Diabetes Mellitus and Enhanced Oxidative Stress: Evidence from Lipid Peroxidation and Coagulation Patterns in Kano, Nigeria

Babagana K., Aliyu D. A., & Ibrahim S. S.*

Department of Biochemistry, Faculty of Basic Medical Sciences,
College of Health Sciences, \Bayero University, P.M.B. 3011, Kano, Nigeria
Corresponding Authour: Ibrahim S. S.
Corresponding Email: ssibrahim.bch@buk.edu.ng

Abstract
Diabetes mellitus is a prevalent non-communicable disease with far-reaching biochemical implications, particularly in Sub-Saharan Africa, where its burden is rapidly increasing. In this study, oxidative stress indices were evaluated in fifty diabetic patients (25 males and 25 females) attending Murtala Muhammad Specialist Hospital in Kano, Nigeria, with results compared to age-matched non-diabetic controls. Patients were stratified into three age groups (21–40, 41–60, and 61–80 years), and hyperglycaemia was confirmed via fasting serum blood glucose measurements. Key biomarkers of oxidative stress were assessed, including serum malondialdehyde (MDA) as a marker of lipid peroxidation, serum ascorbic acid (SAA) as an indicator of antioxidant capacity, and the dry layer oxidative stress test (DLOST) to evaluate alterations in blood coagulation patterns. The diabetic cohort exhibited significantly elevated fasting glucose and MDA levels ($P < 0.05$), together with a marked, age-independent decline in SAA compared to controls ($P < 0.05$). Moreover, DLOST readings revealed abnormal coagulation patterns consistent with heightened oxidative stress. These findings underscore the role of oxidative stress in the pathogenesis of diabetes mellitus and highlight the potential benefit of incorporating antioxidant strategies into therapeutic regimens.

Keywords: *Diabetes Mellitus, Oxidative Stress, Ascorbic Acid, Dry Layer Oxidative Stress Test (DLOST), Northern Nigeria.*

1 Introduction

Diabetes mellitus is a group of metabolic diseases of multiple aetiologies and is characterised by chronic hyperglycaemia resulting from defects in insulin secretion, insulin action or both [1] . The chronic hyperglycaemia leads to long-term damage, dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [2]. At the beginning of the millennium, at least 171 million people worldwide suffered from diabetes, 2.8% of the world's population [3] . By 2013, those numbers had reached a whopping 382 million, with a global prevalence of 8.3%, and an estimated 175 million of these numbers are undiagnosed [4] . The rate of new cases emerging is alarming; it is estimated that by 2030, the number of diabetics would double [3].

Prevalence of diabetes in Nigeria, as with most Sub-Saharan African countries, is at best 'guesstimates' [5] due to the dearth of published studies describing the burden of diabetes in the region [1] . Furthermore, Nigeria has many cultural diversities with about 398 documented ethnic groups [5] and covers the area of 910,770 km^2 and 182,202,000 populations [6] with many different regions and climatic conditions [7] . Both these factors (differences in genetics and environment) affect the susceptibility of developing the disease [1], making generalization from available pockets of different regional studies in the country more difficult. Nevertheless, it was estimated that Nigeria has the highest burden of diabetes in Africa [4].

Diabetes is classified into type 1 diabetes or insulin-dependent diabetes (with onset mostly at young age due to β-cells destruction), type 2 diabetes or non-insulin dependent diabetes (with onset mostly after maturity due to progressive loss of insulin secretion and/or progressive gain of insulin resistance) [8] . There are also type 3 diabetes (diabetes associated with Alzheimer's disease) [9] [2] , as well as other

Date submitted-17/02/2022 Date Accepted-04/05/2022

types of diabetes such as gestational diabetes (carbohydrate intolerance due to pregnancy) [10] . Other types includes specific types of diabetes due to other causes, e.g., monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), diseases of the exocrine pancreas (such as cystic fibrosis), and drug- or chemical-induced diabetes (such as with glucocorticoid use, in the treatment of HIV/AIDS or after organ transplantation) [8] . Untreated or improperly treated diabetes leads to complication [11]. Diabetes mellitus is characterized by symptoms such as thirst (polydipsia), polyuria, blurring of vision and weight loss [8]. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and in absence of effective treatment, death [12]. The long-term effects of diabetes mellitus include progressive development of the specific complication of retinopathy with potential blindness, nephropathy that may lead to renal failure, neuropathy with risk of foot ulcers, amputation, Charcot's joints, cardiomyopathy and features of autonomic dysfunction, including sexual dysfunction [13] [8] essentially affecting practically all major organs in the body. Diabetes is characterized with hyperlipidaemia [14] which theoretically mean higher risk of incidences of lipid peroxidation and oxidative stress.

Oxidative stress occurs when the available supply of the body's antioxidant is insufficient to handle and neutralize free radicals of different types [15] . Oxidative stress in itself is not a disease but a condition that can accelerate existing conditions [16] due to, primarily, the disturbances of various associative redox signalling and control [17] . Oxidative stress have been linked to a wide array of diseases, including; heart disease, hypertension, cancer, acute respiratory distress syndrome, asthma, inflammatory bowel disease, arthritis, diabetes mellitus, etc [18] [19] [20]. In diabetes, there are several sources of free radicals. Among the most popular ones are the Advanced Glycation End Products (AGEs) that are linked to both pathogenesis and complications of diabetes [21] [22] . There is also an increased autoxidation of glucose in a hyperglycaemic state, leading to further generation of free radicals [23] . AGEs are group of heterogeneous compounds formed by Maillard reaction where the carbonyl group of the reducing sugar reacts with the amino group of a protein, lipid or nucleic acid, non-enzymatically, which generates a Schiff bases that rearrange to an unstable Amadori products which eventually leads to the formation of the irreversible AGEs compounds (e.g. glyoxal, 3-deoxyglucosone, methylglyoxal) [21]. In endothelial cells, AGEs can modify intracellular proteins involved in gene regulation or can diffuse out of the cell and alter the signalling proteins (e.g. receptors) or can change/damage circulatory proteins like albumin, or insulin [22] . AGEs can also exert their effect by binding to their various AGE-specific receptors or AGE-binding proteins or the best-characterized molecule - the RAGE, which are associated with inflammatory responses [24] [25] . RAGEs are also known to stimulate NAD(P)H oxidase activity, decrease superoxide dismutase (SOD) activity, decrease catalase activity and indirectly reduce cellular antioxidant defence ammunitions, like GSH and ascorbic acid leading to an increased free radical production [26].

Under hyperglycaemic state and in tissues that do not need insulin for entry glucose into their cells (like lens of the eye and the retina), the level glucose in these tissues becomes very high. The excess glucose will trigger it's on conversion to sorbitol by a NADPH-dependent aldose reductase enzyme making the NADPH to be unavailable for regeneration of reduced glutathione by glutathione reductase. Furthermore, these hyperglycaemic cells can generate more reducing equivalents (NADH and $FADH_2$) due to the high influx of glucose into the Tricarboxylic Acid (TCA) cycle. These reducing equivalents would eventually transfer the electrons they are carrying into the Inner Mitochondrial Membrane's (IMM's) Electron Transport Chain (ETC) [27] . Due to high flux of electrons across the ETC higher voltage is generated across the IMM leading to the blockage of complex III of the ETC [22] . Which means that lipophilic coenzyme Q will be fully reduced in its quinol form and will have nowhere to transfer the electrons it is carrying but to molecular oxygen (O_2), turning it into a superoxide radical ($O_2^{\cdot-}$) [28] . However, the body has an antioxidant enzyme, superoxide dismutase (Mn-SOD), to deal with

complex III blockage, but because of the sheer amount of the electron being fluxed, the body defence will become overwhelmed and the Reactive Oxygen Species (ROS) will eventually leak out causing damage.

One such damage is strand breakage of DNA, which leads to activation of poly(ADP-ribose) polymerase (PARP) – an enzyme that uses NAD^+ to synthesized ADP-ribose that are used in DNA repair [29]. The activity of this polymerase leads to the reduced activity of the glycolytic enzyme, glyceraldehyde 3-phosphate dehydrogenase (G3PD) which normally is shuttled into the nucleus from cytosol to help in DNA repair as well [22]. Decreased activity of G3PD means that its substrate glyceraldehyde 3-phosphate (G3P) concentration would instead increase together with other intermediates leading to glucose synthesis via gluconeogenesis which would eventually activates the polyol pathway (sorbitol synthesis) and higher reducing equivalent synthesis in TCA cycle and the whole process described above begins anew. Increased G3P concentration also activates a pathway leading to AGE, methylglyoxal [22], which could eventually be transformed to more AGE free radicals (like Nε-(carboxyethyl) lysine (CEL), methylglyoxal-derived lysyl dimer (MOLD), argpyrimidine and methylglyoxal-derived hydroimidazolone (MG-H1) [21].

An antioxidant is a molecule capable of slowing, repairing and/or preventing the oxidation of other molecules by free radicals [30]. They can be classified into enzymatic or non-enzymatic, preventative or repair systems, endogenous or exogenous, primary or secondary, hydro-soluble or liposoluble, natural or synthetic [31]. Since free radical reactions proceeds usually by a chain reaction, antioxidant systems tend to terminate these chain reactions by removing free radical intermediates and inhibiting further oxidation reactions [32]. Organisms (including plants and other microorganisms) have multiple complex systems of antioxidants. These complex systems consist of various antioxidant enzymes such as, glutathione peroxidase, glutathione reductase, glucose 6-phophate dehydrogenase [27], superoxide dismutase and catalase, metal-binding proteins such as transferrin, and ceruloplasmin [32]. Various aminothiols (cystine, cysteine, homocysteine, methionine, reduced and oxidised glutathione, N-acetylcysteine) act as antioxidants as well [33]. The expressions of these antioxidant proteins are generally controlled through a 'master switch systems' like NFkB and Nrf2/Keap1 in higher organisms [15]. Vitamins like α-tocopherol, ascorbate and β-carotene also plays a very prominent role as antioxidants by the virtue of their conjugate double bond structures that forms stable radicals when they terminate free radical chain reaction and became oxidized themselves [32]. Other enzymes like proteases, DNAses/RNAases and lipases can also be considered as antioxidant enzymes for they remove damaged cell components of free radical oxidations [21].

The highlight of blood coagulation is when thrombin (activated from prothrombin by a series of coagulating factors) converts a soluble fibrinogen to an insoluble clot of fibrin fibres [34]. Though evidence show that there are no differences in fibrinogen blood-clotting kinetics, its compaction and concentration between diabetic and non-diabetic individuals [35], but fibrinogen is one of the plasma proteins' component which is most susceptible to oxidative modification [36] mostly by methyglyoxal-drived radicals [35]. It ends up generating a deformed fibrin clot with dense matted deposits (DMD) that are not effectively fibrinolized [34]. Activity of free radicals can results in the extracellular matrix holding the cells to become compromised and water soluble fragments (of the matrix) get into the bloodstream and alter the blood clotting cascade leading to imperfect coagulation by creating a polymerized proteins puddle (PPP) disconnecting the coagulating cells [37] [38] [39].

Prior to this study, reliable data on biochemical aberrations due to diabetes mellitus are grossly lacking in Northern Nigeria. Most studies in Nigeria are focused on the prevalence of diabetes in local regions [40] [41] [42] [43] or the effect of mostly plants on diabetes induced experimental animals. Here, the serum glucose concentration was estimated to confirm presence of diabetes in patients attending Murtala Muhammad Specialist Hospital, Kano. Then the indices of oxidative stress was established

in them by determining biochemical markers of stress, including serum malondialdehyde and ascorbate. Finally, the presence of oxidative stress in the diabetic patients compared with the control individuals was validated by studying the clotting pattern in the blood of both the diabetics and the controls.

2 Materials and Methods

2.1 Ethical clearance

Permission was sought and granted by the management of Murtala Muhammed Specialist Hospital (MMSH), Kano, Nigeria, to use patients attending their Diabetic Unit of Medicine Department. The identities of all participants were kept confidential throughout this work.

2.2 Study Subjects

Twenty-five (25) male diabetics and twenty-five (25) female diabetics were randomly selected for this study. The individuals were divided based on age into three tiers: 21-40, 41-60, and 61-80 years. These subjects were established diabetics who were (at the time of the research) undergoing treatment and with the age range of 23 to 73 years. Another 30 non-diabetic individuals with no history of persistent hyperglycaemia were sourced around Kano metropolitan, an area where most of the diabetic objects used in this research reside. Among these individuals, five were used as a control for each group (Table 1).

