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THE AMELIORATIVE POTENTIAL OF AQUEOUS EXTRACT OF *HUNTERIA UNBELLATA* ON ALLOXAN—INDUCED DIABETES AND HYPERLIPIDEMIA IN WISTAR RATS

[1]Emejuru, S.W. *[1]Onyegeme-Okerenta, B.M. [1]Amadi, B.A.
[1]Department of Biochemistry, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria.
*Corresponding Author: e-mail: blessing.onyegeme-okerenta@uniport.edu.ng ; Tel: +234 (0)8035201039

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ABSTRACT

The use of aqueous fruit extract of unripe Hunteria umbellata in the therapeutic management of alloxan-induced diabetic Wistar rats was investigated. Thirty-five Wistar rats divided into 7 groups of 5 rats each were used for the study. Group 1 was the positive control. Diabetes was induced in Groups 2 -7 using 150 mg kg^{-1} body weight of alloxan. Glucose levels were monitored for 72 hrs to confirm diabetes mellitus. Rats in group 2 received no treatment and served as the negative control while rats in Groups 3-7 received 500, 1000, 1500, 2000, and 2500 mg kg^{-1} of aqueous fruit extract of unripe H. umbellata respectively. After 28 days, the animals were anaesthetized with chloroform and blood was collected through cardiac puncture for determination of biochemical and haematological indices. The results showed a significant reduction ($p<0.05$) in the average weights of the rats from 141.4±2.07 g before alloxan treatment to 130.4±8.14 g. However, treatment with 500-2500 mg kg^{-1} of H. umbellata significantly ($p<0.05$) increased the weight of the rats to 141.0±1.41 g. The average fasting blood sugar (FBS) level of the rats before alloxan induction was 5.58±0.73 mmol/l, this increased 72 hours after alloxan treatment to 23.92±5.27 mmol/l. Treatment with 500-2500 mg kg^{-1} of H. umbellata initiated a significant ($p<0.05$) dose-related reduction in FBS levels. There was a significant ($p<0.05$) increase in α-amylase (31.19±2.67 IU) and Glutathione reductase (55.77±9.66 mg/dl) activities in group 6 when compared to group 2 (18.04±0.82 and 18.07±1.94 mg/dl respectively). Total cholesterol (160.51±1.36 mg/dl) in group 2 was reduced to 95.63±1.61 mg/dl in group 5. In conclusion, H. umbellata has the potential to lower blood sugar and may be used in the management of diabetes.

Keywords: Hunteria umbellata, Diabetes mellitus, Hyperlipidemia, α-amylase, Fasting blood sugar, antioxidant

INTRODUCTION

Diabetes Mellitus is a metabolic disease and a leading cause of death worldwide. It occurs when the pancreas does not produce enough insulin or the body cannot effectively respond to insulin, causing abnormally high blood sugar levels (glucose) (Adijat et al., 2021). Failure to properly manage the disease can lead to severe complications in the brain, kidneys, heart, limbs, and even blindness (Solomon et al., 2022). The disease is long-standing and one of the leading causes of death from cardiovascular complications globally. In 2019, the estimated global

prevalence of diabetes was 9.3%, or 463 million people, with a predicted increase of 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 (Sun et al., 2022). Diabetes mellitus is a heterogeneous disorder with varying prevalence among different ethnic groups, and it affects both developed and developing countries.

A current investigation on diabetes revealed that there has been an increase in diabetes mellitus in the people residing in the south-south region of Nigeria (Solomon et al., 2022). In Nigeria, the current International Diabetes Federation (Saeedi et al., 2019) estimation of the prevalence of diabetes mellitus is reported to affect about 3000 patients, ranging from 20 to 69 years in the year 2021 and close to over 7,000 in the year 2045. However, many cases of diabetes in Nigeria are left undiagnosed due to ignorance, poverty, and inadequate access to healthcare in some rural areas, leading to death and complications. Fifty per cent of these victims are unaware of their condition (Adijat et al., 2021).

Modern drugs like insulin and other hypoglycaemic drugs such as Tolbutamide, Phenformin, troglitazone etc. regulate or manage the blood glucose level only when they are consistently administered. However, the exhausting routine of treatment with several unpleasant side effects from these drugs may lead to decreased medication adherence and concordance, including treatment failure (Mohiuddin et al., 2019).

It has been established over the years that herbal medicines taken at the right proportions may be sources of substances with desirable healing potentials than some currently used orthodox medicines (Ighodaro et al., 2009). These drugs are gaining popularity both in developed and developing countries because of their natural origin and fewer side effects when taken in moderation (Onyegeme-Okerenta & Anacletus, 2017).

*Hunteria umbellata*K.Schum (Apocynaceae) is a shrub that grows to a height of 15-22m and is widely distributed throughout West and Central Africa. In southern Nigeria, it is known as "osu" in Edo, "Nkpokiri" in Igbo, and "erin" in Yoruba. It is predominantly found in the sub-Saharan region and is a beneficial medicinal plant with a plethora of domestic and ethno-medicinal uses. The seed, pulp, and bark of *H. umbellata* are commonly prepared as an infusion and have been reported to be effective against fever, leprosy, sores, menstrual disturbances, and infertility. Additionally, the seeds of this plant are used in the local management of diabetes mellitus (Olumide et al., 2021) while the unripe fruit is used in the management of malaria (Onyegeme-Okerenta et al., 2023). From the evaluation made by various scientists and researchers, it can be deduced that numerous bioactive concept with known biological usefulness is present in the extracts of *H. umbellata* and might be responsible for the observed biological and pharmacological activities. This study is aimed at investigating the modulatory potential of unripe *H. umbellata* fruit in the therapeutic management of alloxan-induced-diabetic Wistar rats and to provide a scientific rationale upon which these unripe whole fruit extracts can be used to control hyperglycaemia.

MATERIALS AND METHOD

Collection and Preparation of Plant Sample Material

The plant or whole fruit (*H. umbellata*) used for the present study was purchased locally from Rumuokoro market in Obio/Akpor Local Government Area of Rivers State Nigeria. The plant was first identified by Dr Chimezie Ekeke of the Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Nigeria and deposited at the University Herbarium with the voucher number, UPH/P/294.

The fruits were thoroughly washed with water after which they were cut into little pieces, air-dried and pulverized to powdered form. The powdered sample was then weighed (500

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gm) and dissolved in distilled water (1000 ml). This was allowed to stand for 72 hours and at intervals it was thoroughly mixed, this mixture was then filtered using a muslin cloth and the filtrate was concentrated using a freeze dryer. The dried extract was preserved in an air-tight clean glass container and stored at 4°C in a refrigerator until required.

Experimental animals and Design

Healthy 35 adult rats were procured from the animal house of the Department of Biochemistry at the University of Port Harcourt, Nigeria. The animals were housed in plastic cages and were given food and water ad libitum to acclimatize. The temperature of the animal house was maintained at 27± 1^{o} C temperature. The feed pellets used were grower mash obtained from flour mills in Port Harcourt.

Five (5) rats were randomly assigned to constitute 7 groups for the study. The weights of the rats differed by ±5 g. Group 1, was the positive control and received only water and feed without treatment. A high-fat diet was given to Groups 2 to 7 for four weeks.

Induction of diabetes and treatment with extracts

The rats in Groups 2 to 7 were allowed to fast for 18 hours and then Alloxan monohydrate (150 mg kg^{-1}body weight in cold normal saline) was injected intraperitoneally. The rats were monitored closely for 72for any sign of hyperglycaemia, the glucose level was monitored using a glucometer. After 3 days, rats with consecutive blood sugar levels greater than 15 mmol/l were considered Diabetic. Group 2 rats did not receive any treatment and were tagged negative control. However, rats in Groups 3 - 7 received 500, 1000, 1500, 2000 and 2500 mg kg^{-1}bodyweight of aqueous fruit extract of unripe *H. umbellata* respectively. The study lasted for 8 weeks.

Collection, processing of samples for Laboratory analysis

The fasting blood glucose level of blood samples drawn from the tail vein puncture was determined using a One-touch ultra-easy glucometer. The strip was dipped into animal blood and inserted into the glucometer, which automatically displayed the level of glucose in the blood.

The animals were anaesthetized with chloroform, after which blood was collected from the retro-orbital venous plexus into ethylenediaminotetraacetic acid (EDTA) bottles for immediate determination of haematological indices. Full blood count was determined using an automated Mindray BC-3200 Auto Haematology analyser. Blood for biochemical assays was collected in lithium heparin bottles, separated from plasma and used to assay for the activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), (Reitman & Frankel 1957), and alkaline phosphatase (ALP) (Klein et al. (1960) as outlined in Randox kits, UK. Determination of α-Amylase activity was as outlined by the method of Lorentz, (1998). The measurement of sodium and potassium was done using a flame photometer as described by Chuang et al. (2005). Urea and creatinine levels were measured by the method of Tietz (2004). The methods of Usoh et al. (2005) were adopted for the determination of the activities of oxidative stress markers such as lactate dehydrogenase (LDH), superoxide dismutase (SOD), catalase (CAT), glutathione (GST), and malondialdehyde (MDA). Total Cholesterol, Triglycerides, Low-density lipoprotein (LDL) and High-density lipoprotein (HDL) were analyzed by kinetic methods kits from Randox, (United Kingdom) using a double-beam spectrophotometer.

Histological Analysis

Pancreatic samples for histological studies were surgically removed and placed in a universal bottle containing 10% formal saline. They were subjected to standard routine histological procedures as described by Kiernan (2008). The slides were viewed using the light microscope and histopathological

changes were observed and recorded at X400 magnification, identifying both the normal and the degenerated cells.

