERTIES OF COMBINED AQUEOUS LEAF EXTRACT OF *Vernonia amygdalina* AND *Moringa oleifera* IN EXPERIMENTALLY INDUCED DIABETIC RAT MODEL

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Abstract: **This study was carried out to investigate the anti-hyperglycemic and hepato-protective properties of combined leaf extracts of *Vernonia amygdalina* and *Moringa oleifera* in alloxan-induced diabetic Wistar rats. The study design involved 25 rats weighing 140 to 2100g which were randomly divided into 5 groups of 5 rats each. Group 1 received distilled water and feed; group 2 received no treatment; group 3 received 4% extract combination of *V. amygdalina* and *M. oleifera*; group 4 received 8% extract combination of *V. amygdalina* and *M. oleifera* and group 5 received 16% extract combination of *V. amygdalina* and *M. oleifera*. After 14 days of treatment, the animals were anaesthetized and sacrificed to obtain blood by cardiac puncture. Serum was collected and assayed for alanine aminotransaminase (ALT), aspartate aminotransferases (AST) and alkaline phosphatase (ALP) while the blood glucose levels were determined using hand-held glucometer. The results showed a significant ($p<0.05$) reduction in blood glucose levels of treatment groups with combined extracts of *V. amygdalina* and *M. oleifera* when compared with normal control and negative control groups. Also, the ALT, AST and ALP levels of the treatment groups decreased significantly ($p<0.05$) when compared with the normal control and negative control group. This reduction in blood glucose levels and liver enzyme markers can be attributed to presence of bioactive compounds in both plants working synergistically. Hence, *V. amygdalina* and *M. oleifera* combination may be more potent in management of diabetes.**

Keywords: **Diabetes, *Vernonia amygdalina*, *Moringa oleifera*, Aanti-hyperglycemic, hepatoprotective, Liver enzymes.**

1. INTRODUCTION

Over 50% of plants serve in traditional medicine for their health benefits in combating certain ailments affecting humans such as dysentery, diarrhea, toothache, skin infections and diabetes. Many conventional drugs have been derived from prototypic molecules in medicinal plants. To date, over 400 traditional plant treatments for diabetes have been reported, although only few of the plants have received scientific and medical evaluation to assess their efficacy (Rockwood et al.,

2013). The attributed hypoglycaemic effects of these plants are due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or by the facilitation of metabolites in insulin dependent processes. Hence, treatment with herbal drugs has an effect on protecting β-cells and smoothing out fluctuation in glucose levels. Most of these plants have been found to have chemical constituents like glycosides, alkaloids, phenols, terpenoid, and flavonoids that are frequently connected as having antidiabetic effects (Rotimi et al., 2011).

DM is a global burden, associated with life-threatening complications including stroke, renal failure, and cardiac attack. Life for a person with diabetes mellitus means constant awareness of the illness, one or two insulin shots a day, frequent finger punctures to monitor blood glucose level, a restrictive diet, and concern over complications. Global diabetes prevalence has more than doubled over the last three decades, with prevalence rates far exceeding modelled projections; even after allowing for improved surveillance. Nearly 1 in 10 adults worldwide are now affected with diabetes (Nimenebo-Uadia, 2003; Okoli et al., 2010; Owulade et al., 2004). The World Health Organization estimated that over 86% of the people in developing countries rely on traditional remedies such as herbs for their daily needs and about 855 traditional medicines include crude plant extracts (Nimenebo-Uadia, 2003).

Vernonia amygdalina is a tropical African plant belonging to the Asteraceae family and it is commonly called “Bitter leaf” (Areghore et al., 1997). It is the source of a number of traditional medicines in the West African region (Akah and Okafor, 1992; Alebachew et al., 2014; Amole et al., 2006). The leaves are the plant parts that are most frequently used. The plant's young, succulent, and fresh leaves are typically recommended for laxative and treating conditions like diabetes, kidney function, malaria, fever, constipation, and high blood pressure (Amole et al., 2006).