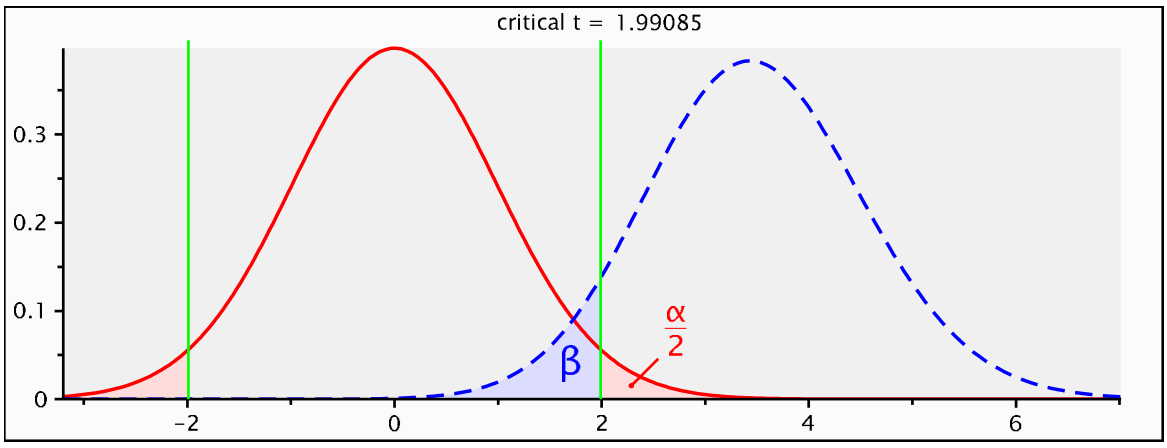
Table 1: Experimental Design (Grouping) of the Diabetic Patients and Respective Controls

Group	Number of male subjects (*n*)	Number of female subjects (*n*)
Group I: (21-40 years)	8	8
Group II: (41-60 years)	9	9
Group III: (61-80 years)	8	8
Control I: (21-40 years)	5	5
Control II: (41-60 years)	5	5
Control III: (61-80 years)	5	5

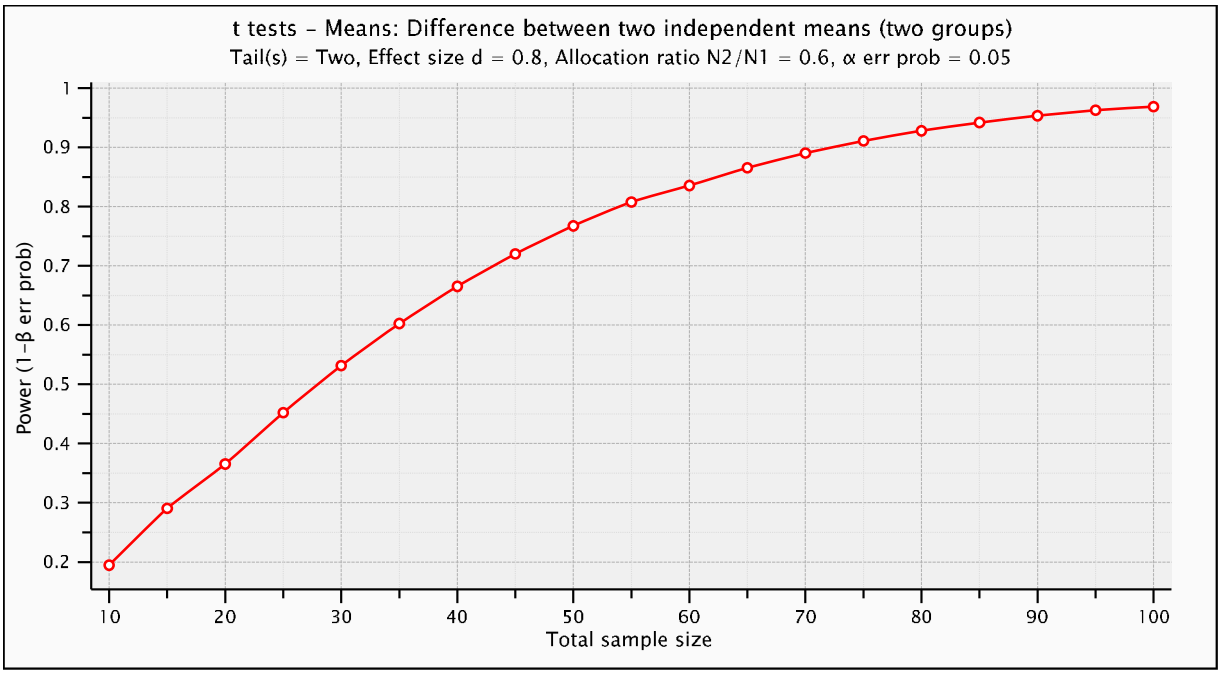
2.3 Sample Power Analysis

The Post hoc sample power analysis was 93% (Noncentrality (δ) = 3.46, Critical = 1.99 and Df = 78) using G*Power (Version 3.1.9.6) using the following input Parameters:

1. **Tail(s):** Two
2. **Effect size d:** 0.8 (large effect)
3. **α:** 0.05
4. **Diabetic sample:** 50
5. **Control sample:** 30



(a)



(b)

Figure 1: *Power Sample Analysis – **(a)** Central and Noncentral Distribution, **(b)** Power probability vs Sample Size*

2.4 Questionnaire administration

To capture the age and sex of the participants, a questionnaire was designed and administered to the patients. The questionnaire captured the gender, age, weight, height of the study subjects, as well as the results of the DLOST. All subjects were made to understand the scope and importance of the study and were allowed to make up their minds independently to participate.

2.5 Sample collection and spot analysis

Venous blood samples were collected by a trained phlebotomist in MMSH. Part of the blood was conveyed to the Haematology Unit of Aminu Kano Teaching Hospital, Kano, Nigeria. A drop of fresh blood was used to make a dried film on a microscope glass slide for oxidative stress analysis. A portion of the blood sample (1 mL) was transferred to a fluoride oxalate container to be used for glucose test and the remaining (4 mL) was placed in a plain container for serum vitamin C (ascorbic acid) and serum malondialdehyde determinations. All samples were centrifuged, and the serum was separated and transferred to appropriate labelled containers individually.

2.6 Estimation of Parameters

Serum glucose concentration was estimated by glucose oxidase-peroxidase method of Barham and Trinder [44]; serum ascorbic acid level was estimated using the method of Roe and Kuether [45] ; serum malondialdehyde was determined using method of Hunter *et al* modified by Gutteridge and Wilkins [46]. Dry layer oxidative stress test (DLOST) (also called Heitan-Le Garde (Bradford) blood test by Manton [47]) was carried out by microscopic examination of dry blood by experts in Haematology Unit of Aminu Kano Teaching Hospital, Kano Nigeria, using the protocol described by Dong and Brown [37]. Results were subjected to statistical analysis by comparing readings from the diabetic patients with their respective age-based controls and their age-gender diabetic counterparts using one-way ANOVA analysis ($p \leq 0.05$).

3 Results

To establish presence of diabetic state independently of the hospital record (since the patients were under diabetic medications) serum fasting blood glucose was determined. For each age cohort, the diabetic patients showed significantly

higher serum glucose concentration (P<0.05) when compared with the control individuals (Figure 2). The different age groups showed no significant differences in fasting blood glucose level between and within the sexes. Serum malondialdehyde (MDA) was also determined as a measure of lipid peroxidation (free radical damage). Not surprisingly, the diabetic patients of both sexes portrayed significantly higher serum MDA concentration (P<0.05) compared with the controls (Figure 3). As with the fasting blood sugar, the different age groups showed no significant differences in the serum MDA level for both between and within the sexes. A similar pattern was observed but in reverse with respect to ascorbate concentration, with diabetic patients exhibiting significantly lower serum ascorbate (P<0.05) compared with the control individuals (Figure 4). Again, as with the first two, no significant differences were observed with respect to their ages in serum ascorbate level for both, between and within, the sexes. For DLOST readings arbitrary numbers were given to samples with evidence of mild, moderate and severe oxidative stress respectively as +1, +2 and +3. Values obtained from the study of the coagulation patterns were then compared with those from control individuals. Statistically significant increase (P<0.05) in dry layer oxidative stress reading was observed when the numerical values of readings from the diabetic subjects were compared with that of respective controls (Figure 5). Here, no significant differences were observed with respect to their ages in the DLOST readings for both between and within the sexes. Figure 6B-D highlights the presence of oxidative stress in the diabetic patients with imperfect coagulation, while Figure 6A for a control individual portrayed the natural coagulation with no DMD or PPP. The degree of oxidative stress is evident in Figure 6B, 6C and 6D showing mild, moderate and severe oxidative stress, respectively from the pattern of coagulation.

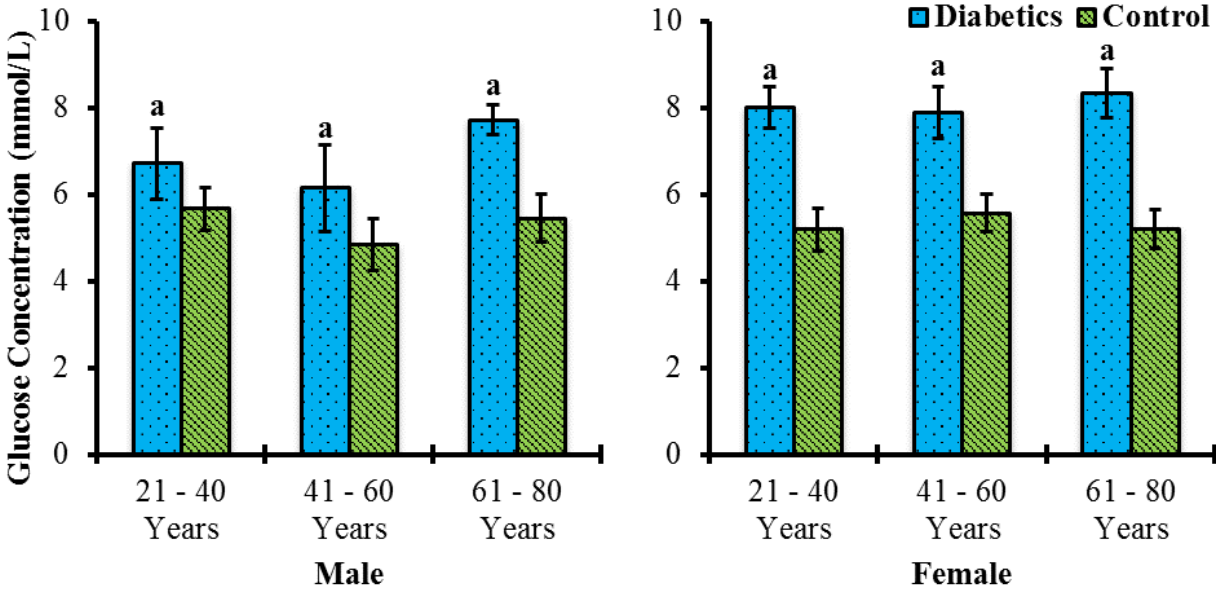


Figure 2: *Serum Fasting Glucose Concentrations for Male and Female Diabetic Patients with their Respective Controls.*

Values are presented as mean ± standard deviation. Readings with '**a**' are significantly different compared with the control group at $p \leq 0.05$.