Statistical Analysis

The data obtained from this study was analysed using SPSS, and the significant level was set at $p< 0.05$. Analysis of Variance (ANOVA) was used to compare the different groups and the results were presented using tables and charts.

RESULTS

The effect of aqueous fruit extract of unripe *H. umbellata* on the body weight (g) of Wistar rats in different groups

The results of the effect of aqueous fruit extract of unripe *H. umbellata*are shown in Figure 1. The results show an overall weight gain across all groups. However, Group 6 rats that were administered 2000 mg kg^{-1} body weight of the aqueous fruit extract of unripe *H. umbellata*were observed to have the highest weight gain when compared to other groups at the end of the experiment.

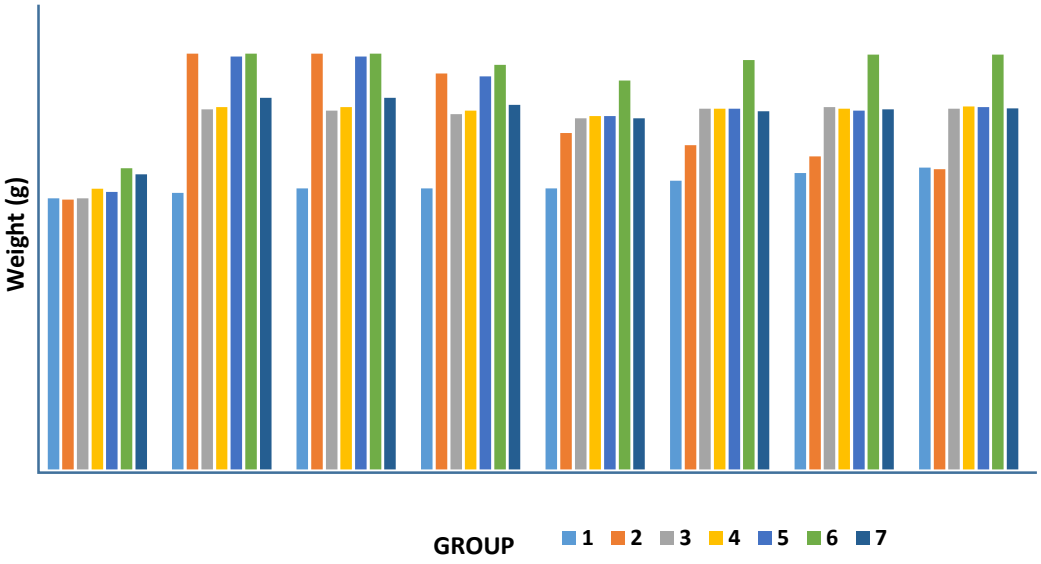

Figure 1: The effect of aqueous fruit extract of *H. umbellata* on the body weight (g) of Wistar rats in different groups.

IW=Initial Weight, WHFD = Weight 28 days after High Fat Diet

The results of the effect of aqueous whole fruit extract of unripe *H. umbellata*on the blood glucose level of Wistar rats from Group 1 showed no significant difference ($p> 0.05$) in sugar level throughout treatment (Figure 2). Additionally, the sugar levels across all groups were significantly not different initially. However, the sugar level of all treated groups statistically increased ($p< 0.05$) after the administration of high-fat feed and diabetes induction with alloxan when compared to the control group.

Scientia Africana, Vol. 22 (No. 3), December, 2023. Pp 299-318
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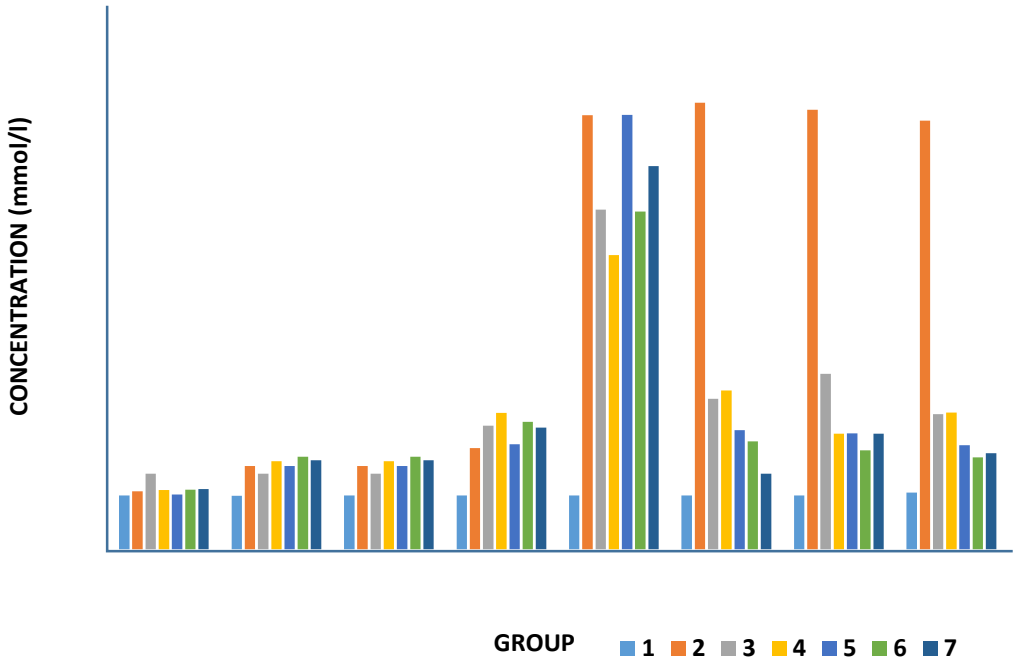

Figure 2: Effect of aqueous whole fruit extract of unripe *H. umbellata* on blood glucose level of alloxan-induced diabetic Wistar rats feed with high fat feed and

ISL= Initial Sugar Level, HFFSL= High Fat Feed (Sugar level)

Effects of the aqueous fruit extract of unripe *H. umbellata* on liver enzymes and α-amylase

The results for ALT, AST and ALP enzymes in Groups 3,4,5,6,7 showed a significant reduction ($p \leq 0.05$) in activities compared to Group 2 indicating that the extract effectively modulated these enzymatic activities in the hepatic cells. Similarly, a significant ($p < 0.05$) decrease in the activity of α-amylase was observed after induction of diabetes with alloxan. However, after treatment with the extract, a total restoration to normal levels was observed. While the level of the diabetic rats in Group 2 showed α-amylase activity of 18.04 ±0.82, it was observed that there was a dose-dependent increase in the level of α-amylase across the treatment groups (3, 4, 5, 6, 7) from 500 to 2000 mg of the extract (Figure 3).

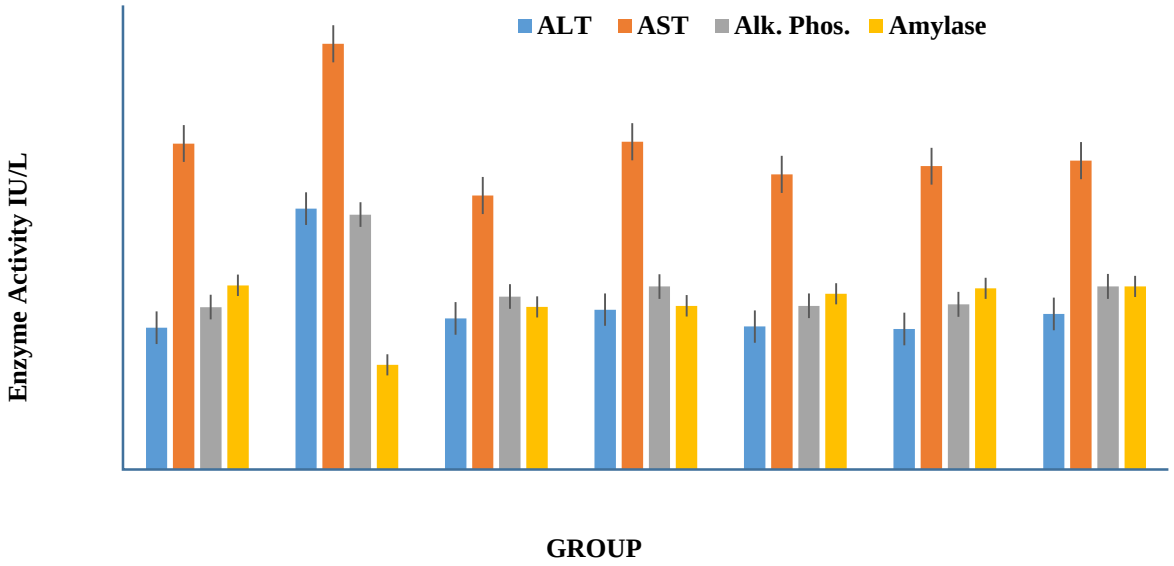

Figure 3: Effect of aqueous whole fruit extracts of unripe *H. umbellata on* alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phospahatase (ALP) and amylase activities of Wistar rats.