Moringa oleifera is the most widely cultivated species of a mono-generic family, the Moringaceae. Native to the sub-Himalayan tracts, it is now widely cultivated and has become naturalized in many locations in the tropics (Szkudelski, 2001). The relative ease with which it propagates through both sexual and asexual means and its low demand for soil nutrients and water after being planted makes its production and management easy (Tasaduq et al., 2003). The plant has also been used extensively for treating inflammation, cardiovascular disease, liver disease and haematological, hepatic and renal function (Duncan, 1955). Moringa leaf extracts have also been shown to have ameliorating effect on chromium-induced testicular toxicity in rats (Fröde and Medeiros, 2008) and to enhance sexual activity in mice (Goji et al., 2009). This study assesses the effect of combined leaf extracts of *V. amygdalina* and *M. oleifera* in alloxan-induced diabetic Wistar rats.

2. MATERIALS AND METHODS

Collection and Identification of Plants

Fresh leaves of Vernonia amygdalina Del. and Moringa oleifera Lam. were collected from a botanical garden at Aluu Community in the Obio/Akpor Local Government Area of Rivers State, Nigeria, and identified in the herbarium of the Department of Plant Science and Biotechnology in the University of Port Harcourt.

Preparation of Plant materials

To get rid of dust and dirt, the leaves were rinsed multiple times with clean tap water and given time to thoroughly drain and air tried for three weeks at room temperature after which the leaves were ground into fine powder using an Electric Blender. The dried leaves were pulverized and soaked in two different bottles containing 50ml of water and were allowed to stand for 48 hours. After a period of 2 days, the mixtures were stirred intermittently and sieved with white handkerchief and filtered with Whitman paper (No. 1). Gel-like extracts were obtained after the filtration. They were later separated into different concentrations and diluted in different volumes of water in plastic bottles as follows; 4% (2% of *V. amygdalina* and *M. oleifera* each) of extract was diluted in 96ml of distilled water; 10% (5% of *V. amygdalina* and *M. oleifera* each) of extracts was diluted in 90ml of distilled water and 16% (8% of *V. amygdalina* and *M. oleifera* each) of extract was diluted in 84ml of distilled water. The extracts were then kept in a refrigerator at a temperature of 2 to 8^{o}C until usage.

Acute toxicity test

The acute toxicity and lethality test of the aqueous plant extracts in rats was estimated using the method of Lorke as described by Okoli et al. (2010), and the animals were observed continuously after every 2 hrs. Under the following profiles:

Behavioural Profile: Alertness, restlessness; Neurological Profile: Pain response, touch response, gait and Autonomic Profile: Defecation and urination. After a period of 24 hrs., the animals were observed for any lethality or death.

Procurement of Animal

For this investigation, adult Wistar rats of either sex weighing 140–210g were used. They were acquired from the Animal House of the Department of Pharmacology at the University of Port Harcourt in River State, Nigeria, and were acclimatized for two weeks. They were kept in a conventional laboratory environment with 28°C temperature (28±2°C), relative humidity (46±6%), a 12-hour light/dark cycle, and adequate ventilation. The animals were given access to water and a commercial feed (Vital Feed Nig. Ltd.) ad libitum. Twelve hours prior to the experiments, food was withheld, although water was always available for free.

Ethical Clearance

According to the recommendations made by the University of Port Harcourt's Research Ethics Committee, all methods used in this study were carried out in compliance with the fundamental principles of animal-based research.

Drug Purchase and Preparation

Alloxan monohydrate, another substance utilized, was also bought from the same pharmacy to cause diabetes in rats

Induction of Diabetes

Alloxan monohydrate, freshly made with distilled water as the vehicle, was diluted to a concentration of 150mg/kg body weight and administered intraperitoneally to rats to cause diabetes according to the principle as described by Okoli et al. (2010). Three days later, diabetes was identified in alloxan-induced rats with Random Blood Glucose (RBG) levels ≥200mg/dL. Glucose levels were monitored using a hand-held glucometer (Accu-CHEK) to test blood samples taken from the tail vein.