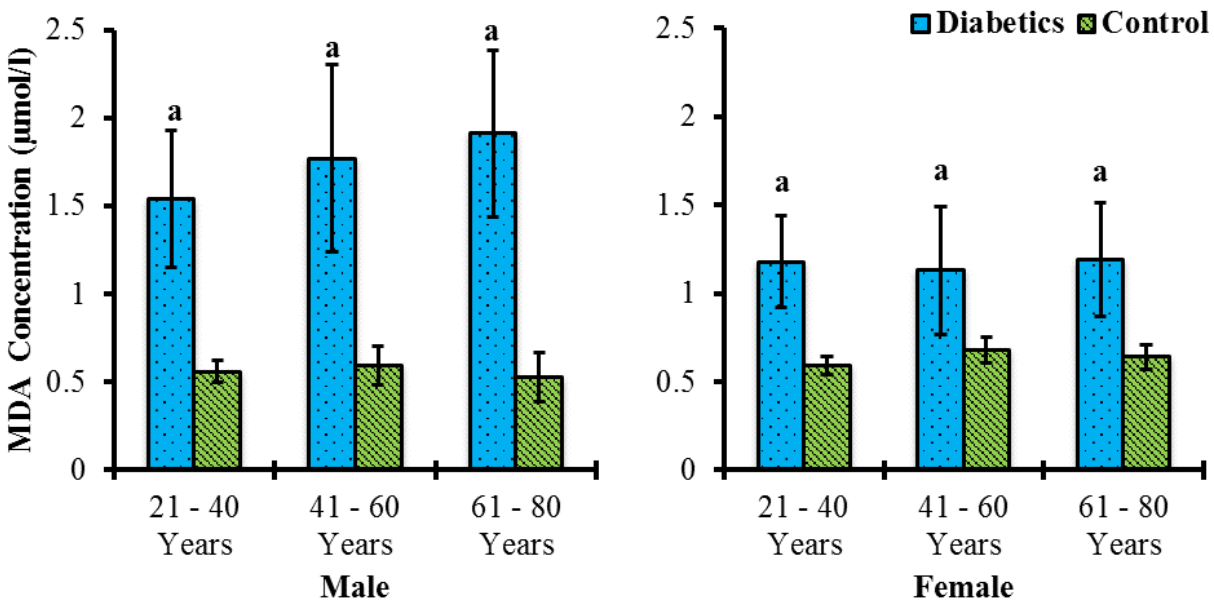


Figure 3: *Serum MDA Concentrations for Male and Female Diabetic Patients with their Respective Controls*.

Values are presented as mean ± standard deviation. Readings with '**a**' are significantly different compared with the control group at $p \leq 0.05$.

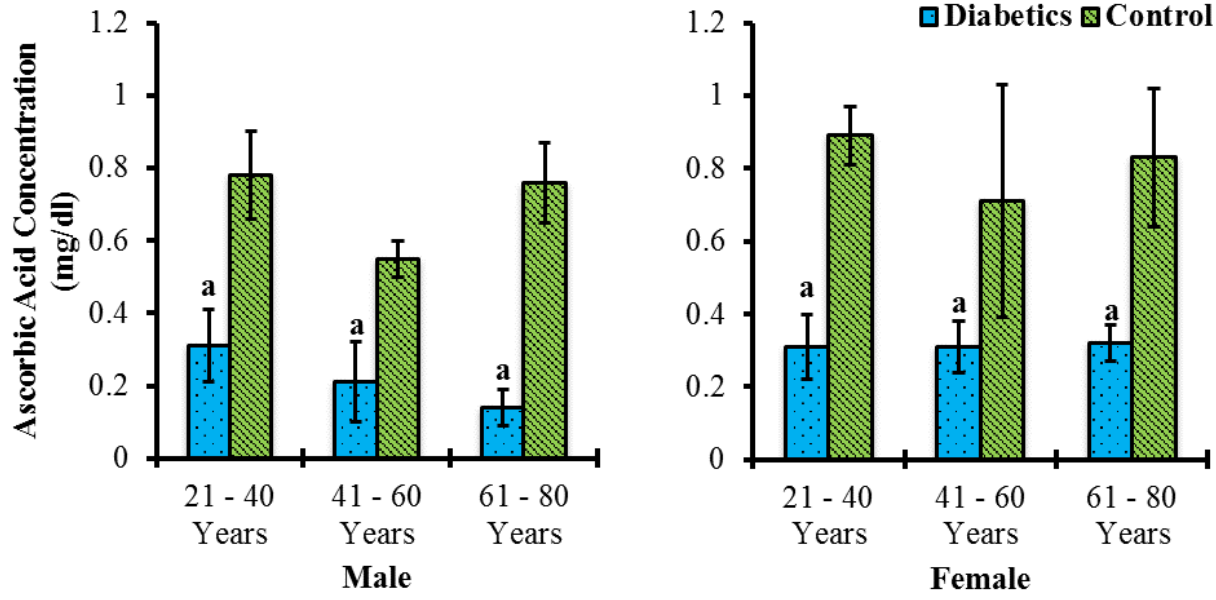


Figure 4: *Serum Ascorbic Acid Concentrations for Male and Female Diabetic Patients with their Respective Controls*

Values are presented as mean ± standard deviation. Readings with '**a**' are significantly different compared with the control group at $p \leq 0.05$.

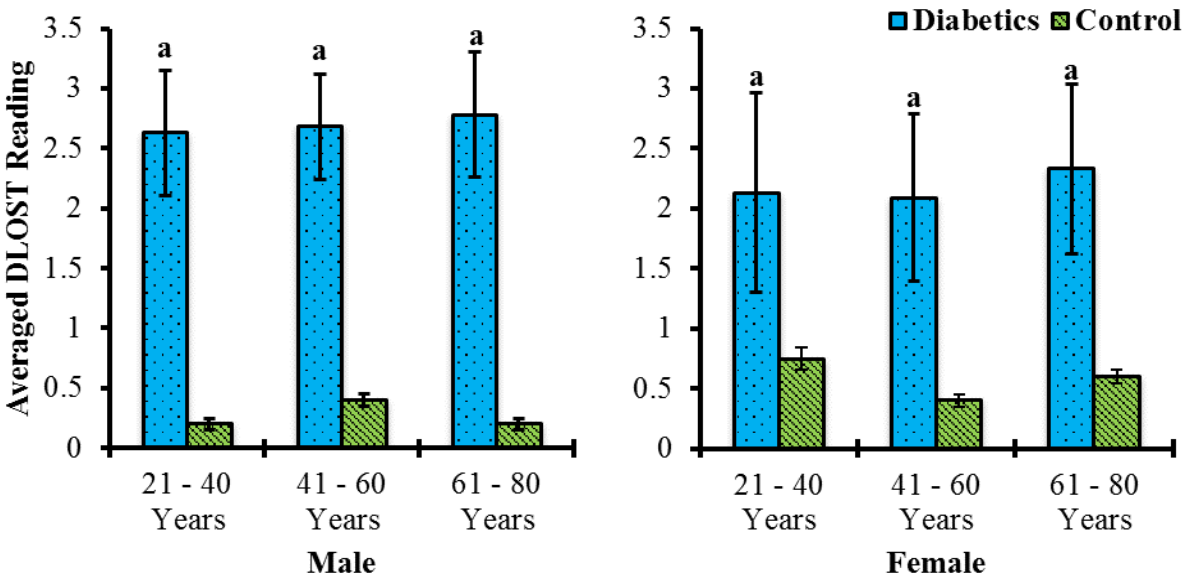


Figure 5: *Averaged DLOST Reading for Male and Female Diabetic Patients with their Respective Controls*
Values are presented as mean ± standard deviation. Readings with '**a**' are significantly different compared with the control group at $p \leq 0.05$.

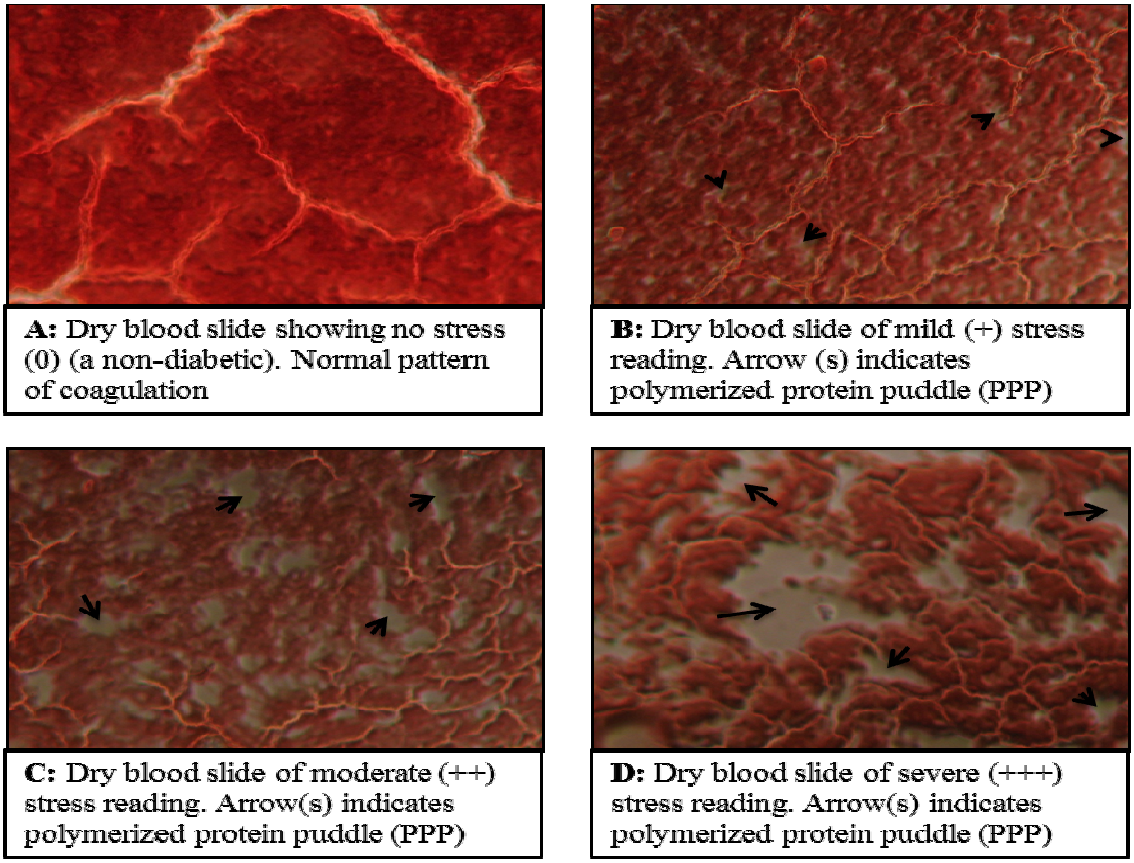


Figure 6: *Dry Layer Oxidative Stress Test*

4 Discussion

Diabetic individuals are believed to generate higher concentration of free radicals then normal individuals [21] , by the virtue of their hyperglycaemia which leads to hyperlipidaemia due to dysfunctional insulin action [14] [48]. This induced hyperlipidaemia means more lipids are present in the plasma, making it an easy attack for free radicals' attack, causing lipid peroxidation [49] . Apart from hyperlipidaemia-induced lipid peroxidation, the diabetic state can also lead to the generation of more free radicals due to a compromised production of NADPH in the pentose phosphate pathway. This is likely due to fact that the two key enzymes – glucose 6-phosphate dehydrogenase and 6-phosphogluconate in the pathway - are both insulin-induced, hence decreasing efficiency of the antioxidant systems requiring NADPH [50] like the glutathione systems [26] . In addition to compromising NADPH cellular production, the diabetic state also greatly depletes the concentration of the already synthesised NADPH due to an increased activity of NADPH-dependent aldose reductase in tissues that uptake glucose independently from insulin action [51] [22].

Malondialdehyde (MDA) is the measure of lipid peroxidation of membrane lipids which is directly proportional to the oxidative stress [52] on primarily the cell membrane's glycolipids, phospholipids and cholesterol [53]. The observed significant increase in serum MDA level of diabetic subjects over their control counterpart (Figure 3) is the consequence of an increased free radical generation in diabetic state which subsequently lead to more lipid peroxidative damages and more MDA productions [54] . In this respect, MDA can not only be considered as the repercussion of free radical oxidation but also a source. Many researchers have made similar findings in which they attributed such observable changes to auto-oxidation of glucose in hyperglycaemic state and AGE/RAGE formation.

These free radicals generated are scavenged by the available antioxidant systems, including ascorbic acid [23], in the plasma, making their concentrations significantly lower than normal as seen in the result. The depleted antioxidant observed (serum ascorbic acid) together with the increased free radical generation described (seen in serum MDA) may have led to the observed oxidative stress seen in the DLOST reading where a DMD-rich, fibrinolysis-resistant, PPP-high blood coagulation was viewed in diabetics over the control subjects.