Effect of aqueous whole fruit extracts of unripe *H. umbellata* on the markers of kidney function

The results obtained for the effects of aqueous extract of unripe *H. umbellata* on the markers of kidney function are presented in Figure 4. The concentration of sodium and potassium in Groups 1,3,4,5,6,7 were significantly higher ($p \geq 0.05$) and lower ($p \leq 0.05$) respectively, when compared to Group 2. The Diabetic control (Group 2) in the present showed a significant increase in the level of urea and the results are displayed in Figure 4. Observations showed that, although the urea levels in the treatment groups were higher than that of the normal control (Group 1), they were all significantly lower than the levels seen in the diabetic control group. Furthermore, ANOVA comparison showed that these values in the treatment group were significantly (p <0.05) lower when compared to the diabetic group (Group 2). In contrast, the values from the normal rats (Group 1) showed that urea levels were of similar ranges. The creatinine levels of Groups 1,3,4,5,6, and 7 were significantly ($p \leq 0.05$) higher when compared with Group 2 (Figure 5).

Scientia Africana, Vol. 22 (No. 3), December, 2023. Pp 299-318
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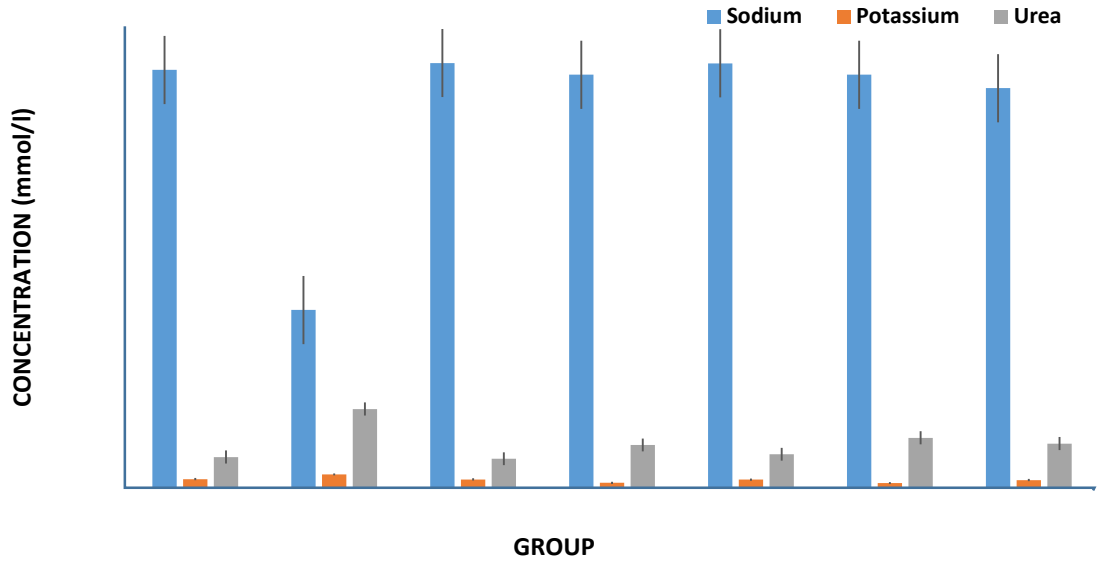

Figure 4: Effect of aqueous whole fruit extracts of unripe *H. umbellata* on serum electrolytes (Na^+, K^+) and urea concentration in Wistar rats.

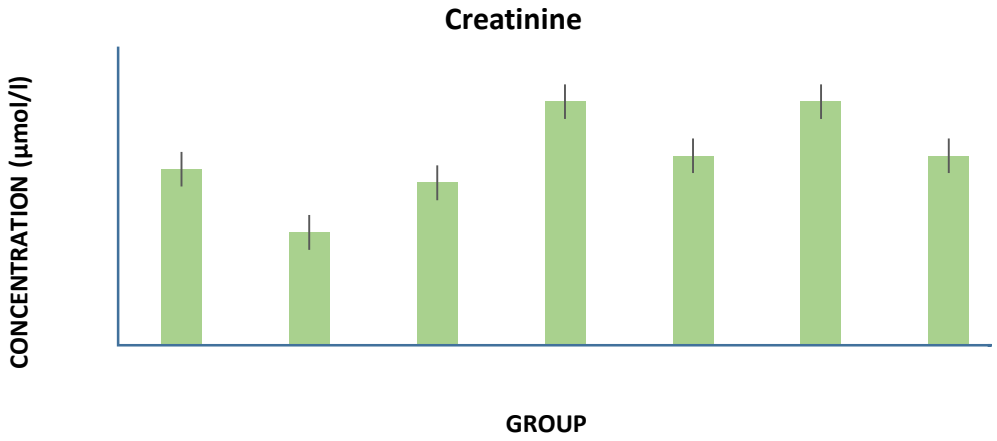

Figure 5: Effect of aqueous whole fruit extracts of unripe *H.umbellata* on creatinine levels in Wistar rats

Effect of aqueous whole fruit extract of unripe H. umbellata the lipid profile of experimental animals

The effect of the aqueous extract of unripe H. umbellata fruit on the lipid profile of alloxan-induced diabetic Wistar rats is presented in Figure 6. The triglyceride, total cholesterol, low-density lipoproteins, and very low-density lipoproteins levels of the positive control (Group 1) and the different treatment groups (3, 4, 5, 6 and 7) were significantly ($p \leq 0.05$) lower, while their HDL level was significantly ($p \leq 0.05$) higher when compared with the negative control (Group 2). These results implied that the extract was effective in modulating the lipid levels of the experimental animals.

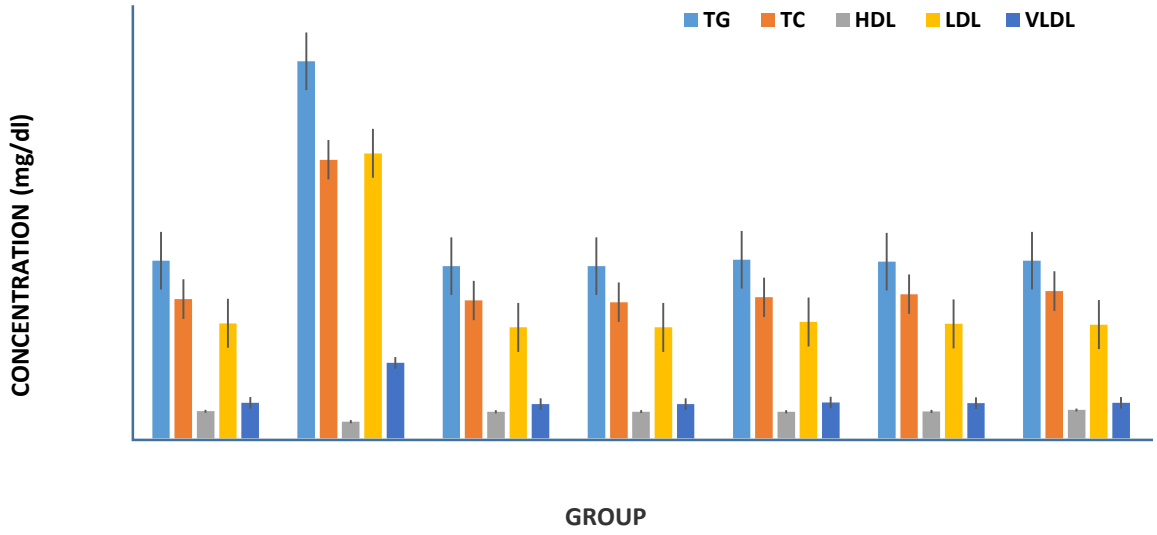

Figure 6: Effect of aqueous whole fruit extracts ofunripe*H. umbellata* on the lipid profile of alloxan-induced diabetic Wistar rats

Effect of aqueous whole fruit extract of unripe H. umbellata *fruit on markers of oxidative stress in alloxan-induced diabetic Wistar rats*

From the results obtained, it was observed that there was a decrease in catalase activity in Group 2 (Figure 7). However, treatment with the different doses of the extract resulted in a 7-fold increase in the activity of the enzyme as observed in Groups 3–7. These increases were significantly higher ≤ 0.05) when compared to the diabetic control (Group 2), but it was observed that the restoration of catalase activity was not up to the level of activity in the Group 1 rats. It was also observed that the SOD (Figure 7) and GSH (Figure 8) activities of Groups 1,3, 4, 5, 6, 7, were significantly ($p \leq 0.05$) higher when compared with Group 2. The results for MDA obtained from the study showed an increased peroxidation in the alloxan-induced diabetic groups (Figure 9). After treatment with the extract, the level of peroxidation was observed to be significantly ($p \leq 0.05$) reduced.

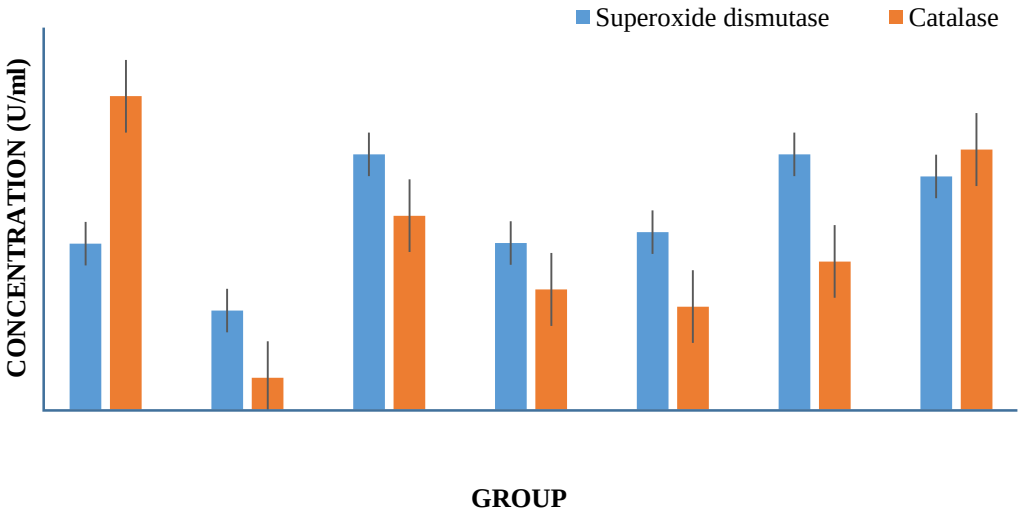

Figure 7: Effect of aqueous whole fruit extract of unripe *H. umbellata* on superoxide dismutase (SOD) and catalase (CAT) activities in Wistar rats.