Experimental Design

Twenty-five (25) rats were divided into five different experimental groups of 5 animals each. Group 1 (Positive control group) received distilled water and feed; group 2 (diabetic control group) received no treatment; group 3 (Low extract concentration of *V. amygdalina* and *M. oleifera*) received 4% extract combination of *V. amygdalina* and *M. oleifera* (2% of *V. amygdalina* and *M. oleifera* each); group 4 (Medium extract concentration of *V. amygdalina* and *M. oleifera*) received 8% extract combination of *V. amygdalina* and *M. oleifera* (4% of *V. amygdalina* and *M. oleifera* each) and group 5 (High extract concentration of *V. amygdalina* and *M. oleifera*) received 16% extract combination of *V. amygdalina* and *M. oleifera* (8% of *V. amygdalina* and *M. oleifera* each). Upon administration, experimental rats were allowed access to feed and water. The plant extracts were administered orally. The duration of treatment was 14 days after induction of diabetes.

Blood glucose Determination

Blood glucose levels were monitored in order to assess the effect of combined leaf extract of *V. amygdalina* and *M. oleifera* extracts in treated diabetic rats. The blood glucose levels were monitored using a hand-held glucometer (Accu-CHEK) to measure the blood glucose level from the tail vein using a tail clip. Experimental animals had their blood glucose levels checked before starting therapy, on day 7 and 14, before (pre-treatment) and after inducing diabetes.

Biochemical Assay

Glucose was determined using methods as described by Tietz (1996), while serum lipids including total cholesterol, total protein and triacylglycerol were determined using the method of Tietz (1990). The method of Reitman and Frankel (1957) was adopted for the ALP, ALT and AST assay. ALP was estimated using the method of King and King (1954) as adapted by Cheesbrough (2000).

Method of Data Analysis

Results were analyzed using Statistical Package for Social Sciences (SPSS Inc. Chicago IL) version 23.0. All data were reported as mean ± SEM. Multiple comparison tests and analysis of variance (ANOVA) were used to differentiate differences between means. Differences were considered to be statistically significant if $P<0.05$. The mean ± SEM blood glucose values were given in mmol/L.

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3. RESULTS

Table 1: Effect of *Vernonia amygdalina and Moringa oleifera* leaf extracts combination on blood glucose levels of diabetic Wistar rats.

Group	Pre-treatment	Day 7	Day 14
Normal Control	4.8±0.52	4.9±0.08	4.6±0.08
Negative Control (150mg/kg Alloxan)	5.6±0.35	14.8±1.98*	15.2±1.74*
Alloxan + 4% Extract Combination of VAMO	4.4±0.09	12.6±1.02*	10.4±1.03*#
Alloxan + 8% Extract Combination of VAMO	4.7±0.11	13.1±0.95*	11.0±1.05*#
Alloxan + 16% Extract Combination of VAMO	5.1±0.13	10.5±1.01*#	7.5±0.61*#

Values are represented in mean±SEM, values marked with (*) differ significantly from normal control value (*p≤0.05) while those marked (#) differ significantly from Negative control group (#p≤0.05). VEMO: *Vernonia amygdalina and Moringa oleifera*