The results showed a clear effect of free radical activity, even though all the diabetic subjects used in this research were under treatment. This is likely due to fact that for most, initially, hyperglycaemic condition remain asymptomatic and individuals become aware of their situation only after when one or more of its complications drove them to the hospital [1] . Some of those free radicals generated during such period have the tendency to accumulate in tissues and organs over time [48] and the damage may have already been done by the time they want to the hospitals. Even at the hospital, the available protocol in place is just to lower the blood sugar level [55] rather than reversing, repairing and/or preventing further damages that may have already been caused by the free radicals. This could probably explain why diabetic complications still occur in patients even when their blood sugar levels are apparently controlled [56].

Limitations of the study: This study has several limitations that should be acknowledged. First, the cross-sectional design restricts the ability to establish causal relationships between hyperglycaemia, oxidative stress markers and altered coagulation patterns. Although the sample size provided adequate statistical power for our primary comparisons, the number of subjects—50 diabetic patients and 30 control individuals—remains relatively small and may not fully capture the heterogeneity of the diabetic population in Northern Nigeria. Additionally, participants were recruited from a single tertiary hospital, and the use of convenience sampling may limit the generalizability of our findings to other settings and ethnic groups within the region.

Another limitation concerns the fact that all diabetic subjects were under treatment at the time of sample collection. This therapeutic intervention could have modulated oxidative stress indices, including serum malondialdehyde (MDA) and ascorbic acid levels, thereby potentially confounding the true biochemical profile associated with uncontrolled

hyperglycaemia. Moreover, the dry layer oxidative stress test (DLOST) employed in this study is a qualitative assay; while it provided valuable insights into coagulation patterns and oxidative damage, more precise quantitative methodologies might yield a more detailed assessment of oxidative stress. Finally, investigation was confined to a limited panel of biomarkers, and a more comprehensive evaluation of both enzymatic and non-enzymatic antioxidant defences would further elucidate the complex redox imbalance in diabetes.

Future Direction: Future research should aim to overcome the limitations of the current study by employing a longitudinal design. Such an approach would facilitate the exploration of temporal changes in oxidative stress markers and clarify their causal relationship with the development of diabetic complications. Expanding the study to include multiple centres and a larger, more diverse cohort would enhance the representativeness and generalizability of the results across different populations within Nigeria.

In addition, it is recommended that subsequent investigations incorporate a broader range of oxidative stress biomarkers. Quantitative assessments of key enzymatic antioxidants—such as superoxide dismutase, catalase, and glutathione peroxidase—along with non-enzymatic antioxidants could offer a more comprehensive profile of the redox status in diabetic patients. Refining the methodology for evaluating coagulation patterns, perhaps by utilising advanced quantitative assays, would also provide more nuanced insights into the interplay between oxidative stress and blood coagulation.

Finally, future studies should examine the potential therapeutic implications of antioxidant supplementation. Investigating whether the inclusion of antioxidants—either through diet or as adjunctive therapy—can ameliorate oxidative damage and improve clinical outcomes in diabetic patients could have significant implications for the development of comprehensive treatment protocols for diabetes mellitus.

5 Conclusion

This study demonstrates that diabetes mellitus in patients from Kano, Northern Nigeria, is associated with a substantial state of oxidative stress. Diabetic subjects exhibited significant increases in serum malondialdehyde and corresponding decreases in ascorbic acid levels, along with pronounced abnormalities in blood coagulation patterns as determined by the DLOST. Notably, these oxidative alterations were consistent across all age groups, suggesting that such biochemical disturbances are intrinsic to the diabetic state rather than a function of ageing. These findings underscore the importance of addressing oxidative stress in clinical management, advocating for the integration of antioxidant interventions alongside conventional glycaemic control strategies. Future research should focus on longitudinal studies and include a broader range of oxidative biomarkers to further elucidate the mechanistic links between oxidative stress and diabetic complications, as well as to assess the therapeutic potential of antioxidant supplementation.

6 Recommendation

Based on our findings, it is recommended that the management of diabetes mellitus in this population should extend beyond glycaemic control to include strategies aimed at mitigating oxidative stress. Specifically, the incorporation of antioxidant supplementation—either through dietary means or pharmacological formulations—should be considered as an adjunct to standard antidiabetic therapies.

A comprehensive approach may involve:

1. **Dietary Modifications:** Encouraging the consumption of antioxidant-rich fruits and vegetables, such as citrus fruits (oranges, grapefruits, limes, lemons), grapes, pomegranates, apples, and dates; as well as green and yellow vegetables (peppers), cabbage, strawberries, carrots, dark leafy greens, and bananas. These food items are known to provide essential vitamins and other phytochemicals that help neutralize free radicals [57].

2. **Inclusion of Additional Antioxidants:** Consideration should also be given to

incorporating herbs, spices, nuts, and other natural products, known for their high antioxidant content [58] . Such dietary adjustments may help restore the balance between oxidant production and antioxidant defence.

3. **Clinical Evaluation of Supplementation:** Further clinical investigation is warranted to determine the optimal type, dosage, and duration of antioxidant supplementation. This could lead to the development of evidence-based guidelines that ensure antioxidant interventions are both safe and effective for diabetic patients.

Implementing these recommendations could potentially reduce oxidative damage, delay the progression of diabetic complications, and improve overall clinical outcomes in this population.

References

1. Jamison TD, Feachem RG, Makgoba MW, Bos ER, Baingana FK, Hofman JK, Rogo KO. Disease and Mortality in Sub-Saharan Africa. In: Ramiaya K, Mbanya J. *Diabetes Mellitus*. 2nd ed. Washington DC: The World Bank, 2006. p. 270.
2 Martin IJ. Diabetes and Organ Dysfunction in the Developing and Developed World. *Global Journal of Medical Research*. 2015;15(1):1-9.
3 Wild S, Roglic G, Green A, Sincree R, King H. Global Prevalence of Diabetes: Estimates for the Year 2000 and Projections for 2030. *Diabetes Care*. 2004;5:1047-1053.
4 Oputa RN, Chinenye S. Diabetes in Nigeria - a translational medicine approach. *African Journal of Diabetes Medicine*. 2015;23(1):7-10.
5 Ogbera AO, Ekpebegh C. Diabetes mellitus in Nigeria: The past, present and future. *World Journal of Diabetes*. 2014 December 15;5(6):905-911.
6 United Nations. *World Population Prospects: The 2015 Revision, Key Findings and Advance Tables*. New York: United Nations; 2015. ESA/P/WP.241.
7 Mobuogwu M. Rural Land and Credit Access in Nigeria. *Focus on Land in Africa*. 2013 1-7.
8 American Diabetes Association. *Classification and Diagnosis of Diabetes*. Diabetes Care. 2016;39(1):S13-S22.
9 Mittal K, Katare DP. Shared links between type 2 diabetes mellitus and Alzheimer's disease: A review. Diabetes & Metabolic Syndrome: *Clinical Research & Reviews*. 2016.
10 Mishra S, Rao CR, Shetty A. Trends in the Diagnosis of Gestational Diabetes Mellitus. *Scientifica*. 2016 1-7.
11 Kautzky-Willer A, Harreiter J, Pacini G. Sex and Gender Differences in Risk, Pathophysiology and Complications of Type 2 Diabetes Mellitus. *Endocrine Reviews*. 2016;37(3):278-316.
12 Almalki MH, Buhary BM, Khan SA, Almaghamsi A, Alshahrani F. Clinical and Biochemical Characteristics of Diabetes Ketoacidosis in a Tertiary Hospital in Riyadh. *Clinical Medicine Insights: Endocrinology and Diabetes*. 2016;9:7-11.
13 World Health Organisation. *Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications: Report of a WHO Consultation. Part 1: Diagnosis and Classification of Diabetes Mellitus*. Geneva: World Health Organisation; 1999. WHO/NCD/NCS/99.2.
14 King P, Peacock I, Donelly R. The UK Prospective Diabetes Study (UKPDS): clinical and therapeutic implications for type 2 diabetes. *British Journal of Clinical Pharmacology*. 1999;48(5):643-648.
15 Sies H. Oxidative Stress: A Concept in Redox Biology and Medicine. *Redox Biology*. 2015;4:180-183.
16 Holly JL. Your Life Your Health - Oxidative Stress: Why is it important to me? [Internet]. 2005 [cited 2016 August 29]. Available from: http://www.setma.com/Your-Life-Your-Health/oxidative-stress-why-is-it-important-to-me.
17 Sies H, Jones D. *Oxidative Stress*. Encyclopedia of Stress (Second Edition). 2007 45-48.
18 Halliwell B. Oxidative Stress and Cancer: Have

We Moved Forward? *Biochemical Journal*. 2007;401(1):1-11.

19 Volka M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free Radicals and Antioxidants in Normal Physiological Functions and Human Disease. *The International Journal of Biochemistry & Cell Biology*. 2007;39(1):44-84.

20 Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options. *Current Neuropharmacology*. 2009;7(1):65-74.

21 Nowotny K, Jung T, Hohn A, Weber D, Grune T. Advanced Glycation End Products and Oxidative Stress in Type 2 Diabetes Mellitus. *Biomolecules*. 2015;5:194-222.

22 Brownlee M. *The Pathobiology of Diabetic Complications - A Unifying Mechanism. Diabetes* (Banting Lectures 2004). 2005;54:1615-1625.

23 Oleniuc M, Secara I, Onofriescu M, Hogas S, Voroneanu L, Siriopol D, Covic A. Consequences of Advanced Glycation End Products Accumulation in Chronic Kidney Disease and Clinical Usefulness of Their Assessment Using a Noninvasive Autofluorescence. *Mædica - A Journal of Clinical Medicine*. 2011;6(4):298-307.

24 Shoji T, Koyama H, Morioka T, Tanaka S, Kizu A, Motoyama K, Mori K, Fukumoto S, Shioi A, Shimogaito N, et al. Receptor for Advanced Glycation End Products Is Involved in Impaired Angiogenic Response in Diabetes. *Diabetes*. 2006;55:2245-2255.

25 Goldin A, Beckman JA, Schmidt AM, Creager MA. Advanced Glycation End Products: Sparking the Development of Diabetic Vascular Injury. *Circulation*. 2006;114:597-607.

26 Gkogkolou P, Böhm M. Advanced glycation end products: Key players in skin ageing? *Dermato-Endocrinology*. 2012;4(3):159-170.

27 Nelson DL, Cox MM. *Lehninger Principles of Biochemistry*. 5th ed. New York (New York): W. H. Freeman and Company, 2008.

28 Murphy MP. How Mitochondria Produce Reactive Oxygen Species. *Biochemical Journal*. 2009;417:1-13.

29 Herceg Z, Wang Z. Functions of poly(ADP-ribose) polymerase (PARP) in DNA repair, genomic integrity and cell death. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*. 2001;477(1-2):97-110.

30 Li YR, Jia Z, Trush MA. Defining ROS in Biology and Medicine. *Reactive Oxygen Species*. 2016;1(1):9-21.

31 Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. *European Journal of Medicinal Chemistry*. 2015;97:55-74.

32 Rahman K. Studies on Free Radicals, Antioxidants and Co-factors. *Interventions in Ageing*. 2007;2(2):220-236.

33 Hannan PA, Khan JA, Iqbal Z, Ullah I, Rehman WU, Rehman M, Nasir F, Khan A, Muhammad IS, Hassan M. Simultaneous Determination of Endogenous Antioxidants and Malondialdehyde by RP-HPLC Coupled with Electrochemical Detector in Serum Samples. *Journal of Liquid Chromatography & Related Technologies*. 2015;38(10):1052-1060.

34 Pretorius E, Bester J, Vermeulen N, Lipinski B. Oxidation Inhibits Iron-Induced Blood Coagulation. *Current Drug Targets*. 2013;14:13-19.

35 Singh VP, Bali A, Singh N, Jaggi AS. Advanced Glycation End Products and Diabetic Complications. *Korean Journal of Physiology and Pharmacology*. 2014;18:1-14.

36 Štikarová J, Kotlín R, Riedel T, Suttnar J, Pimková K, Chrastinová L, Dyr JE. The Effect of Reagents Mimicking Oxidative Stress on Fibrinogen Function. *The Scientific World Journal*. 2013 1-8.

37 Dong JL, Brown B. Dry Layer Oxidative-Stress Test. [Internet]. 2016 [cited 2016 September 8]. Available from: http://naturalhealingcentermb.com/oxidative_stress_test.html#.V9G_yPkrLIU.

38 BiosynergyPro. Dry Layer Oxidative Stress Test. [Internet]. 2016 [cited 2016 September 8].

Available from: http://www.biosynergypro.com/oxidative-stress-test.html.