Scientia Africana, Vol. 22 (No. 3), December, 2023. Pp 299-318
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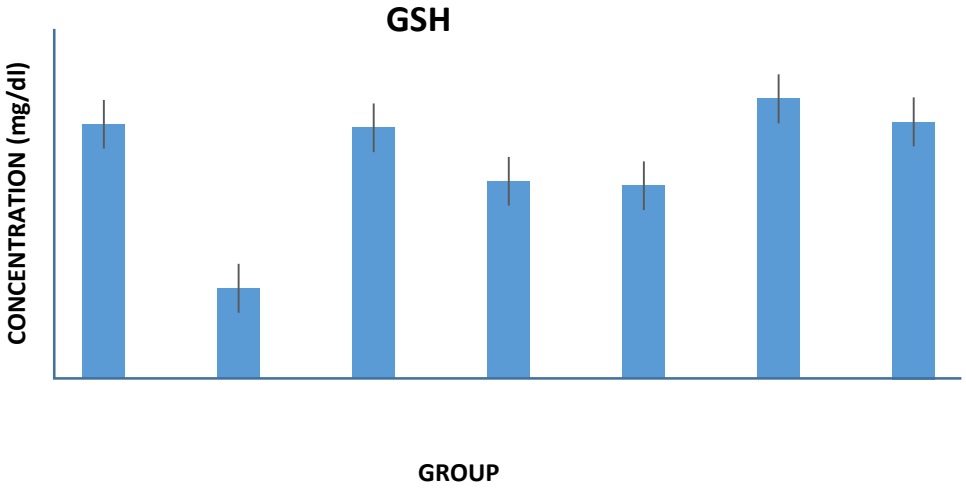

Figure 8: Effect of aqueous whole fruit extracts of unripe *H. umbellata* on glutathione (GSH) Concentration in Wistar rats.

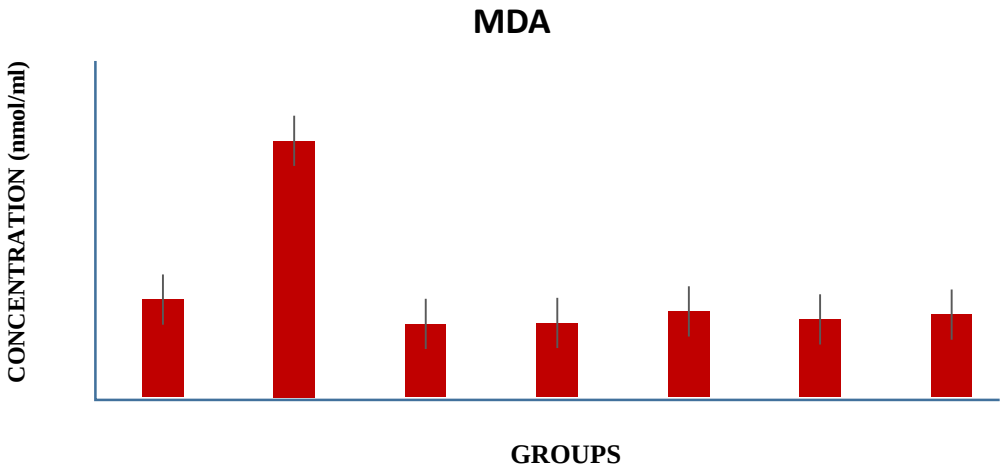

Figure 9: Effect of aqueous whole fruit extracts of *H. umbellata* on lipid peroxidation (MDA) level on Wistar rats.

The results for nitric oxide level obtained showed that the values recorded in Group 2 rats were almost twice the levels observed in all the other groups. This value was significantly (p <0.05) higher when ANOVA comparisons were carried out (Figure 10). Furthermore, observations showed that after treatment with the extract for four weeks, there was a significant reduction in NO level when the groups were compared to Group 2. These results imply that the extract had antioxidant-modulating activities.

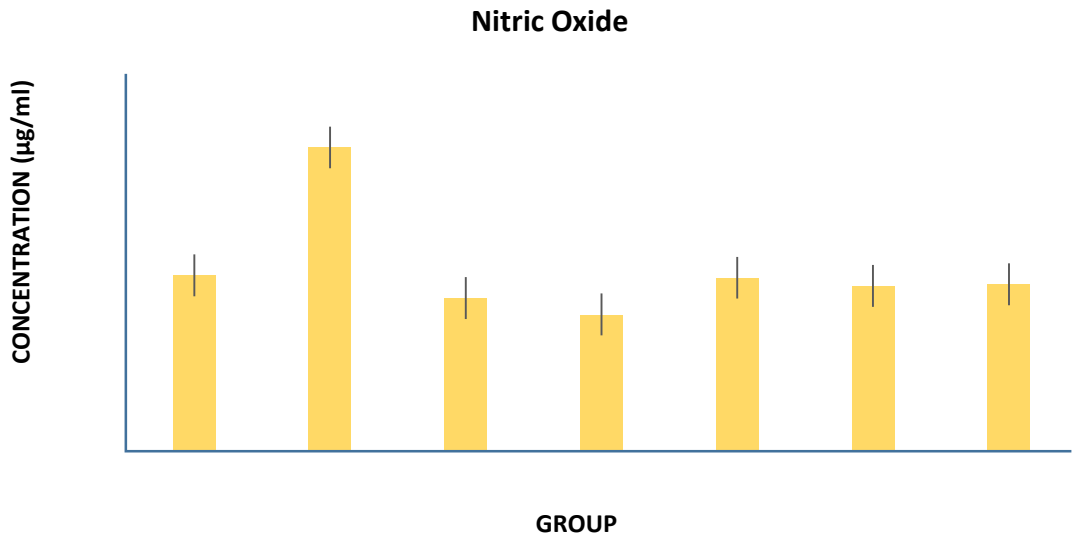

Figure 10: Effect of aqueous whole fruit extracts of unripe *H. umbellata* on nitric oxide (NO) concentrations in Wistar rats.

Effect of aqueous whole fruit extract of unripe H. umbellata *on Haematological indices of Wistar rats.*

Table 1A demonstrates the effect of aqueous whole fruit extract of unripe *H. umbellata* against alloxan-induced toxicity on haematological parameters in Wistar rats. In rats treated with various doses of aqueous fruit extract of *H. umbellata* showed no significant differences ($p > 0.05$). in PCV, Hb, and RBCs when compared to the control In addition, as presented in Table 1B, rats treated with 500, 1000, 1500 and 2000 mg kg^{-1} of *H. umbellata* demonstrated no significant differences ($p > 0.05$) in NUT, LYMP, EOS, MON, MCHC, MCH, and MCV compared to normal negative controls ($p > 0.05$).

Table 1A: Effect of aqueous whole fruit extract of unripe *H. umbellata* on haematological indices of Wistar rats

Group	PCV (%)	Hb (g/dl)	RBC (g/dl)	WBC (per mm^3)	PLT (X 10^5/µL)
1	34.00 ± 6.08^a	11.6 ± 1.85^a	5.63 ± 1.12^a	15.57 ± 9.11^a	425.33 ± 230.01^a
2	34.00 ± 1.00^b	9.97 ± 1.00^b	6.03 ± 1.00^b	4.00 ± 1.00^b	173.00 ± 1.00^b
3	40.67 ± 1.53^a	13.47 ± 0.64^a	7.07 ± 0.45^a	11.33 ± 4.43^a	455.00 ± 215.37^a
4	37.67 ± 8.08^a	12.43 ± 2.44^a	6.17 ± 1.29^a	10.50 ± 4.18	514.00 ± 296.99^a
5	40.00 ± 3.61^a	13.4 ± 0.96^a	6.6 ± 0.46^a	13.07 ± 4.06^a	456.34 ± 395.12^a
6	41.67 ± 3.79^a	13.87 ± 1.06^a	7.27 ± 0.55^a	13.53 ± 3.46^a	614.33 ± 110.82^a
7	40.33 ± 4.16^a	13.27 ± 1.97^a	6.93 ± 0.55^a	17.00 ± 2.05^a	721.00 ± 95.14^b

Values represent the mean ± SD for (n = 5 rats)

Values in the same columns bearing similar superscript letters are not significantly different from each other ($p > 0.05$).RBC = Red Blood Cells, PCV = Packed Cell Volume, Hb = Hemoglobin, WBC = White, PLT = Platelets