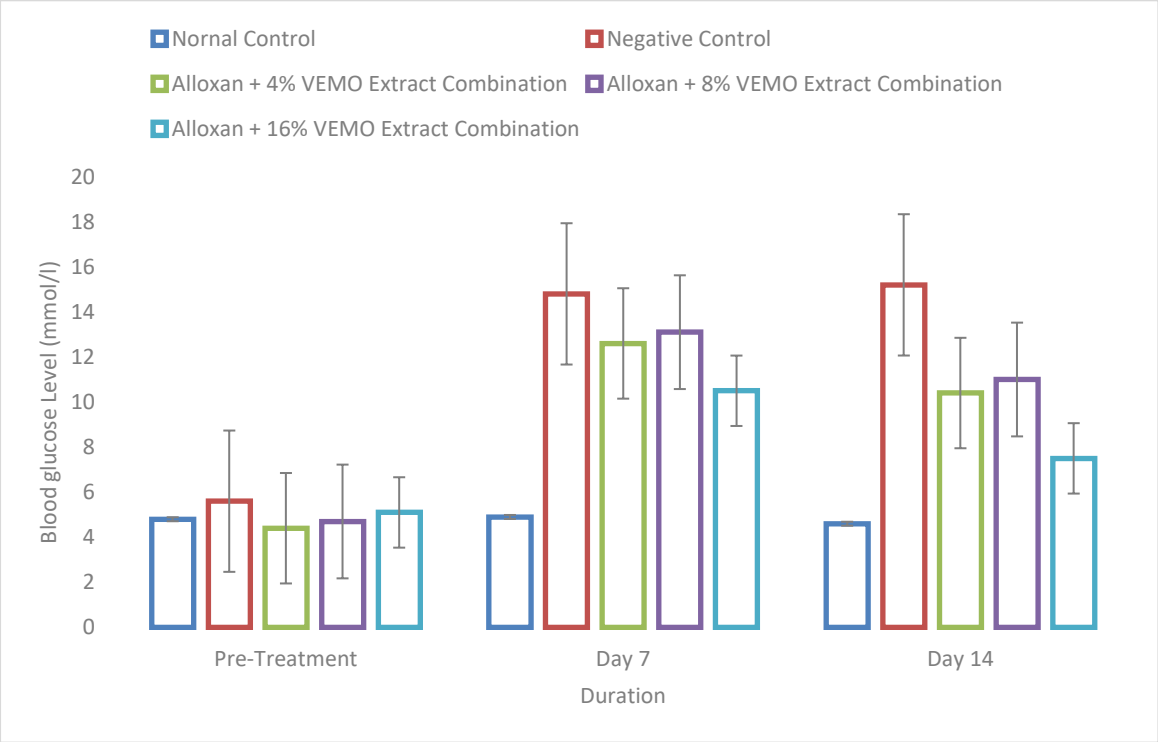

Figure 1: Bar chart showing the effect of *Vernonia amygdalina and Moringa oleifera* leaf extracts combination on blood glucose levels of diabetic Wistar rats.

Table 2: Effect of *Vernonia amygdalina and Moringa oleifera* leaf extracts combination on some liver enzyme markers of diabetic Wistar rats.

Group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Normal Control	10.2±1.26	9.8±1.60	12.6±1.02
Negative Control (150mg/kg Alloxan)	21.2±2.04*	18.5±2.17*	32.4±4.17*
Alloxan + 4% Extract Combination of VAMO	18.4±1.69*	15.1±1.42*	24.9±2.78*#
Alloxan + 8% Extract Combination of VAMO	14.5±2.01*#	17.3±2.05*	21.2±2.52*#
Alloxan + 16% Extract Combination of VAMO	11.6±1.06*#	10.8±1.04*#	16.5±2.63#

Values are represented in mean±SEM, values marked with (*) differ significantly from normal control value (*p≤0.05) while those marked (#) differ significantly from Negative control group (#p≤0.05). VEMO: *Vernonia amygdalina and Moringa oleifera*

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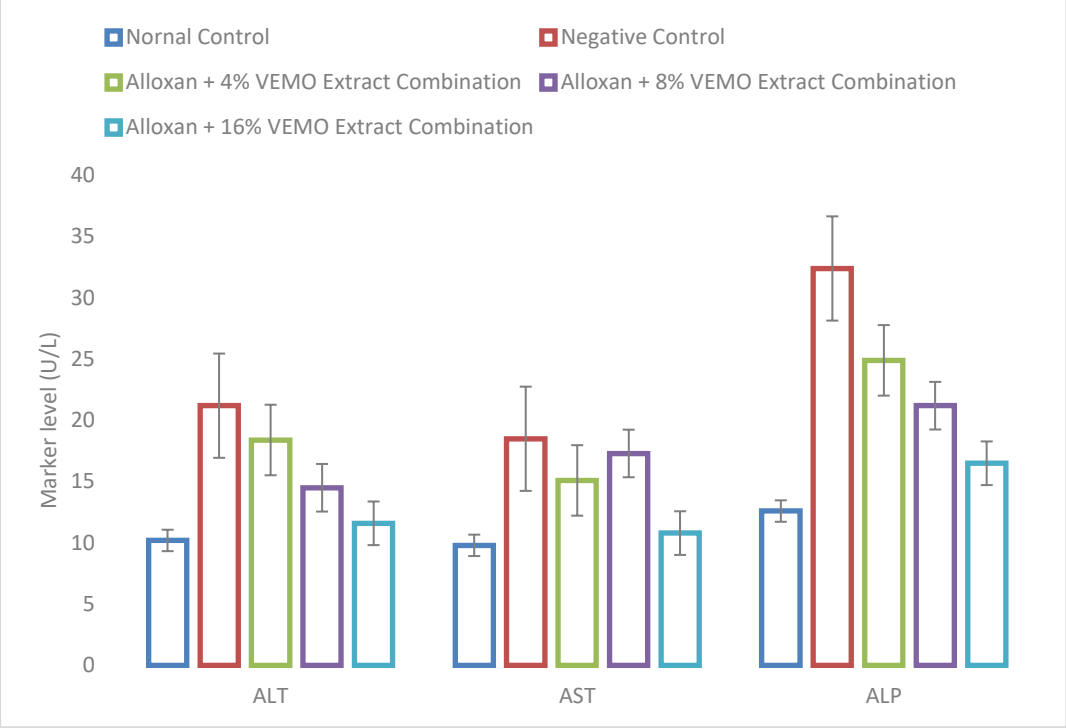