39 Arndt U. Reading in the "Book of the Body". [Internet]. 2009 [cited 2016 September 8]. Available from: http://www.horusmedia.de/1998-buch/buch-en.php.

40 Uloko AE, Adeniyi AF, Abubakar LY, Yusuf SM, Abdu A, Gezawa ID, Uloko AT. Pattern of diabetes admissions in a Northern Nigerian tertiary health centre. *African Journals Online*. 2013;7(1).

41 Ejike CECC, Uka NK, Nwachukwu SO. Diabetes and Pre-diabetes in Adults Nigerians: Prevalence and correlations of blood glucose concentrations with measures of obesity. *African Journal of Biochemistry Research*. 2015;9(3):55-60.

42 Sabir A, Ohwovoriole A, Isezuo S, Fasanmade O, Abubakar S, Iwuala S. Type 2 diabetes mellitus and its risk factors among the rural Fulanis of northern Nigeria. *Annals of African Medicine*. 2013;12(3):217-222.

43 Isara AR, Okundia PO. The burden of hypertension and diabetes mellitus in rural communities in southern Nigeria. *PanAfrican Medical Journal*. 2015 1-7.

44 Barham D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst*. 1972;97:142-145.

45 Roe J, Kuether C. Estimation of Ascorbic Acid. *The Journal of Biological Chemistry*. 1943;147:3999.

46 Gutteridge JMC, Wilkins S. Copper-dependent hydroxyl radical damage to ascorbic acid: Formation of a thiobarbituric acid-reactive product. *FEBS Letters*. 1982;137(2):327-330.

47 Manton, C. Dry Layer Blood Testing. [Internet]. 2008 [cited 2016 September 8]. Available from: http://healthdetective.com.au/services/dry-layer-blood-testing.html.

48 Semba RD, Nicklett EJ, Ferrucci L. Does Accumulation of Advanced Glycation End Products Contribute to the Ageing Phenotype? Journal of Gerontology: *Medical Sciences*. 2010;65A(9):963-975.

49 Acton QA. *Hypercholesterolemia: New Insight for the Healthcare Professional*. 2011th ed. Atlanta (Georgia): Scholarly Edition; 2011.

50 Caimi G, Carollo C, Lo Presti R. Diabetes mellitus: oxidative stress and wine. *Current Medical Research and Opinion*. 2003;19(7):581-586.

51 Mathebula SD. Polyol pathway: A possible mechanism of diabetes complications in the eye. *African Vision and Eye Health*. 2015;74(1):1-5.

52 Thakur M, Javarappa D. Adenosine Deaminase and Malondialdehyde Levels in Type-2 Diabetes Mellitus – a Short Study. *Global Journal of Medical Research: F Diseases*. 2014;14(4):6-9.

53 Ayala A, Muñoz MF, Argüelles S. Lipid Peroxidation: Production, Metabolism, and Signalling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. *Oxidative Medicine and Cellular Longevity*. 2014 1-31.

54 Singh Z, Karthigesu IP, Singh P, Kaur R. Use of Malondialdehyde as a Biomarker for Assessing Oxidative Stress in Different Disease Pathologies: a Review. *Iranian Journal of Public Health*. 2014;43(3):7-16.

55 Chinenye S, Ofoegbu EN, Uloko AE, Ogbera A, Olorogun SK, Johnson TO, Oli JM, Onyemelukwe GC, Puepet F, Mijinyawa B, et al. *Clinical Practice Guidelines for Diabetes Management in Nigeria*. 2nd ed. Port Harcourt (Choba Park): Diabetes Association of Nigeria; 2013.

56 Anonymous. New Scientific Review Supports Role of Vitamin K Supplements in Diabetes. [Internet]. 2016 [cited 2016 September 2]. Available from: http://www4.dr-rath-foundation.org/Newsletter/articles/new-scientific-review-supports-role-of-vitamin-k-supplements-in-diabetes.html.

57 Jideani AIO, Silungwe H, Takalani T, Omolola AO, Udeh HO, Anyasi TA. Antioxidant-rich Natural Fruits and Vegetable Products and Human Health. *International Journal of Food Properties*. 2021;24(1):41-67.

58 Carlden MH, Halvorsen BL, Holte K, Bohn SK, Dragland S, Sampson L, Willey C, Senoo H, Umezono Y, Sanada C, et al. The Total Antioxidant Content of More than 3100 Foods, Beverages, Spices, Herbs and Supplements used Worldwide. *Nutrition Journal*. 2010;9(2):1-11.

Anxiolytic Evaluation of Ethanol Extract of *Tapinanthus Globiferus* A. Rich Mistletoe on Vitellaria Paradoxa Host in Mice

*Abdullahi MH.[1] & Shuaibu AB.[1]

[1]Department of Pharmacology and Therapeutics, Bayero University, Kano. Nigeria

***Corresponding Author:** Abdullahi MH

***Corresponding Email:** mahuguma.pha@buk.edu.ng

Abstract

Anxiety disoder manifest with symptoms such as intense fear, panic, sweating, etc., resulting in disabling conditions that usually begin during the period between childhood to early adulthood, and Females are more affected than their male counterparts. Several factors, like biological, psychological and social factors, play their etiopathologic role. Anxiety is a physiological and psychological state characterised by emotional, physical, cognitive, and behavioural components. Its management involved the use of both orthodox and herbal medicine. *Tapinanthus globiferus* commonly is referred to as mistletoe, and its activity depends on the host tree. *Tapinanthus* species have been scientifically validated for sedative, antimicrobial, antidiabetic, antihypertensive and among others. This study aimed to evaluate the anxiolytic activity of the ethanol extract of *Tapinanthus globiferus* (ETg) grown on *Vitellaria paradoxa* in mice. The anxiolytic property of the extract was evaluated using the elevated plus-maze, staircase and open field test in mice. The extract produced no effect in mice on the number of entries and time spent in open arms of the elevated plus-maze at doses of 150 and 300 mg/kg, but at a dose of 75 mg/kg it produced a significant ($p \leq 0.05$) increase in the number of entries into the open arm. ETg did not produce an effect in the number of rearing at doses of 75 and 150 mg/kg; hence, ETg reduced the number of steps climbed in the staircase at a dose of 300 mg/kg. However, ETg significantly ($p \leq 0.05$) reduced the number of lines crossed and rearing at doses of 150 and 300 mg/kg in the open field. The study revealed that the extract of *T. globiferus* may have an anxiolytic effect in mice at a lower dose, as indicated in the elevated plus-maze and staircase test at a higher dose, as well as CNS depressant activity as observed in the open field test. The extract possesses anxiolytic and sedative properties, which may suggest its use in the management of anxiety.

Keywords: *Tapinanthus globiferus, Vitellaria paradoxa,* anxiolytic, staircase, elevated plus-maze

Introduction

Anxiety belongs to the group of mental disorders which are characterised by a sudden feeling of intense fear, panic, shortness of breath, chest pain, insomnia, fatigue, sweating e.t.c.[1,2] Anxiety disorders are disabling and commonly seen in clinical conditions that usualy begins during the period of childhood, and early adulthood.[3] The global prevalence of anxiety disorders ranges from 2.5 to 7 percent. About 284 million people were found to have experienced an anxiety disorder around the globe in 2017, making it the most prevalent mental health or neurodevelopmental disorder.[4] The etiologic factors associated with anxiety disoder include: (1) Biological factors; heredity, medications, nutrition and neurotransmitter imbalance such as gamma-aminobutyric acid, dopamine, serotonin, norepinephrine etc. (2) Psychological factors; personality traits, low self-esteem, cognitive dissonance, negative emotions, inter/intra-personal conflicts and perception of situational factors among others (3) Social factors; adverse life experiences, lack of social support, work stress, lack of social skills, conflict of societal norms and natural calamities.[5]

The pathologic hallmark of the disorder is hyper-responsiveness of the amygdala and limbic system, which is connected to the prefrontal cortex in the brain.[1,2] The neurochemicals that play a role in anxiety disorders include: gamma-aminobutyric acid (GABA), monoamines (Dopamine,

Date submitted-25/05/2022 Date Accepted-13/07/2022

noradrenaline, serotonin), neuropeptides (galanin, neuropeptide Y, arginine vasopressin, tachykinin, substance P), neurosteroids and cytokines.[7,8]

Anxiety disorders are managed by drugs which include: benzodiazepines, barbituates and buspirone.[9] Antidepressants are also found to be very effective in the management of a range of anxiety disorders. There are some limitations as well as unfavourable risks with the use of these anxiolytic drugs. These include drug abuse, dependence, withdrawal symptoms, anterograde amnesia, abstinence syndrome, paradoxical reaction in humans and decay of psychomotor functions.[5,9] Herbal medicines, on the other hand, are well known and popularly used as remedies for most diseases and other clinical conditions, and also play an important key role in the human health care services, as a result of which the world's populations rely on the use of traditional medicine, which is predominantly based on plant materials.[9] Plant products and/or their extracts may produce similar pharmacological activity to orthodox medicine on the CNS. Therefore, substances that can be able to act on the CNS may selectively relieve pain, depression, anxiety, insomnia and other neuropsychiatric and neurological disorders.[10] The leaves, flowers, stem and root barks of the plants are used to prevent, relieve and treat illnesses and also to improve one's sense of wellbeing, but the mechanisms by which herbal medicine acts in the CNS have not been fully elucidated. However, herbal medicines are believed to be more potent, safe and affordable.[11] *Tapinanthus globiferus* is widespread in the Savannah regions across Africa from Senegal to Ethiopia and tropical areas of Nigeria,[12] and it belongs to the family *Loranthaceae*. It is commonly attached to the branches of *kola, citrus, combretum, acacia, aloe* and *Terminalia* host tree.[13] *T. globiferus* has been used traditionally in the treatment of hypertension, epilepsy, anxiety and arthritis.[12,14] Several studies on *T. globiferus* were reported to possess a number of therapeutic uses for managing a wide range of diseases such as diabetes mellitus and hypertension,[25,26] oxidative stress and inflammation, and trypanosomiasis.[27,28] The objective of this study is to evaluate the anxiolytic activity of the ethanol extract of *Tapinanthus globiferus* mistletoe on *Vitellaria paradoxa* (host) in mice.

Materials and Methods

Preparation of the plant materials

Fresh *Tapinanthus globiferus* grown on *Vitellaria paradoxa* was collected in February 2022 from Huguma Village of Takai Local Government Area, Kano State, Nigeria. *T. globiferus* was identified and authenticated at the Herbarium unit, Department of Botany, Ahmadu Bello University, Zaria, Nigeria, by comparing with the existing voucher specimen number 1052. The plant material was dried under shade until constant weight, crushed and pounded into fine powder. The extraction was conducted with ethanol (75% in water) using soxhlet extractor, and the extract was concentrated on a water bath at a temperature of 60 ˚C.

Drugs, Chemicals and Equipment

Diazepam (F. Hoffmann-La Roche Ltd, Basel, Switzerland), ethanol (Sigma Aldrich Inc., USA), normal saline (Dana Plc, Nigeria) and Soxhlet extractor (Franz von Soxhlet apparatus).

Preparation of drug solutions

The ethanol extract of *Tapinanthus globiferus* was prepared by dissolving the powdered plant in normal saline before administration, and diazepam is supplied in ampoules, and appropriate dilutions were made with normal saline. The drug solutions were usually prepared fresh during the experiment to maintain the stability of the drugs.

Experimental Animals

Swiss Albino mice (20 – 25 g) aged between 8 – 9 weeks were obtained from Animal House, Department of Pharmacology and Therapeutics, Bayero University, Kano and kept in a well-ventilated room, fed with pelletized grower's mash (Vital feeds, Plc Jos) with available of *ad-libitum* water and allowed to acclimatise prior to the experiment.

Elevated plus-maze test in mice for anxiolytic behaviour

The method described by Lister [15] was adopted for this study. The elevated plus-maze (EPM) apparatus consists of 2 open arms (35 x 5 cm) and 2 closed arms (35 x 5 x 20 cm) connected together with a central square (5 x 5 cm). The apparatus was elevated to a height of 25 cm in a dimly illuminated room. Thirty mice were divided into five groups of six each. First and second groups were treated with normal saline (10 mL/kg body weight *i.p.*) and diazepam (0.25 mg/kg body weight *i.p.*) respectively, while third, fourth and fifth groups were treated with 75, 150 and 300 mg/kg body weight *i.p.* of the ethanol extract of *T. globiferus.* Thirty minutes after *i.p.* treatment, mice were placed individually into the centre of EPM facing the closed arms. The mouse was allowed to spend a maximum of 5 minutes in both open and closed arms. The number of entries and time spent in the open and closed arms were counted and recorded during 5 minutes. An entry into an open or closed arm was defined as having all four paws within the arm.