Scientia Africana, Vol. 22 (No. 3), December, 2023. Pp 299-318
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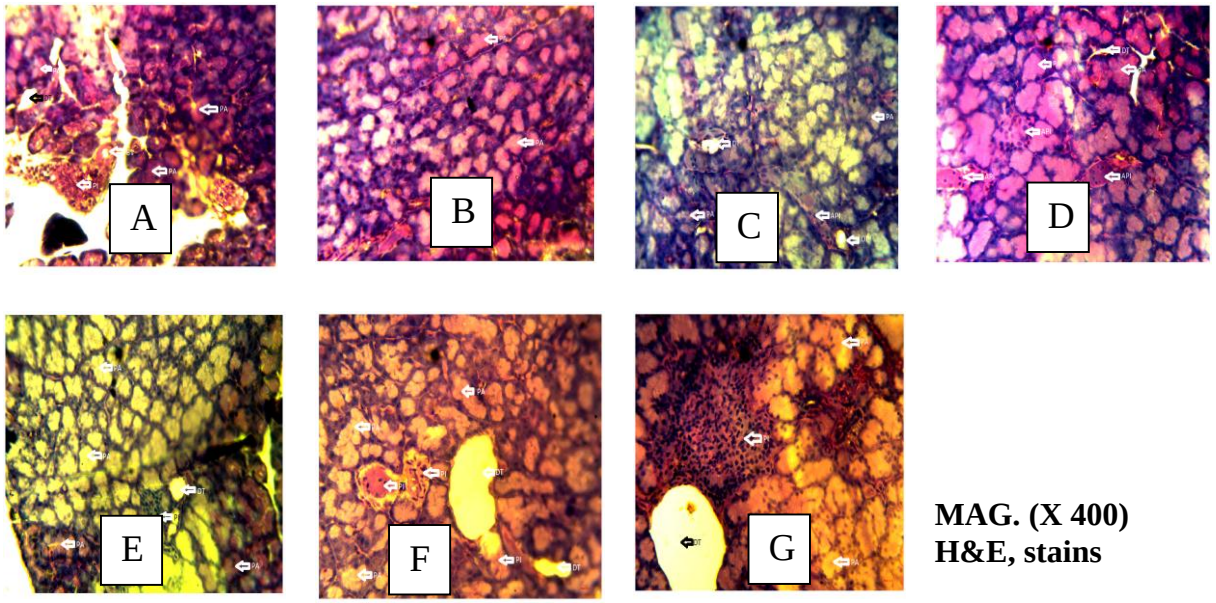
Table 1B: Effect of aqueous whole fruit extract of unripe *H. umbellata* on haematological indices of Wistar rats

Group	NUT (%)	LYMP (%)	EOS (%)	MON (%)	MCHC (g/dl)	MCH (PG)	MCV (FL)
1	12.33±3.21	81.00±7.81^{a}	2.00±1.73^{a}	4.00±2.00	33.8±0.53^{a}	19.73±1.81^{a}	58.73±5.35^{a}
2	13.00±1.00^{a}	82.33±2.52^{a}	1.67±0.58^{a}	3.00±1.73^{a}	33.43±0.42^{a}	19.17±0.55^{a}	51.33±12.54^{b}
3	10.00±1.00	86.67±1.53^{b}	1.33±0.58^{a}	1.33±0.58^{a}	286.00±1.00^{b}	15.73±1.55^{b}	55.87±0.81^{a}
4	14.67±2.52^{a}	81.00±3.61^{a}	1.67±0.58^{a}	2.67±0.58^{a}	32.63±0.75^{a}	18.9±0.35^{a}	57.83±2.73^{a}
5	14.33±1.15^{a}	80.33±2.52^{a}	2.00±0.00^{a}	3.33±1.53^{a}	32.23±0.35^{a}	19.8±1.22^{a}	61.73±2.80^{a}
6	14.00±1.73^{a}	80.33±4.93^{a}	2.00±1.00^{a}	3.67±2.31^{a}	32.67±1.08^{a}	19.77±0.32^{a}	60.07±2.80^{a}
7	16.67±0.58	74.33±4.51^{a}	2.33±1.15^{a}	6.67±3.06^{b}	31.93±2.21^{a}	18.80±1.61^{a}	58.40±0.85^{a}

Values represent the mean ± SD for (n = 5 rats).Values in the same columns bearing similar superscript letters are not significantly different from each other (p> 0.05).NUT = neutrophils, LYMP = lymphocytes, EOS = Eosinophils, MON = monocytes, MCHC = Mean Corpuscular Haemoglobin Concentration, MCH = Mean Corpuscular Haemoglobin, MCV = Mean Corpuscular Volume

Histology Result



MAG. (X 400)
H&E, stains

Plate 1**A**: Photomicrograph of the pancreas of Group 1 rats showing normal pancreas, pancreatic acini (PA), pancreatic islet (PI) containing alpha, beta & delta cells.Ducts (DT) and blood vessels (BV). **B**: Photomicrograph of the pancrease of Group 2 rats showing abnormal pancrease, PA, atrophic pancreatic islet (API) containing alpha, beta and delta cells.**C**:Photomicrograph of the pancrease of Group 3 rats showing abnormal pancrease, PI, API containing alpha, beta, deltaand DT.**D**: Photomicrograph of the pancrease of Group 4 rats showing abnormal pancreas, PA, API containing alpha, beta, delta cells and DT.**E**:Photomicrograph of the pancrease of Group 5 rats showing abnormal pancreas, PI, API containing alpha, beta, delta cells and DT.**F**: Photomicrograph of the pancrease of Group 6 rats showing abnormal pancrease, PI, API containing few alpha, beta, delta cells and ducts DT. **G**: Photomicrograph of the pancrease of Group 7 rats showing normal pancreasePA, PI containing alpha, beta, delta cells and ducts.

DISCUSSION

Finding safer and more potent anti-diabetic medications is necessary because the pharmacological regimen now used to manage diabetes mellitus has some downsides (Grover et al., 2000). Long-term diabetes mellitus is linked to several problems, including nephropathy, atherosclerosis, and myocardial infarction. The chronically increased blood glucose level have long been thought to be a factor in these problems (Beppu et al., 2003). By destroying the β-cells in the islets of Langerhans, alloxan significantly reduces the amount of insulin released, leading to hyperglycemia (Szkudelski, 2001). In addition to having a necrotic effect on the pancreas, it also hurts the other organs of the experimental animals, altering the haematological and biochemical parameters of organ function and ultimately impairing the normal operation of those organs. Alloxan has been researched on and confirmed to induce diabetes at certain doses; 120mg/kg and 150mg/kg and this is done by forming highly reactive free radicals which destroy the insulin-producing beta cells of the pancreas.

In the present study, Wistar rats were treated with intraperitoneal injections of cold alloxan monohydrate in normal saline to induce type 1 diabetes, which is in line with the study of Adeneye et al. (2014) when they induced the Wistar rats with type 1 diabetes using alloxan monohydrate. The intracellular accumulation of alloxan in pancreatic beta-cells via the GLUT2 glucose transporters through the ROS mechanism, which leads to pancreatic beta-cell degeneration, is known to cause diabetes and oxidative/nitrosative stress (Lenzen, 2008). The intrinsic antioxidative defence mechanisms of pancreatic cells are weak, making them vulnerable to being overpowered by redox imbalance from reactive oxygen and nitrogen species, which may lead to damaging consequences like lipid peroxidation, protein oxidation, and DNA damage (Djordjevic et al., 2010). In addition, pancreatic -cells are known to be susceptible to oxidative stress. The physiological system is designed in a way to balance the oxidant and anti-oxidant system, but when an imbalance arises due to the overproduction of free radicals, and underproduction of anti-oxidants a state of oxidative stress occurs and induces cell, protein oxidation, Lipid peroxidation and DNA damage (Murray et al., 2009).

The overall weight difference of the entire groups in weeks 1, 2, 3 and 4 was compared with the weight per group during the four weeks. There was a significant ($p<0.05$) increase in the mean weight of Groups 2, 3, 4, 5, 6 and 7 after being fed with a high-fat diet over 28 days. Subsequently, there was a significant decrease in the weight of the animals in Groups 2, 3, 4, 5, 6, and 7 after alloxan induction in 72 hrs when compared to Group 1 (which was the normal group which received no treatment from both alloxan and the aqueous extract). A significant ($p<0.05$) observation from this study is the significant reduction in the mean weight in group 2 animals. Another significant finding of this study is the improvement in the average body weight following repeated oral treatment with 500, 1000, 1500, 2000 and 2500 mg kg^{-1} body weight of unripe *H. umbellata fruit* extract in the treatment groups 3, 4, 5, 6, which showed a significant increase in weight remarkably in week 4 when compared to the diabetic group 2 which received no extract treatment, this study is in line with the works of Gamde et al. (2023). Results of the current study confirmed earlier findings that alloxan causes significant weight loss along with metabolic complications consisting of hyperglycemia, dyslipidemia, hyperketonemia, and lactic acidosis. The fact that untreated alloxan-induced diabetic rats also exhibited significant weight loss, shows that this study is in agreement with the works of Adeneye, (2014) and Gamde et al. (2023).