Figure 2: Bar chart showing the effect of *Vernonia amygdalina and Moringa oleifera* leaf extracts combination on some liver enzyme markers of diabetic Wistar rats.

4. DISCUSSION

The health and quality of a person's life are seriously threatened by diabetes. Achieving proper glycemic control is important for managing diabetes since it has higher mortality and complications rates. This study investigated the anti-diabetic efficacy of *Vernonia amygdalina* and *Moringa oleifera* leaf extract combinations in alloxan-induced diabetic rats. Oral administration of the extract decreased fasting blood glucose levels and prevented the rise in blood glucose in normal rats. Researchers have demonstrated the hypoglycemic effects of coumarin, flavonoid, terpenoid, and a variety of other secondary plant metabolites, including arginine and glutamic acids, in a variety of experimental animal models (Rockwood et al., 2013).

However, in accordance with Marles and Farnsworth's (1995) hypothesis, which states that plants containing terpenoids have hypoglycemia effects on both diabetic and healthy mammals, it can be deduced that *Vernonia amygdalina* and *Moringa oleifera* have hypoglycemic activity in their aqueous extracts. This result is consistent with that of Akoko et al. (2022), who report in their study that co-administration of various combination doses of *V. amygdalina* and *M. oleifera* considerably lowered blood glucose in experimental rats when compared to diabetic control rats in a dose-dependent manner.

The daily oral treatment of the extract for 14 days during the anti-diabetic activity trials showed a slow but sustained decrease in blood glucose levels in diabetic rats. According to Akoko et al. (2022) and Oyedepo et al. (2013), aqueous extracts of Vernonia amygdalina and Moringa oleifera and extract mixtures of *Vernonia amygdalina*, *Moringa oleifera*, and *Ocimum gratissimum* have shown to normalize the high blood glucose level in diabetic rats and are effective after 28 days of experimental use. The extract treatment also increased the survival and decreased hyperglycemia-related mortality in diabetic rats. In this investigation, nearly every animal in the negative control group died on day 14 after the onset of diabetes, but the group that received the extract survived beyond the duration of the trial. It is likely that the combination of aqueous extracts from Vernonia amygdalina and Moringa oleifera has some direct effects because improved activity has been seen in severely diabetic rats with damaged islets.

The degree of an attack and the toxicity of a chemical compound on organs and tissues can be determined by measuring the activity of biomarkers in bodily fluids (Yakubu et al., 2003; Akanji, 1986). Additionally, these measures can be utilized to predict tissue cellular damage brought on by a chemical molecule before it is detected by histological methods (Omeodu, et al., 2022). When compared to the normal control, the observed significant rise in the activities of ALT, AST, and ALP in the diabetic untreated rats (negative control) is evidence of cytotoxic injury to the liver. In contrast, when *Vernonia*

amygdalina and *Moringa oleifera* combined leaf extract treated diabetic rats were compared to the diabetic untreated rats (negative control), a significant decrease in the activities of these liver marker enzymes was seen. This suggests a reduction in the rate and magnitude of tissue cell injury and is also consistent with the observed protective effect of plant extracts against alloxan-induced diabetes in rats (Aleme et al., 2022; Akoko et al., 2022; Ayobami et al., 2020; Malomo, 2000).