Staircase test in mice for anxiolytic behaviour

This test was conducted according to the method described by Simiand *et al.* [16]. The staircase was made up of wood and consisted of five identical steps, 2.5 cm high, 10 cm wide and 7.5 cm deep. The staircase was surrounded by walls of transparent Plexiglas, the height of which was constant along the whole length of the staircase. Thirty mice were divided into five groups of six each. First and second groups were treated with normal saline (10 mL/kg body weight *i.p.*) and diazepam (0.25 mg/kg body weight *i.p.*), respectively, while third, fourth and fifth groups were treated with 75, 150 and 300 mg/kg body weight *i.p.* of the ethanol extract of *T. globiferus*. Thirty minutes after *i.p.* treatment, mice were placed individually on the floor of the box with their backs on the staircase. The number of steps climbed and rear climbed was counted and recorded during 3 minutes, but the number of steps descended was not considered. A mouse is considered to have climbed when the mouse places all four paws on the step, while rearing is when the hind legs are either on the step or against the wall to sniff air. The box was cleaned after each trial in order to eliminate olfactory cues which might modify the behaviour of the next animal.

Open field test in mice for locomotor activity

The method of Brown *et al*. [17] was adopted in this study. The arena of the open field consists of a 72 x 72 cm wooden box, 36 cm high, in which the floor is divided into 16 squares (15 x 15 cm). Thirty mice were divided into five groups of six each. First and second groups received normal saline (10 mL/kg) and diazepam (0.25 mg/kg), respectively (*i.p.*), while third, fourth and fifth groups were treated with 75, 150 and 300 mg/kg, body weight, *i.p.* of the ethanol extract of *T. globiferus*. Thirty minutes after *i.p.* treatment, each mouse was placed at the centre of the open field and allowed to explore the apparatus for 5 minutes. After the 5-minute test, mice were returned to their cage, and the open field was carefully cleaned with 70 % ethanol solution after every test. Each mouse was then given a score for locomotor activity. The number of lines crossed and rearing was counted and recorded for each mouse.

Statistical Analysis

Statistical analysis was carried out using SPSS software (verssion 23), and data were analysed using One Way Analysis of Variance (ANOVA) followed by Dunnett's *post-hoc* test, in which values of $p \leq 0.05$ were considered statistically significant. Results were presented as Mean ± Standard Error of the Mean (Mean ± SEM) in tables.

Results

Effect of ethanol extract of *T. globiferus* on the elevated plus-maze test in mice

Elevated plus-maze was conducted to evaluate the anxiolytic behaviours of the ethanol extract of *T. globiferus* in mice. The extract significantly ($p \leq 0.05$) increased the number of entries into the open arm at a dose of 75 mg/kg when compared with the negative control group treated with 10 mL/kg normal saline. The standard drug (diazepam, 0.25 mg/kg) significantly ($p \leq 0.05$) increased both the time spent in the open arm and the number of entries into the open arm of the elevated plus-maze test in mice compared to the control (Table 1).

Effect of ethanol extract of *T. globiferus* on the staircase in mice

Staircase was carried out to investigate the anxiolytic activity of the *T. globiferus* ethanol extract in mice, the extract produced a significant ($p \leq 0.05$) decrease in the number of steps climbed and rearing by mice in staircase test at dose of 300 mg/kg and produced no effects in both the number of climbed and rearing at tested doses (75 and 150 mg/kg) compared to negative control (10 mL/kg normal saline). However, diazepam used as a positive control significantly ($p \leq 0.05$) decreased the number of steps climbed as well as the number of rearing (Table 2).

Effect of ethanol extract of *T. globiferus* on the open field test in mice

An open field test was carried out to evaluate the locomotor activity of the extract in mice. The ethanol extract of *T. globiferus* caused a significant ($p \leq 0.05$) decrease in the number of lines crossed and number of rearing in the open field test at doses of 150 and 300 mg/kg, respectively, and showed no effects on central square entries and central square duration at the tested doses compared to the control treated with 10 mL/kg normal saline. However, diazepam (standard drug) at a dose of 0.25 mg/kg significantly ($p \leq 0.05$) reduced the number of lines crossed, rearing, central square entries and central square duration compared to control treated with normal saline (Table 3).

Table 1: Effect of Ethanol Extract of *Tapinanthus globiferus* on Number of Entries and Time Spent in Open and Closed Arms of the Elevated Plus-maze Test in Mice

Treatment (mg/kg)	Number of Entries		Time spent (sec)	
	Open	Closed	Open	Closed
NS 10mL/kg	6.00 ± 0.52	13.50 ± 1.38	75.83 ± 7.43	164.17 ± 16.31
ETg (75)	11.50 ± 0.76[a]	8.33 ± 1.12	82.50 ± 9.21	141.83 ± 9.64
ETg (150)	8.83 ± 1.54	7.83 ± 0.87[a]	103.50 ± 10.67	106.00 ± 15.22[a]
ETg (300)	9.00 ± 1.44	8.00 ± 1.55	106.00 ± 4.82	103.67 ± 13.17[a]
DZP (0.25)	12.00 ± 1.03[a]	8.33 ± 2.20	129.17 ± 16.00[a]	70.17 ± 7.55[a]

[a]$p \leq 0.05$ compared to NS, One Way ANOVA followed by Dunnett's *post-hoc* test. n = 6, Data = Mean ± SEM, route of administration = intraperitoneal, ETg = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline, DZP = Diazepam.

Table 2: Effect of Ethanol Extract of *T. globiferus* on Number of Steps Climbed and Rearing in Staircase test in Mice

Treatment (mg/kg)	Number of steps climbed	Number of rearing
NS 10 mL/kg	27.17 ± 3.24	15.50 ± 2.78
ETg (75)	25.00 ± 2.80	14.33 ± 3.57
ETg (150)	24.17 ± 3.13	13.33 ± 3.42
ETg (300)	13.50 ± 2.09[a]	5.83 ± 1.74 [a]
DZP (0.25)	11.83 ± 4.38[a]	2.83 ± 2.29[a]

[a]$p \leq 0.05$ compared to NS, One Way ANOVA followed by Dunnett's *post-hoc* test, n = 6, Data = Mean ± SEM, route of administration = intraperitoneal, ETg = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline, DZP = Diazepam.

Table 3: Effect of Ethanol Extract of *Tapinanthus globiferus* on Behaviour of Mice in Open Field Test in Mice

Treatment (mg/kg)	LC	Rr	CSE	CSD (sec.)
NS 10 mL/kg	82.00 ± 19.15	23.83 ± 7.57	2.50 ± 0.76	0.08 ± 0.03
ETg (75)	77.17 ± 18.74	22.50 ± 8.04	3.33 ± 1.23	0.05 ± 0.01
ETg (150)	47.83 ± 4.14[a]	8.67 ± 3.76 [a]	1.17 ± 0.31	0.08 ± 0.05
ETg (300)	53.17 ± 18.54[a]	6.50 ± 0.83[a]	1.67 ± 0.76	0.09 ± 0.25
DZP (0.25)	59.83 ± 2.38[a]	7.00 ± 0.10[a]	1.33 ± 0.07[a]	0.01 ± 0.01[a]

[a]$p \leq 0.05$ compared to NS, One Way ANOVA followed by Dunnett's *post-hoc* test. n = 6, Data = Mean ± SEM, route of administration = intraperitoneal, ETg = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline, LC = Line crossed, Rr = Rearing, CSE = Central square entries, CSD = Central square duration, DZP = Diazepam.

Discussion

The ethanol extract of *T. globiferus* produced an increase in the number of entries into open arms of EPM at a lower dose after intraperitoneal administration, which may suggest the anxiolytic activity of the extract at a low dose of 75 mg/kg, and this is buttressed by the observed decrease in the number of steps climbed and the number of rearing in the staircase. An increase in the number of entries and time spent in the open arms of the maze is a measure of anxiolytic property.[18] Hence, anxiolytic agents produce a significant ($p \leq 0.05$) decrease in the number of rearing and no effect on the number of steps climbed in mice. Reduction in mice rearing is a measure of anxiolytic property.[19] Similarly, diazepam (0.25 mg/kg) reduced the number of both steps climbed and rearing behaviour in mice. In the staircase model, step climbing serves as an index of exploratory or locomotor activity while rearing serves as an index of anxiety [20], and the model is used to detect behavioural effects of agents active at the $GABA_A$ receptors.[16] The extract reduced the number of lines crossed and rearing in the open field, and the reduction observed in these parameters may be due to CNS depression. Measurement of several parameters in an open field allows to identify various types of sedative or stimulant agents.[20] Study showed that reduction in the number of lines crossed, grooming, rearing and central square entries predict the anxiolytic and hypnotic properties. [21] Increase in number of lines crossed (locomotion) can be considered a stimulant effect, while decreased locomotion is related to sedation [22]; hence, the extract decreased the number of lines crossed. This could be as a result of the possible sedative effect of the extract, and it could have anxiolytic properties at lower doses.

Staircase and open field tests are useful in identifying specific and non-specific effects of drugs and assessing anxiety, as well as aspects of sedation.[19] Mice's preference to stay close to the walls in an open field is a natural phenomenone hence the tendency to be anxious. The number of entries into the central and the time spent in the central square are measures of anxiolytic activity.[23] The pathophysiology of anxiety disorders has been observed to be due to the neurobiological abnormalities of endogenous neurotransmitters in serotonergic, noradrenergic, glutamatergic and GABAergic transmission.[7] Activation of GABA receptors directly or indirectly would have an anxiolytic effect, given that $GABA_A$ receptors are implicated in anxiety disorders.[8] However, the anxiolytic activity of *T. globiferus* extract may be due to sedative activity on one of the receptors of the endogenous neurotransmitters. Probably, the extract may produce its effect through $GABA_A$ receptor mediation. Agents that are active at the $GABA_A$ receptor complex have been shown to reduce rearing at doses that do not reduce climbing in the staircase test.[16] The previous study on *T. globiferus* extracts revealed its safety and also demonstrated the presence of phytochemical constituents such as flavonoids, saponins and tannins. The phytochemicals present in the extracts are likely to be responsible for their anxiolytic activity. Natural and synthetic flavonoids are found

to be potent anxiolytic agents without sedative effects.[24]

Conclusion

This study showed that the ethanol extract of *Tapinanthus globiferus* possessed anxiolytic activity as seen in the elevated plus maze and elevated staircase tests in mice. These may suggest the probable use of *Tapinanthus globiferus* plant extract in the treatment of anxiety.

Conflict of interest

The authors declared no conflict of interest

Acknowledgement

We acknowledge all those who have contributed in one way or another towards making this piece of work. We appreciate your hard work, dedication, commitment and discipline.