Daily administration of aqueous fruit extract of unripe *H. umbellata for* 28 days at different doses (500, 1000, 1500, 2000 and 2500 mg kg^{-1}body weight) resulted in

Scientia Africana, Vol. 22 (No. 3), December, 2023. Pp 299-318
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decrease in blood glucose levels in alloxan-induced diabetic Wistar rats. Oral administration of the extract at different doses of 500,1000,1500,2000 and 2500 mg $kg^{-1}$of aqueous fruit extract of unripe*H. umbellata*led to significant reduction (p<0.05) in blood glucose levels of alloxan-induced diabetic Wistar rats when compared to the diabetic control group after the treatment period which is in line with the works of Longe & Momoh., (2014). Similar report has been documented in another study using the ethanolic leaf extract of *Senna alata* (Onyegeme-Okerenta & Anacletus, 2017). Ogunlana et al. (2021) also recorded a decline in glucose level of the diabetic animals after treatment with methanolic seed extract of *H. umbellata*on streptozotocin induced diabetic Wistar rats. Also, at the highest dose (2500mg kg^{-1}) there was a less but significant reduction on the blood glucose level of the rats, which is in agreement with the findings of Nwaogwugwu et al. (2022). In their work on the hypoglycaemic effect of aqueous seed extract of *H. umbellata* in streptozotocin induced diabetic Wistar rats, it was observed that on administration of the extract at higher doses the glucose level was seen to decline with increased concentration which was revealed also in the acute toxicity test carried out by Nwaogwugwu et al. (2022) and this may imply that the extract at higher concentrations could inhibit its absorption by saturating the cells in the gastrointestinal tract (Onyegeme-Okerenta & Anacletus, 2017).

The liver plays a crucial role in maintaining carbohydrate balance, it also regulates glucose and insulin degradation. There has always been a link between diabetes and liver pathology, therefore, Hepatic impairment is one problem of diabetes mellitus. Increases in these liver biomarkers, such as ALT, ALP, and AST activities, will provide a reliable or good indicator of the functional integrity of the liver as well as the success of treatment because hepatic impairment is one issue associated with diabetes mellitus (Shittu et al., 2017). In this study, high ALT activity levels in untreated diabetics is a sign of liver and plasma membrane dysfunction, which will negatively affect amino acid and carbohydrate metabolism as well as affecting the level of ATP production. This observation of the ALT and AST activities suggests that diabetes affects transaminase activities specifically (Yusuf et al., 2018).The extracts were able to regulate the liver-associated enzymes. The liver parameters in this study showed that the untreated rats had values above normal liver functions, indicating an increased susceptibility to liver damage and liver dysfunction. These liver challenges caused by diabetes were restored upon treatment with the extract, it was further observed that all the doses of the extract ranged from 500 to 2500 mg kg^{-1} exerted similar effects and their values were all like those of the normal rats. These results agrees with the findings of Wild et al. (2004) and Oboh et al. (2017). Most likely these liver parameters may have been regulated by the flavonoids since their presence regulates liver enzymes.

During the hydrolysis of dietary starch in the metabolism of carbohydrates, alpha-amylase is usually a crucial enzyme. *H. umbellata* would be beneficial in reducing blood glucose levels by delaying the digestion of carbohydrates and lowering the concentration of postprandial plasma glucose, as shown in the current study by its strong inhibitory effects on α-amylase activity. This inhibitory activity of the extract may be attributed to the presence of antioxidant phytochemicals, such as flavonoids, tannins, and saponins, which have been shown to inhibit α-amylase activity and maintain cell integrity, preventing the development of insulin resistance, especially in type 2 diabetic patients (Nickavar &Yousefian., 2009; Ogboyeet al., 2018).

Kidney function markers are used to evaluate the rate at which the kidney is functioningand some of these markers include urea, creatinine, uric acid and electrolytes. Diabetes causes electrolyte imbalance as well as renal dysfunction such as nephrosclerosis and acute

glomerulonephritis leading to an abnormal excretion of creatine and urea which automatically results in a dysfunction in the serum levels of urea, creatinine and nitrogen in the body (Mehrdad & Mozhgan 2011; Onyegeme-Okerenta et al., 2018)

Electrolyte imbalance is always associated with diabetic states and as such monitoring the electrolyte balance and levels is key to the management of diabetes. This study observed that after treatment with the extracts, a dose-dependent change was observed. The extracts at 500 mg showed a promising restoration potential. On the other hand, the values of the electrolytes under consideration, notwithstanding the dose of the extract used, had similar results to the normal rats; these values therefore imply that this extract could be exploited to manipulate renal function positively. The results from this study conform to results obtained by Aguwamba, (2022) and Oboh et al. (2019). Furthermore, Ajibola et al. (2018) also experimented and got similar results. The renal modulating potential may have been due to Phenols found in this plant as this is known to improve renal function (Ganesan et al., 2018).

In diabetic patients, lipid abnormalities are typically present and termed diabetic dyslipidemia. It is usually characterized by increased total cholesterol (TC), high triglyceride, reduced HDL and LDL may be moderately increased or high. These lipid changes are the major link between diabetes and the possible increase in cardiovascular risk for individuals with diabetes (Wu et al; 2014). Although these characteristics exist, they may be present in different patterns based on ethnicity, economy and access to health care. One of the very prominent complications seen in Diabetes is weight loss, this is a consequence of increased lipolysis since the body depends more on tissues for energy.

The diabetic rats treated with the extract showed dose-dependent reductions in their levels of total cholesterol, triglycerides, and LDL as well as an increase in their levels of HDL and there was no significant difference when compared to group one which is the group that received no extract treatment as well as alloxan induction. This outcome is consistent with the findings of Claudia et al. (2006) and Coresh et al. (2006) that lipid problems are frequently linked to diabetes, especially in people with type 2 diabetes. According to the research, the most typical lipid abnormalities in these patients are hypertriglyceridemia and low levels of high-density lipoprotein (HDL) cholesterol. With better glycemic control, lipid abnormalities often become better, but normalisation seldom happens. The outcome of this study supports the conclusions of other researchers who claimed that numerous plant extracts may be useful in managing atherosclerosis, one of the main problems of diabetes by lowering serum lipids particularly total cholesterol, triglyceride and low-density lipoproteins (Luka & Tjjani 2013)

In this study, untreated Diabetic rats in group 2 showed a two-fold increase in the level of TG and TC, while a twofold decrease in the HDL was observed. The different concentrations of the extracts exerted similar promising Lipid profile managing properties, these results are similar to results published by Ajiboye et al. (2017) where aqueous seed extract of *H. umbellata* significantly caused a reduction in the levels of TG, TC and LDL as well as increasing the level of HDL in high - fructose diet induced metabolic syndrome in Wistar rats.

Hyperglycemia leads to oxidative stress in muscle, liver, eye and kidney tissues (Korkmaz et al., 2013). The direct consequence of ROS when they cause lipid peroxidation is malondialdehyde (MDA) which is an unstable aldehyde that is used as a biomarker of oxidative stress, MDA is also known to modify the bases of DNA in the cell (Murray et al., 2009). When this damage occurs to cells, the body produces substances to counter it, these substances include catalase and superoxide dismutase (SOD) which are enzymes and glutathione a non-

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enzymatic anti-oxidant. SOD activities in cells help in controlling levels of ROS and reduce the damage the toxicity of ROS may cause in cells (Di Meo & Venditti, 2020). Catalase protects pancreatic beta cells, and elevated levels of H_2O_2 which has been observed in people with untreated diabetes have a corresponding deficient catalase activity (Valsala et al., 2020). Diabetes reduces the ability of the body to produce Glutathione invariably leading to the unchecked damage of free radicals which finally contributes to insulin insensitivity (Shen et al., 2021)

Nitric Oxide is an important signalling molecule that controls numerous physiological processes like vasodilation, neurotransmission and immune response. In this study, a significant (p<0.05) increase in the peroxidation level was observed in the diabetic rats which agrees with Esteghamati et al. (2013). Also, diabetes models were seen to have a depleted glutathione concentration and decreased activity of antioxidant enzymes (CAT & SOD), which corresponds to results obtained by Olubunke et al. (2021).

The extract for this study showed anti-oxidant modulating potentials. The extracts in the different doses caused a restoration of the depleted glutathione as seen in the increase of glutathione levels when treated for weeks. Furthermore, SOD and CAT activities which were reduced due to the effect of diabetes were observed to have increased after treatment with the extract which was similar to the study of Ajiboye et al. (2017).

It was also observed that the anti-oxidant potential of the extract under study was most effective at the doses of 2000 and 2500 mg kg^{-1}. At 2000 mg kg^{-1} the SOD activity was at its optimum level while at 2500 mg kg^{-1} the catalase and glutathione were at optimum levels, which corresponds with the work of Nwaogwugwu et al (2022) that yielded a positive effect of the extracts at different doses causing an increase in the oxidative stress markers.

The antioxidant-modulating properties of this extract may have occurred as a result of the phenols and flavonoids found in the plant. Phenols and flavonoids play significant roles in reducing oxidative stress and potentiating the activities of antioxidants (Eze et al., 2022; Kingsley et al., 2022; Ogulana et al., 2021)

Complete blood counts White blood cell (WBC) and WBC differentials are used to assess the level of cellular immunity, while red blood cells (RBC), Packed cell volume (PCV), and haemoglobin (Hb), are all indicators of anaemia. Anaemia may be used to indicate the risk of heart failure and risk of death in patientsGraham et al., 2022). White blood cells are very important and key players in living cells and are saddled with the responsibility of protecting the entire system and this protection is against foreign threats that may enter from the external environment or threats from within, the internal environment (Obia et al., 2023). Diabetes is accompanied by a chronic inflammatory state, and this state is a result of the aberrant responses associated with dysfunctional WBCs. Low WBC may result in a risk of infections while increased WBC may cause damage to organs (Hamuel, 2012). In the present study, aqueous fruit extract of unripe *H. umbellata* treatment for 28 days produced progressive elevations in RBC, PCV and Hb levels indicating the haematopoietic effect of unripe *H. umbellata*. Thus, the significant elevations in RBC, PCV and Hb levels suggest that unripe *H. umbellata* could be useful in the management of anaemia. This study showed similar patterns to the findings of Gabriel et al. (2022); Longe et al. (2015) and Aguwamba, (2022).