The combined administration of both plant extracts demonstrated a potential hepatoprotective and anti-hyperglycemic effect, which may be the result of a synergy of the two plants' bioactive secondary metabolites. This is in line with research by Atangwho et al. (2009) and Effiong et al. (2013), which showed that these plants' leaves appear to have a complement of bioactive compounds that may be responsible for their hypoglycemic and hepatoprotective effects.

5. CONCLUSION

In the treatment of diabetes, the combined leaf extracts of *Vernonia amygdalina* and *Moringa oleifera* showed anti-hyperglycemic and liver protective effect against alloxan-induced hepatotoxicity on serum levels of liver enzymes, thereby confirming their uses as hepatoprotective and antidiabetic agents. This study shown that the combination extract of *V. amygdalina* and *M. oleifera* can guard against the toxicities brought on by alloxan. The current research supports the traditional usage of *Vernonia amygdalina* and *Moringa oleifera* as liver disease treatments. Thus, it can be said that *Vernonia amygdalina* and *Moringa oleifera* combined in a 16% extract are powerful enough to reverse oxidative stress and hyperglycemia in diabetic rats.

Declaration

Authors declared that this is an original research and no conflicts of interest exist.

REFERENCES

[1] Akah PA, Okafor CI. (1992). Blood sugar lowering effect of *Vernonia amygdalina* (Del) in an experimental rabbitmodel. Phytother. Res.; 6:171-3.21.

[2] Akanji MA. (1986). A comparative biochemical study of the interaction of some trypanocides with rat tissue cellular system. Ile-Ife Nigeria: University of Ife; Ph. D. Thesis

[3] Akoko S, Aleme BM, Uahomo PO. (2022). The Effect of Addition of Extracts of *Vernonia amygdalina* and *Moringa oleifera* in the Nutrition of Alloxan- Induced Diabetic Wistar Rats. International Journal of Pharma Research and Health Sciences, 10(4): 3455-3462.

[4] Alebachew M, Kinfu Y, Makonnen E, Bekuretsion Y, Urga K. and Afework M. (2014). Toxicological evaluation of methanol leaves extract of *Vernonia bipontini* Vatke in blood, liver and kidney tissues of mice. African Health Sciences, 14(4): 1012–24.22.

[5] Aleme BM, Omeodu SI, Akoko S and Uahomo PO. (2022). Alloxan-induced Diabetes: The Synergistic effects of combined ethanolic leaf extract of *Dacryodes edulis* and *Psidium guajava*. International Journal of Pharmaceutical Research and Applications, 7(4), 1391-1398

[6] Amole OO, Izegbu MC, Onakoya JAA. and Dada MO. (2006). Toxicity studies of the aqueous extract of *Vernonia amygdalina*. Biomedical Research, 17: 39–40.

[7] Areghore EM, Makkar HPS, and Becker K. (1997). Chemical composition and tannins in leaves of some browse plants from Delta (Central Nigeria) eaten by ruminants. Proceedings of the Society of Nutrition Physiology, vol. 5, pp. 11–1.20.

[8] Atangwho IJ, Ebong PE, Eyong EU, Williams IO, Eteng MU, Egbung GE (2009). Comparative chemical composition of leaves of some antidiabetic medicinal plants: *Azadirachta indica*, *Vernonia amygdalina* and *Gongronema latifolium*. Afr. J. Biotechnol. 8(18):4685-4689.

[9] Ayobami AL, Kade EA, Oladimeji KA, Kehinde S, Gurpreet K. (2020). Anti-diabetic Potential of Aqueous Extract of *Moringa oleifera, Ocimum gratissimum* and *Vernonia amygdalina* in Alloxan-Induced Diabetic Rats. J Diabetes Clin Res.; 2(3):78-85.

[10] Cheesbrough M. (2000). Clinical chemistry in: District and Laboratory Practice in Tropical Countries, Part 2, Cambridge University Press, Cambridge p. 318.

[11] Duncan DB. Multiple range and multiple F tests. Biometrics. 1955 Mar 1;11(1):1-42.

[12] Efiong EE., Igile GO, Mgbeje BIA., Otu EA. and Ebong PE. (2013). Hepatoprotective and anti-diabetic effect of combined extracts of Moringa oleifera and Vernonia amygdalina in streptozotocin-induced diabetic albino Wistar rats. Journal of Diabetes and Endocrinology, 4(4), 45-50

[13] Fröde TS, Medeiros YS. (2008). Animal models to test drugs with potential antidiabetic activity. Journal of Ethnopharmacology, 115(2):173-83.