References

1. Martinez RCR, de Oliveira AR, Brandão ML. Serotonergic mechanisms in the basolateral amygdala differentially regulate the conditioned and unconditioned fear organised in the periaqueductal gray. *Eur. Neuropsychopharmacol.* 2007; **17**: 717-724.
2. Keeton CP, Kolos AC, Walkup JT. Pediatric Generalised Anxiety Disorder Epidemiology, Diagnosis, and Management. Pediatric Drugs. 2009; **11**: 171-183.
3. Craske M. G. And Stein M.B. 2016; Anxiety. Lancet online
4. Saloni D, Hannah R, Max R. "Mental Health". *Published online at OurWorldInData.org.*2021; Retrieved from: 'https://ourworldindata.org/mental-health' [Online Resource].
5. Milind P, Monu P, Sharma K. Medicinal plants possessing anxiolytic activity: A brief review. Pelagia Research Library. *Der Pharmacia Sinica.* 2015; **6**(5):1-7.
6. Kumar R, Murugananthan G, Nandakumar K, Talwar S. Isolation of anxiolytic principle from ethanolic root extract of *Cardiospermumhalicacabum*. Phytomedicine. 2011; 18219‑223.
7. Nutt DJ, Malizia AL. New insights into the role of the GABA (A) benzodiazepine receptor in psychiatric disorder. *British Journal of Psychiatry.* 2001; **179**(1): 390 – 396.
8. Vogel HG, Vogel WH. Drug Discovery and Evaluation. Heidelbery Springer, New York, 2002; 401 – 461.
9. Thakur P, Rana AC. Anxiolytic Potential of Medicinal Plants. A review. *Int J Nutr Pharmacol, Neurol Dis*. 2013; **3**(4): 325 – 331.
10. Pandy V, Narasingam M, Mohamed Z. Antipsychotic-like activity of Noni (*Morinda citrifolia* Linn.) in mice. *Journal of Traditional, Complementary and Alternative Medicine.* 2012; **19**(12): 186 – 192.
11. Cragg GM, Newman DJ. Natural products: a continuing source of novel drug leads. *Biochim Biophys Acta.* 2013; 3670 – 3695.
12. Adesina SK, Illoh HC, Johnny II, Jacob IE. African mistletoes (Loranthaceae); Ethanophamacology, Chemistry, and Medicinal Values: An Update. *African Journal of Traditional Complementary Alternative Medicine.* 2013; **10**(4): 161- 170.
13. Burkill HM. *Useful Plants of West Tropical Africa*, Vol. 5, (2nd ed.) Royal Botanic Gardens, Richmond, Survey, England, 2000; 548-560.
14. Abedo JA, Jonah OA, Abdullahi RS, Mazadu MR, Idris HY, Muhammed H, Shettima FT, Ombugadu S, Dauda M, Garba J, Abdulmalik U, Kugu BA, Usman AO. Evaluation of Trypanosomal Activity of *Tapinanthus globiferus* and *Gongronema latifolium* on *Trypanosoma congolense. Bioscience Research.* 2013; **10**(1): 20 - 28.
15. Lister RG. The use of elevated plus-maze to measure anxiety in the mouse. *Psychopharmacology.* 1987; **92**(2): 180 – 185.
16. Simiand J, Keane PE, Morre M. The staircase test in mice: a simple and efficient procedure for primary screening of anxiolytic agents. *Psychopharmacology.* 1984; **84**(1): 48 – 53.
17. Brown RE, Corey SC, Moore AK. Differences in Measure of Exploration and Fear in MHC-congenic C57BL/6J and BG-H-2K Mice. *Journal of Behaviour Genetics.* 1999; **29**(4): 263 – 271.
18. Kavyasree K, Vijusha M, Rajani A, Hemamalini K, Sundari EGR. Screening of behavioural, muscle co-ordination and anxiolytic activities of methanolic extract of

Tabebuia rosea (Bertol). *Asian Journal of Pharmaceutical and Clinical Research.* 2013; **6(5):** 187 – 190.
19. Ya'u J, Abdulmalik UA, Yaro AH, Chindo BA, Anuka JA, Hussaini IM. Behavioural properties of *Balanites aegyptiaca* in rodents. *Journal of Ethnopharmacology.* 2011; **135**(3): 725 – 729.
20. Vogel, G. H. Psychotrophic and neurotrophic activity In: Vogel, G. H. (Ed) *Drug discovery and Evaluation: Pharmacological Assays,* Springer-Verlag Berlin Heidelberg New York, 2008; 566 – 874.
21. Hosseinzadeh H, Shahandeh S, Shahsavand S. Anxiolytic and Hypnotic Effects of Aqueous and Ethanolic Extracts of Aerial Parts of *Echium italicum* L. in mice. *Journal of Natural Pharmaceutical Products.* 2012; **7(2):** 71 – 79.
22. Belzung C, Prut L. The open field paradigm to measure the effects of drugs on anxiety-like behaviours: A review. *European Journal of Pharmacology.* 2003; **40**(3): 3 – 33.
23. Sethi A, Das BP, Baja BK. The Anxiolytic Activity of Gabapentin in Mice. *Journal of Applied Research.* 2005; **5**(3): 415 – 422.
24. Herberlain H, Tscheirsch KP, Schazer HI. Flavonoids from *Leptospermum scoparium* with affinity to the benzodiazepine receptors characterized by structure activity relationship and in vivo studies of plant extract. *Journal of Pharmazie.* 1994; **49**(12): 912 – 922.
25. Akanji MA, Ayorinde BT, Yakubu MT. (2009). Anti-lipidaemic Potentials of Aqueous Extract of *Tapinanthus globiferus* Leaves in Rats. *Ethnopharmacology, Chemistry and Medicinal Values.* 2009; **25:** 1 – 8.
26. Ogbonnia SO, Anyika EN, Mbaka GO, Utah P, Ugwu D, Nwakakwa N, Ota DA. antihyperglycaemic and antihyperlipldaemic effects of aqueous ethanol extract of *Tapinanthus globiferus* leaves and Treculia africana root bark and their mixture on alloxan diabetic rats. *Agriculture and Biology Journal of North America.* 2012; **3(6):** 237 – 246.
27. Adekunle AS, Oyewo BE, Afolabi OK. Therapeutic Efficacy of *Tapinanthus globiferus* on Acetaminophen-induced Nephrotoxicity, Inflammatory reactions and oxidative stress in albino rats. *International Research Journal of Biochemistry and Bioinformatics*. 2012; **2(2):** 41 – 45.
28. Abedo JA, Jonah OA, Abdullahi RS, Mazadu MR, Idris HY, Muhammed H, Shettima FT, Ombugadu S, Dauda M, Garba J, Abdulmalik U, Kugu BA, Usman AO. Evaluation of Trypanosomal Activity of *Tapinanthus globiferus* and *Gongronema latifolium* on *Trypanosoma congolense. Bioscience Research.* 2013; **10(1):** 20 – 28.

Age And Side Variations of Breast Lump Cases Submitted to Histopathology Laboratory of AKTH, Kano State, Nigeria

*Gudaji A[1],Salma IA [1], Amin SM[2], Ashiru HY[1], Bashir MA[1], Idris AT[1], Abdullahi AY[1], Musa A[1], Saad D[1],Abdulrashid S[1], Maigari, YK[1],Tahir A[1], Aliyu SA[1], Ahmed AA[1], Gaya AA[1], Maryam IS[1], Maharazu MM[1], Rabiu IF[1], & Mukhtar M.[1]

[1]Department of Anatomy, Bayero University, Kano, Nigeria.
[2]Department of Histopathology, National Hospital, Abuja, Nigeria.
***Corresponding Author:** Gudaji A
***Corresponding Email:** agudaji.ana@buk.edu.ng

Abstract

Globally, breast tumours are among the most common human neoplasms and the second leading cause of cancer death among women. The incidence and histological subtypes of these tumours differ among regions of the world and across races. Breast cancer mortality remains alarmingly high in Nigeria, and most patients are detected at late stages of the disease due to the lack of awareness and inadequacy of screening programs. Furthermore, the marked heterogeneity of the tumour in terms of inter-racial, intra-locality, intra-patient and even intra-tumour variation is quite challenging to the scientific world. This study was aimed at determining the relationship of type of breast lesions, patient age and lesional laterality among the samples of breast lumps submitted to the Department of Histopathology, Aminu Kano Teaching Hospital (AKTH) within one year. This study was a retrospective study conducted in AKTH, Kano State, Nigeria. Histological report registers were reviewed, and relevant clinical information was extracted using a simple data proforma. A total of 368 breast lesions were obtained, of which 84 (23%) were malignant, and 273 (76%) were benign. With respect to gender, 11 cases were male, of which only one was malignant. Conclusively, age was found not influence lesion side, but variation was observed between age groups and lesional laterality among the samples of the breast lumps. The incidence of breast malignancies among patients with breast lesions is high. This signals the urgency for the implementation of breast screening programs.

Keywords: Breast Masses, Patient Age, Lesional Laterality, Histopathologic Analysis, AKTH.

Introduction

Globally, breast tumours are among the most frequent human neoplasms occurring almost exclusively in females of various age groups.[1] Breast cancer (BC) is considered to be the second most common cancer subtype investigated[2], and it is estimated that 2.3 million new cases are diagnosed every year worldwide [3].In Nigeria, breast cancer is the most common cancer in women [4], accounting for 23%.[5] Despite higher incidences of breast cancer in the developed world, the mortality rate is higher in low and medium-income countries (LMICs), including Nigeria [6]. Indeed, various studies have shown that the death rate in Nigeria from breast cancer is about three times that of America [6]. Some studies have reported BC to be a leading female cancer in Jos [7] and Kano in northern Nigeria [8]. The incidence and histological subtypes of these tumours differ across regions, with a rising incidence particularly in LMICs. [9].

It has been opined that there is a significant variation in the presentation of different breast lesions among various age cohorts [10]. Undoubtedly, the most frequent presentations of breast disorders are pain and palpable breast lumps [11]. Furthermore, regardless of the efficiency of mammography and fine needle aspiration cytology, tissue biopsy remains the gold standard for diagnosis, and this can be obtained by different techniques such as needle core, incisional or excisional biopsies.[12]

A breast lump is a growth of tissue that develops within the breast. Different types of breast lumps can vary in the way they look and feel [13]. It has

been consistently reported that women are slightly more likely to be diagnosed with breast cancer in the left breast than in the right [14][15].

Despite the consistency of this predominance of left-sided breast cancer, meaningful underlying characteristic differences between the two sides have not been well identified. Numerous studies conducted in Nigeria and elsewhere focused on breast lesion laterality, providing important information about this symptom. However, there is no clear explanation about the correlation between breast lesions and patients' age with the laterality of the lesion. This justifies the need for this study.

Materials and Methods

This is a one-year descriptive retrospective study extending from 1st June 2017 to 30th June 2018, conducted in a Nigerian tertiary health centre, Aminu Kano Teaching Hospital (AKTH), a major histopathological services provider in the densely populated Kano city. The centre receives samples for histopathological and cytologic evaluation from most of the government and private hospitals in the state and the neighbouring Bauchi, Jigawa, Katsina and Kaduna states [16]. Laboratory request forms and the register of histological reports were retrieved, and the relevant clinical information was extracted. Data were then collated using a simple data proforma and summarised using frequencies and percentages. IBM SPSS version 24.0 was used for the analyses. Chi-square was used to determine the association between the categorical variables, and a p-value of less than 0.05 was considered significant.

Group 1: Fibroadenoma, Fibrocystic changes, Inflammatory lesions; Invasive ductal carcinoma (NST), Invasive ductal carcinoma.

Group 2: Benign phylloides tumour, Gynecomastia, Lactational Adenoma, Tubular adenoma, Sclerosing adenosis; Invasive lobular carcinoma, Metaplastic Carcinoma, Metastatic Carcinoma

Others: Secretory Carcinoma, Sebaceous Carcinoma, Apocrine Carcinoma, Papillary Carcinoma, Mucinous Carcinoma, Medullary Carcinoma, Malignant phylloides tumor, Intermediate grade ductal carcinoma in-situ, Cystic tissue carcinous, Metastatic carcinoma and Invasive Apocrine Carcinoma; Reactive lymphoid hyperplasia, Reactive hyperplasia, Plexiform Neurofibroma, Lipoma, Blunt duct adenosis, Adenomyoepithelial adenosis, Lactational Adenoma with Fibrocystic changes, Fibrocystic changes with Fibroadenomatoid hyperplasia, Fibrocystic changes with Fibroadenoma, Fibrocystic changes with chronic inflammation, Fibrocystic changes with adenosis, Fibroadenoma with Lactational changes, Fibroadenoma with Fibrocystic changes and adenohyperplasia.

Results

Table 1 shows the distribution of breast lumps in females according to tumour type, with the majority being benign tumours (76%) and malignant cases were (24%). Table 2 shows the distribution of breast lumps in males according to tumour type, with the majority being benign tumours (10, 91%) and a lesser number of malignant cases (1, 9%). Table 3 shows the distribution of breast lumps based on histological subtype. Group 1 was found to be the majority (267, 75%), followed by group 2 (55, 15%) and then group 3 (35, 10%).