Photomicrographs of the pancreas of Group 1 showed normal pancreatic islets when compared to Group 2 which showed an abnormal pancreas with significant damage to the beta cells of the islets of Langerhans, causing atrophic pancreatic islets and absence of ducts. Groups 3, 4, 5 and 6, showed significant damage to the beta cells of the islets of Langerhans as compared to group

one. On the other hand, Group 7 showed a remarkable improvement (normal pancreatic features) on administration of the extract at a dose of 2500 mg kg^{-1}bodyweight. It is possible that stable cells with the capacity to regenerate exist in the islets of the animals in Group 7 after being treated with 2500 mg kg^{-1} day^{-1} of fruit extract. This implies that the plant extract, at this dosage, can promote quiescent cells to proliferate to regenerate the cells that have been lost. Although the precise process is unknown, it is known that the flavonoid component of this fruit extract lowers blood glucose and enhances the number of beta cells (Eliakim-Ikechukwu et al 2012). At the end of the extract treatment period, there was no significant regeneration of the pancreatic cells in groups 3, 4, 5, and 6 due to the duration of the experiment, also probably the dose at these levels was not able to restore the cells to normal and the lack of a long wash-off period where the animals are free of extracts and have access to water and food, ad libitum (Onyegeme-Okerenta & Anacletus 2017). The loss of beta cells and insulinitis that were noticed could be signs of type 1 diabetes. A significant amount of lymphocytic infiltration within and around the islet is a sign of insulinitis. This is a regular occurrence in islets that still include beta cells, and it lends support to the idea that type 1 diabetes may result from a specific, immunologically mediated death of beta cells.

CONCLUSION

Conclusively, the extract from the plant under investigation after this experimental study showed that it has the potential and should be explored for diabetic therapy. The extract showed promising results as regards its effect on lipid profile, renal, liver function, haematological and finally pro and anti-oxidant status. Histological studies were conducted to support the safety profile of the plant and the dosage used for this study.

REFERENCES

Adeneye, A.A., Adenekan, S.O., Adeyemi, O.O& Agbaje, E.O. (2014). Glucose utilization and antioxidative mechanisms of aqeous *Hunteria umbellata* seed extract in Alloxan induced diabetic rats. *Nigerian Journal of Physiological Sciences: Official Pulication ofthe Physiological Society of Nigeria,* 29(1), 71-79

Adijat, O. A, Folakeme, E. A & Adesola, A. J. (2021). The prevalence and risk factors of diabetes mellitus among civil service workers in Osogbo, osun, state, Nigeria. *Journal of Hypertension and Management,* 7(1), 2474-3690

Aguwamba, C. (2022). Effect of *Hunteria umbellata* ethanolic seed extracts on streptozotocin-induced diabetic albino rats. *Asian Journal of Biochemistry, Genetics and Molecular Biology,* 12(3), 14-27.

Ajibola, O.O.E., Akinmegha, T.S., Nwaiwu, O & Balogun, O.J. (2018). Biometric analysis of *Hunteria umbellata* (K.schum) hallier f and metformin in the treatment of diabetes. *Journal of Applied Sciences and Environmental Management,* 22(4),561-564

Ajiboye, T. O., Hussaini, A. A., Nafiu, B. Y & Ibitoye, O. B. (2017). Aqueous seed extract of *Hunteria umbellata (K. Schum.)Hallier f.* (Apocynaceae) palliates hyperglycemia, insulin resistance, dyslipidemia, inflammation and oxidative stress in high-fructose diet-induced metabolic syndrome in rats. *Journal of Ethnopharmacology*, 198(1), 184-193.

Beppu, H., Shimpo, K., Chihara, T., Kaneko, T., Tamai, I., Yamaji, S., Ozaki, S., Kuzuya, H & Sonoda, S. (2003). Antidiabetic effects of dietary administration of *Aloe arborescens* Miller components on multiple low-dose streptozotocin-induced diabetes in mice: Investigation on hypoglycemic action and systemic absorption dynamics of aloe components. *Journal of Ethnopharmacology*,103(3), 468–477.

Chuang, F.S., Sarbeck, J.R. & Winefordner, J.D. (2005). Flame spectrometric

Scientia Africana, Vol. 22 (No. 3), December, 2023. Pp 299-318
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

determination of sodium, potassium and calcium in blood serum by measurement of microsamples. *Clincal Chemistry*, 21:16-23.

Claudia, E.N.M., Julias, E.O., Dagobert, T & Etienne, D.(2006). Anti-diabetic and hypolipidemic effect of Laportea ovaliolia (Urticaceae) in alloxan induced diabetic rats. *African Journal of Traditional, Complementary and Alternative Medicines*, 42:87-95.

Coresh, J., Levey, A. S., Greene, T., Stevens, L. A., Zhang, Y., Hendriksen, S., Kusek, J. W & Van Lente, F. (2006). Using standardized serum creatinine values in the modification of diet in renal disease study Equation for estimating glomerular filtration rate. *Annals of Internal Medicine*,145(4), 247–254.

Di Meo, S & Venditti, P. (2020). Evolution of the knowledge of free radicals and other oxidants. *Oxidative Medicine and Cellular Longevity*,2020: 1–32.

Djordjevic, J., Djordjevic, A., Adzic, M., Niciforovic, A., & Radojcic, M. B. (2010). Chronic stress differentially affects antioxidant enzymes and modifies the acute stress response in liver of Wistar rats. *Physiological research*, 59(5), 729–736.

Eliakim-Ikechukwu, C., Edem, A., William, U., Okori, S & Ihentuge, C. (2012). Phytochemical Composition of *Cassia Alata* Leaf Extract and its Effect on the Histology of the Pancreas of Diabetic wistar Rats. *IOSR Journal of Pharmacy and Biological Sciences*, 5:7-13.

Esteghamati, A., Etemad, K., Koohpayehzadeh, J., Abbasi, M., Meysamie, A., Noshad, S., Asgari, F., Mousavizadeh, M., Rafei, A., Khajeh, E., Neishaboury, M., Sheikhbahaei, S & Nakhjavani, M. (2013). Trends in the prevalence of diabetes and impaired fasting glucose in association with obesity in Iran: 2005–2011. *Diabetes Research and Clinical Practice*,*103*(2), 319–327.

Eze, K. C., Ugwu, C. E., Enyum, I. O., Odo, F. S & Njoku, G. C. (2022). Antidiabetic and wound healing potential of biologically fabricated chitosan nanoparticles of aqueous seed extract of *Hunteria umbellata*. *GSC biological and pharmaceutical sciences*, 20(1), 182-196.

Ganesan, D., Al-Sayed, E., Albert, A., Paul, E., Singab, A. N. B., Selvam, G. S & Saso, L. (2018). Antioxidant activity of phenolic compounds from extracts of *Eucalyptus globulus* and *Melaleuca styphelioides* and their protective role on D-glucose-induced hyperglycemic stress and oxalate stress in NRK-49Fcells. *Natural Product Research*,*32*(11), 1274–1280.

Graham, F. J., Masini, G., Pellicori, P., Cleland, J. G., Greenlaw, N., Friday, J., Kazmi, S & Clark, A. L. (2022). Natural history and prognostic significance of iron deficiency and anaemia in ambulatory patients with chronic heart failure. *European Journal of Heart Failure*, 24(5), 807–817.

Grover, J., Yadav, S & Vats, V. (2000). Medicinal plants of India with anti-diabetic potential. *Journal of Ethnopharmacology*,81(1), 81–100.

Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B. B., Stein, C., Basit, A., Chan, J. C. N., Mbanya, J. C., Pavkov, M. E., Ramachandaran, A., Wild, S. H., James, S., Herman, W. H., Zhang, P., Bommer, C., Kuo, S., Boyko, E. J., & Magliano, D. J. (2022). IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes research and clinical practice*, 183, 109119.

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A. A., Ogurtsova, K., Shaw, J. E., Bright, D., Williams, R., & IDF Diabetes Atlas Committee (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition.

Diabetes research and clinical practice, 157, 107843.

Ighodaro, Igbe; Raymond, I; Ozolua, Steve, O. Okpo & Osahon Obasuyi (2009). Antipuyretic and analgesic effects of the ageous extract of the fruit pulp of *Hunteria umbellata*, K. Schum (Apocynacea e). *Tropical Journal of pharmaceutical research.* 8(4), 331-336.

Hamuel, J. D. (2012). Phytochemical Extractions methods, basics structures and mode of action as potential Chemotherapeutic Agents. Intechopan books published online 21st March, 2012. doi: 10.5772/26052

Kiernan, J. A., Histological and Histochemical Methods: Theory and Practice. 4th Edn. Scion, Bloxham, UK. 2008.

Kingsley, J. I., Omorogie, M. O., Bayode, A. A., Adeleye, A. T & Helmreich, B. (2022). Environmental microplastics and their additives—a critical review on advanced oxidative techniques for their removal. *Chemical Papers*. 77(2), 657–676.

Klein, B., Read, P.A., Babson, L.A. (1960). Rapid Method for Quantitative Determination of Serum Alkaline Phosphatase. *Clinical Chemistry*, 6:269-275.

Korkmaz, G. G., Altınoglu, E., Civelek, S., Sözer, V., Erdenen, F., Tabak, O & Uzun, H. (2013). The association of oxidative stress markers with conventional risk factors in the metabolic syndrome. *Metabolism*,62(6), 828–835.

Lenzen, S. (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*. *51*(2), 216–226.