[14] Goji AD, Dikko AA, Bakari AG, Mohammed A, Ezekiel I, Tanko Y, et al. (2009). Effect of aqueous-ethanolic stem bark extract of *Commiphora africana* on blood glucose levels on normoglycemic Wistar rats. International Journal of Animal and Veterinary Advances; 1(1):22-4.

[15] King EJ, King PR. (1954). Estimation ofserum phosphatase by determination of hydrolysed phenol with amino – antipyrene. J. Clin. Path., 7: 322 – 327

[16] Malomo SO. 2000). Toxicological Implication of Ceftriaxone Administrationin Rats. Nig J Biochem & Mol Biol., 15(1), 33–38.

[17] Marles RJ, Farnsworth NR. (1995). Antidiabetic plants and their active constituents. Phytomedicine; 2(2):137-89.

[18] Nimenibo-Uadia R. (2003). Effect of aqueous extract of *Canavalia ensiformis* seeds on hyperlipidaemia and hyperketonaemia in alloxan-induced diabetic rats. Biokemistri; 15(1):7-15.

[19] Okoli CO, Ibiam AF, Ezike AC, Akah PA, Okoye TC. (2010). Evaluation of antidiabetic potentials of Phyllanthus niruri in alloxan diabetic rats. African Journal of Biotechnology; 9(2):248-259.

[20] Omeodu SI, Uahomo PO, Porobe VL. (2022). Antidiabetic and HepatoprotectiveEffects of Combined Aqueous leaf extract of *Azadirachta indica* and *Mangifera indica* in Alloxan Monohydrate-induced Diabetic Wistar Rats. EPRA International Journal of Multidisciplinary Research, 8(10):249-256

[21] Owulade MO, Eghianruwa KI, Daramola FO. (2004). Effects of aqueous extracts of Hibiscus sabdariffa calyces and *Ocimun gratissimum* leaves on interstinal transit in rats. African Journal of Biomedical Research; 7(1):31-34.

[22] Oyedepo TA, Babarinde SO, Ajayeoba TA. (2013). Evaluation of anti-hyperlipidemic effect of aqueous leaves extract of Moringa oleifera in alloxan induced diabetic rats. International Journal of Biochemistry Research & Review; 3(3):162-170.

[23] Reitman S and Frankel S. (1957). A Colorimetric Method for the Determination of Serum Glutamic Oxalacetic and Glutamic PyruvicTransaminases. American Journal of Clinical Pathology, 28, 56-63

[24] Rockwood JL, Anderson BG, Casamatta DA. (2013). Potential uses of Moringa oleifera and an examination of antibiotic efficacy conferred by M. oleifera seed and leaf extracts using crude extraction techniques available to underserved indigenous populations. International Journal of Phytotherapy Research; 3(2):61-71.

[25] Rotimi SO, Omotosho OE, Rotimi OA. (2011). Persistence of acidosis in alloxan-induced diabetic rats treated with the juice of *Asystasia gangetica* leaves. Pharmacognosy Magazine.; 7(25):25-30.

[26] Szkudelski T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiological Research.; 50(6):536-46.

[27] Tasaduq SA, Singh K, Sethi S, Sharma SC, Bedi KL, Singh J, et al. (2003). Hepatocurative and antioxidant profile of HP-1, a polyherbal phytomedicine. Human & Experimental Toxicology.; 22(12):639-45.

[28] Tietz NW. (1990). Clinical Guide to Laboratory Tests; 2nd Edition (Philadelphia: W B Saunders Company)pp: 554-556

[29] Tietz NW. (1996). Lever Function Tests, Nitrogen Metabolites and Renal Function In: Fundamentals of Clinical Chemistry. 3rd ed. W.B. Saunders, Philadelphia pp. 476 – 576.

[30] Yakubu MT, Bilbis LS, Lawal M, Akanji MA. (2003). Effect of Repeated Administration of Sildenafl citrate on selected enzyme activities of liver and kidney of male albino rats. Nig J Pure & Appl Sci.; 18:1395–1400.