Longe, A.O & Momoh, J. (2014). Effects of methanolic seed extract of *Hunteria umbellata*(abeere) on blood glucose level, hematological and lipid profile parameters in alloxan- induced diabetes in male rats. *Pinnacle of Medicine & Medical Sci*ence. *1*(2), 225- 230.

Longe, O. A, Momoh, J., Adepoju, P. A & Akoro, S. M. (2015). Hypoglycemic effects of the methanolic seed extract of *Hunteria umbellata*(Abeere) and its effect on liver, hematological and oxidative stress parameters in alloxan-induced diabetic male albino rats. *International Journal of Current Research and Bioscience Plant Biology*. 2(6), 27–34.

Lorentz, K. (1998). Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzymes. Part 9. IFCC method for amylase (1,4-a-D-glucan 4-glucanohydrolase, EC 3.2.1.1). International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). Committee on enzymes, *Clinical Chemistry and Laboratory Medicine*,36: 185–203.

Luka, C. D., & Tijjani, H. (2013). Comparative studies of the aqueous extracts of *Ocimum gratissimum, Aloe vera, Brassica oleracea and Ipomoea batatas* on some biochemical parameters in diabetic rats. *IOSR Journal of Pharmacy and Biological Sciences*, 3:23-29

Mehrdad, M & Mozhgan, G. P. (2011).Effect of zingiber extract on histopathologic changes in mice kidneys. *Research journal of applied sciences*. 6(5), 1-3

Mohiuddin, A.S; Palain, S. Shankar, P.R; Sam K.G & Kumar, M. (2019). Uncommon side effects of commonly used anti-diabetes: *Time to monitor them. International Journal of Pharmaceutical Sciences and Research.* 10(9),45-48.

Murray, J. A., Farrington, D. P., Sekol, I., & Olsen, R. F. (2009). Effects of parental imprisonment on child antisocial behaviour and mental health: a systematic review. *Campbell Systematic Reviews*. *5*(1), 1–105.

Nickavar, B & Yousefian, N. (2009). Inhibitory effects of six *Allium species* on α-amylase enzyme activity. *Iranian Journal of Pharmacology Research.* 8:53-57.

Scientia Africana, Vol. 22 (No. 3), December, 2023. Pp 299-318
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

Nwaogwugwu, J. C., Nosiri C. L., Aguwamba, C., Aaron, C.F & Ike, U. W. (2022)."Effect of H. umbellata Methanolic Seed Extracts on Streptozotocin-induced Diabetic Albino Rats." *Asian Journal of Biochemistry, Genetics and Molecular Biology.*12 (3), 14-27

Obia, C., Nwuke, P.C& Okereke, G.O. (2023). Haematological effects of methanol and –hexae seed extracts of *Hunteria umbellata*(abeera) on alloxan induced diabetic wistar rats. *British Journal of Health Care Ad Medicial Research*. 10(4), 11-126

Oboh, G., Adebayo, A. A., Ademosun, A. O.& Aegunde, O.A. (2019).Aphrodisiac effect of *Hunteria umbellata*seed extract: modulation of nitric oxide level ad arginase activity in vivo. *Pathophysiol*. 26(1), 39-47

Oboh, G., Olasehinde, T. A., & Ademosun, A. O. (2017). Inhibition of enzymes linked to type-2 diabetes and hypertension by essential oils from peels of orange and lemon. *International Journal of Food Properties*. 20(1), S586–S594.

Ogboye, P. O., Onyegeme-Okerenta B.M., Monago-Ighorodje C. (2018). Proximate Composition, Antioxidant and Hypoglycaemic Potential of Aqueous Extracts of Seeds of Delonix regia on High Fat Diet and Streptozotocin-Induced Diabetes in Female Wistar Rats. *Clinical and Experimental Medical Sciences*, 6(1), 33 – 46.

Ogunlana, O.O., Adetuyi, B.O., Rotimi, M., Esalomi, L., Adeyemi, A., Akinyele, J., Ogunlana, O.E., Adetuyi, O.A., Adebisi, O.A., Opata, E.K & Baty, R.S. (2021). Hypoglycemic and antioxidative activities of ethanol seed extract of *Hunteria umbellata(Hallier F.)* on streptozotocin-induced diabetic rats. *Clinical Phytoscience*. 7(1), 55.

Olubunke,O.O., Babatude, O.A., Miracle,R., Lohor,E., Ala,A., Julie, A., Oluseyi,E.O., Oluwatosi, A.A., Oluwaseun,A.A., Edward,K.O., Roua, S.B & Gaber El-Saber, B. (2021). Hypoglycemic and atioxidative activities of ethanol seed extract of *Hunteria umbellata*(hallier f) on streptozotocin-induced diaetic rats. *International Journal of Phytomedicie Ad Phytotherapy.Clinical Phytoscience.* 7:5

Olumide, S. F., Adegbola, P.I., Olubukola, S.O., Temitayo, I.O., David, O.A. & Ayodeji, Z.A. (2021). Ethno-medicinal phytochemistry and pharmacological importance of *Hunteria umbellata(K. Schum) Hallier F. (Apocynaceae)*: a useful medicainal plant of sub-saharan Africa. International Journal of phytochemistry and phytotherapy. *Clinical Phytoscience.* 7 (54), 2-17

Onyegeme-Okerenta, B. M & Anacletus, F. (2017). Hypoglycaemic and hypolipidaemic potentials of *Sennaalata* and its effect on the pancrease of Alloxan –diabetic induced Albino – Rats. *Journal of Applied Life Sciences International*. 11(1), 1-10.

Onyegeme-Okerenta, B. M., Ogboye, P. O., Monago-Ighorodje, C. (2018). Ameliorative Effect of Aqueous Extracts of Seeds of Delonix regia (Hook) Raf on the Liver, Kidney and Spleen of High-fat Diet Streptozotocin-induced Diabetes in Female Wistar Rats. *European Journal of Medicinal Plants*, 25(2), 1-14.

Onyegeme-Okerenta, B.M., Minadoki, D.M & Amadi, B.A. (2023). Phytochemical Composition and anti-plasmodia potential of fruit extract of *Hunteria umbellata*on Chloroquine sensitive plasmodium berghei berghei (NK 65)-infected albino mice. *Asian Journal of Emerging Research.* 5(1), 9-21.

Reitman, S & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*. *28*(1), 56–63.

Shen, B., Li, Y., Sheng, C., Liu, H., Hou, T., Xia, N., Sun, S., Miao, Y., Pang, Y., Gu, K., Lü, X., Wen, C., Cheng, Y., Yang, Y., Wang, D., Zhu, Y., Cheng, M.,

Harris, K., Bloomgarden, Z. T & Shi, Y. (2021). Association between age at diabetes onset or diabetes duration and subsequent risk of pancreatic cancer: results from a longitudinal cohort and Mendelian randomization study. *The Lancet Regional Health - Western Pacific*, 30: 100596.

Shittu, R. O., Kasali, F. O., Biliaminu, S. A., Odeigah, L. O., Aderibigbe, S., Sule, A & Yusuf, M. (2017). Prevalence of diabetes and pre-diabetes in Oke-Ogun region of Oyo State, Nigeria. *Cogent Medicine*,4(1), 1326211.

Gamde, S. M., Ugwah-Oguejiofor, C. J., Garba, A., Avwioro, G. O., Moronkeji, A., & Jimoh, A. A. (2023). Histologic and Biochemical Effect of Balanite aegyptiaca Fruit Extract on Alloxan-Induced Diabetes in Wistar Rats. *Ethiopian Journal of Health Sciences*, 33(3), 441–450.

Solomon, M., Uvoh, K.E & Nwafor, C. C (2022). *Hunteria umbellata*geaes fruit extract hypoglycemic potentials on normoglycemic and alloxan induced diabetes in Wistar rats. *World journal of pharmacy and pharmaceutical sciences*, 1:105-116.

Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological research*, 50(6),537–546.

Tietz, N.W.(2004). Clinical guide to laboratory tests 3rd Edition, W B. Saunders Co, Philadelphia, 187-216.

Usoh,.I.F., Akpan, E.J., Etim, E.O., Farombi, E.O.(2005). Antioxidant Actions of Dried Flower extract of Hibiscus sabdariffa L. on Sodium arsenite-induced oxidative stress in rats. *Pakistan Journal of Nutrition*,4(3),135-141.

Gabriel E, D. K., Uvoh, D. S., & Ngaikedi, D. C. (2023). Haematological restoration effect of *Hunterial umbellata* fruit extract on phenylhydrazine induced anemia in wistar rats. *GPH-International Journal of Applied Science*, 5(12), 01-13.

Valsala, V., Sreeush, M. G & Chakraborty, K. (2020). The IOD impacts on the Indian Ocean carbon cycle. *Journal of Geophysical Research: Oceans*, 12:11.

Wild, S. H., Roglić, G., Green, A., Sicree, R & King, H. (2004). Global prevalence of diabetes. *Diabetes Care*, 27(5), 1047–1053.

Wu, Y., Ding, Y., Tanaka, Y & Zhang, W. (2014). Risk factors contributing to Type 2 diabetes and recent advances in the treatment and prevention. *International Journal of Medical Sciences*,11(11), 1185–1200.

Yusuf, A. J., Baiyewu, O., Bakari, A., Garko, S. B., Jibril, M. E., Suleiman, A. M., Muktar, H. M & Amedu, M. A. (2018). Low education and lack of spousal relationship are associated with dementia in older adults with diabetes mellitus in Nigeria. *Psychogeriatrics*,18(3), 216–223.