There is a statistically significant association between breast tumour type and age groups ($p<0.001$) as shown in Table 4. The majority (78%) of the patients with benign breast tumours are within the 11 to 29-year group; conversely, those in the 30 to 50 years group account for the majority of patients with malignant breast lesions. Only 5% of the patients with malignant tumour type and 3% of the patients with the benign tumour type account for 11 to 29 years and 51 to 90 years, respectively. Table 5 shows the association between age and breast lesions, grouped based on histology. There is a statistically significant association across the breast tumour type and its subgroups (p-value; benign = 0.04 and malignant = 0.0009). Of the malignant lesions, occurrence was found in group 1 and others constituting group 3 lesions among all the age groups, while in group 2 lesions, no occurrence was observed below the age of 30 years. Table 6 above shows the association between age and side of breast lesions across the tumour types. Occurrence of breast lesions was observed mostly on the right side for both benign and malignant type though not statistically significant.

In general, breast lumps were found more

commonly on the right side than the left side of the body in both benign and malignant types, and only 9 cases and one case were found to be bilateral in the benign and malignant types, respectively, though not statistically significant.

Table 1: Distribution of Breast Lumps in Females According to Tumour Type

	Frequency	Percent (%)
Benign	273	76
Malignant	84	24
Total	357	100

Table 2: Distribution of Breast Lumps in Males According to Tumour Type

	Frequency	Percent (%)
Benign	10	91
Malignant	1	9
Total	11	100

Table 3: Distribution of Breast Lumps in Females Grouped According to Subtype

Lesion type	Frequency	Percent (%)
Group 1	267	75
Group 2	55	15
others	35	10
Total	357	100

Table 4: Distribution of Breast Lumps in Females Based on Histological Subtype

Codition	Frequency (n)	Percentage (%)
Benign	179	50
Malignant	83	23
Inflammatory	14	4
Tumor-like	81	23
	357	100

Table 5: Test of Association between Types of Breast Lumps and Age Group in Female Subjects

Age Category(yrs)	Benign	Malignant	Chi-square	p-value
11-29	213(98.2%)	4(1.8%)		
30-50	53(48.6%)	56(51.4%)		
51-90	7(22.6%)	24(77.4%)	15	0.0008

Table 6: Test of Association between Age Group and Histological Subtypes of Breast Lumps among Females

Tumor Type	Lesion type	Age Group			X^2	p-value
		11-29	30-50	51-90		
Benign	Group 1	165	34	7	10.197	0.04
	Group 2	36	8	0		
	Others	12	11	0		
Malignant	Group 1	1	35	19	18.768	0.0009
	Group 2	0	17	1		
	Others	3	4	4		

Table 7: Test of Association between Tumour Site and Age Group in both Malignant and Benign Breast Lesions

Tumor Type	Site	Age Group(yrs)			X^2	P-value
		11-29	30-50	51-90		
Benign	Right	143	42	4	5.66	0.23
	Left	64	9	3		
	Both	6	2	0		
Malignant	Right	3	32	17	2.95	0.57
	Left	1	23	7		
	Both	0	1	0		

Chi-square (X^2)

Discussion

A study reported over two decades ago in Nigeria, suggested that breast cancer was present in the right breast in 53% of the patients, the left breast in 45%, and bilateral in 2% [22], which differs significantly from the result of this study and most other studies. However, it was also cited that 'although a slight right-sided trend was observed among the black patients in this series, the number of cases was too few to approach significance [23]. This may possibly suggest a racial factor.

When it comes to age at diagnosis of breast lumps, Kramer's study suggests a possible explanation for the higher incidence of left-breast cancer in American women. A higher percentage of left-handed women developed breast cancer before age 45, but the overall incidence of unilateral breast cancer was not greater in left-handed women than in right-handed or ambidexterous subjects [24]. While in another literature, the t-test revealed no significant difference in mean ages according to laterality [23]. In this study, the p-value (0.22) revealed no association between age and lesion side for the occurrence of breast lumps, meaning that age does not influence the side of the body in which breast tumours occur. Although age and side variation among the types of breast tumors was observed, the variation was not observed among their age groups. On the other hand, benign breast lumps were observed to be more common in the right side (189 cases) than the left side (76 cases), with only 8 cases being bilateral in all age groups. Similarly, malignant cases, were located predominantly on the right side (52 cases) than the left (31 cases) with only 1 case being bilateral in all age cohorts and the following literature might be the explanation for such findings as cited: The t-test revealed no significant difference in mean ages according to laterality [23]; but the over-all incidence of unilateral breast cancer was not greater in left- handed women than in right-handed or ambihanded subjects [24].

Breast tumours are very common, and in most studies within and outside Nigeria, the frequency of benign breast tumours outnumbered that of malignancies [25], but the proportions, however, vary. This study also found benign breast tumours to be overwhelmingly preponderant over malignant tumors comprising two-thirds (76%) of 273 cases of breast neoplasms, and is higher than 66.3% earlier reported study in this centre (AKTH) about a decade ago, while the remaining 84 cases (23%) were malignant lumps (cancerous).

The primary risk factor for breast cancer is being female and older age [26]. It was described as the most common cancer in women worldwide, as it affects about 12% of women worldwide, and it is 100 times less common in men as compared to women, and affects predominantly older populations with a peak incidence at approximately 60 years of age [27, 28]. In Nigeria, the cancer statistics record: 91.3% for females and 8.3% for males [29]. This study also found the number of female cases (329 cases) overwhelmingly preponderant over male cases (11 cases) out of the total 357 cases found, as widely advocated in the literature by numerous studies.

An interesting finding, the number of male patients with breast lumps outnumbered that found about a decade ago in this centre (AKTH), in which gynecomastia was the only benign breast tumour

recorded with 1.4%. However, in this study, the record of 11 cases of male patients with breast tumours was found, in which 10 cases were benign, and the remaining 1 case had a malignant tumour and was found to be above the age of 50 years. These results are supported by the literature as cited: Breast cancer is not common in men (approximately 2,400 new cases diagnosed per year in the U.S.), but it typically has a significantly worse outcome. Although it can occur at any age, male breast cancer usually occurs in men over 60 years of age [30]. This might be the explanation for the finding of only 1 case of a male diagnosed with carcinoma, who was above the age of 50 years.

The present study also showed that 50% of breast lesions were benign, and 23% were malignant, while inflammatory and tumour-like lesions constituted the remaining subjects. The primary risk factor for breast cancer is being female and older age [26]. The rapid rate of increase before menopause (ages 40-50) slows down after that, probably owing to diminishing levels of circulating estrogens [31]. African women with breast cancer are more likely to be pre-menopausal, with the incidence peaking between 35 and 40 years. This is almost 10-15 years earlier than the peak incidence for Western countries [32, 33]. The disease appears one decade earlier in Nigeria than in Western countries. Quite at variance with the findings in Europe and the US, breast cancer in Nigeria does not increase with age after menopause [22].

In our study, the age range of the patients with breast lumps was found to be 11-90 years with a mean age of 30 years and about 66.70%, 28.20% and 5.10% of the female cancer cases were detected at age ranges 30-50years, 51-90years and 11-29years respectively. These results indicate that the risk for breast cancer increases with an increase in age, which slows at approaching menopause, and are supported by the literature.
Of the malignant cases, invasive carcinoma (NST) and intraductal carcinoma were found to be the most common types of breast cancer, with the highest occurrence (55 cases altogether of the total 84 malignant cases found), and these lesions, according to literature, are also the major types of breast cancer as cited[25]. The three most common histopathological types collectively represent approximately three-quarters of breast cancers: Invasive ductal carcinoma, 55% of breast cancers; Ductal carcinoma in situ, 13%; and Invasive lobular carcinoma, 5% [34]. While Invasive lobular carcinoma, Mataplastic Carcinoma and Metastatic Carcinoma are the second most common types of breast cancer found, representing 14 cases altogether of the total 84 cases found, in which there was no case found to occur below the age of 30 years, 13 cases at the age range 30-50years and only1 case found at the age range 51-60 years. However, according to literature, the range of breast carcinoma generally is 31-83 years [35, 36]. So it might be the explanation for which no case was found below the age of 30 years. Secretory Carcinoma, Sebaceous Carcinoma, Apocrine Carcinoma, Papillary Carcinoma, Mucinous Carcinoma, Medullary Carcinoma, Malignant phyllodes tumor, Intermediate grade ductal carcinoma in Situ, Cystic tissue carcinoma, Metastatic carcinoma and Invasive Apocrine Carcinoma were the third group with least occurrence in which altogether represent 11 cases of all the breast cancer found (3 cases below 30 years, 4 cases at age range 30-50 years and 4 cases above 50 years). These results indicate that breast cancer was more common among premenopausal older patients, carcinoma of breast was common in the 30-50 year age group, and there was also a sharp rise in the number of carcinomas up to 50 years of age, and all are supported by the literature as cited. African women with breast cancer are more likely to be pre-menopausal, with the incidence peaking between 35-40years. This is almost 10-15years earlier than the peak incidence for Western countries [32, 33].

Furthermore, the occurrence of some of the breast cancer types at a younger age (below 30years) is also in line with the literature for the age range as cited. The reported age range for apocrine carcinoma is from 19 to 86 years [37]. Many of the histologic patterns of breast carcinoma that occur in adults have also been reported in patients younger than 20 years of age [38].

Furthermore, of the benign cases, fibroadenoma, fibrocystic changes and inflammatory lesions were

found to be the most common benign breast lesions, which is in line with the literature. In the current study, these lesions represent 202 cases of all identified benign conditions. While Benign phlyllodes tumour, Gynecomastia, Lactational Adenoma, Tubular adenoma, and Sclerosing adenosis are the second most common benign lesions found, representing 40 cases (35 cases at 11-29years, 5 cases at 30-50years and no case found at 51-60 years). Reactive lymphoid hyperplasia, Reactive hyperplasia, Plexiform Neurofibroma, Lipoma, Blunt duct adenosis, Adenomyoepithelial adenosis, Lactational Adenoma with Fibrocyctic changes, Fibrocyctic changes with Fibroadenomatoid hyperplasia, Fibrocystic changes with Fibroadenoma, Fibrocyctic changes with chronic inflammation, Fibrocystic changes with adenosis, Fibroadenoma with Lactational changes, Fibroadenoma with Fibrocystic changes and adenohyperplasia, Fibroadenoma with Fibrocystic changes constitute the third group with the least occurrence representing 20 cases altogether of all the identified benign breast lesions (12 cases at 11-29years, 8 cases at 30-50years and no case found at 51-90years). These results indicate that the risk of benign breast lump is more common among younger patients, which is in line with the literature as widely advocated by numerous studies. Many breast lumps are benign, especially in younger patients [39].

Conclusion

The results of this study show that age has no influence on the lesion site for the occurrence of breast lumps. Age and side variations among the samples of the breast lumps were observed. Breast cancer has an equal chance to occur on either side of the body (left/right), and benign breast lesions occur more on the right side than the left side. In contrast, the variation was not observed to occur among their age groups. Similarly, the results revealed an association between the patients' ages and breast lesions. The prevalence of breast cancer among the samples of breast tumours is high. However, heightened awareness among women and enlightenment drives may have contributed to the increase in the frequency of presentation and diagnosis.

Recommendation

Self-breast exam; although BSE was the most commonly practised early detection technique in most of the studies in this review, evidence for its effectiveness is disappointing. A Cochrane Review of two large RCTs (in Shanghai and in Russia) found no beneficial effect from BSE screening compared with no intervention, and there were twice as many biopsies of benign lesions in the BSE group.

Acknowledgements: We really appreciate the staff of the Histopathology department of Aminu Kano Teaching Hospital for permitting us to use their records in conducting this study.

References

1. Lakhani SR , Ellis IO, Schnitt SJ, Tan PH, Van de Vijver MJ, eds. WHO Classification of Tumours of the Breast. Fourth ed. IARC, Lyon. 2012. ISBN.13.
2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics. CA Cancer J Clin. 2022; 72(1):7-33. doi: 10.3322/caac.21708. Epub 2022. PMID: 35020204.
3. Łukasiewicz S, Czeczelewski M, Forma A, Baj J, Sitarz R, Stanisławek A. Breast Cancer-Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies-An Updated Review. *Cancers* (Basel). 2021; 13(17):4287. doi: 10.3390/cancers13174287. PMID: 34503097; PMCID: PMC8428369.
4. Ferlay J, Héry C, Autier P, Sankaranarayanan R. Global burden of breast cancer. Breast Cancer Epidemiology: Springer; 2010. pp. 1-19.
5. Eheazu CL, Uzoagu IF. The place of environmental literacy education in cancer prevention among rural Nigerian communities. *International Journal of Cancer, Clinical Inventions and Experimental Oncology*. 2021. 3(1):1-23. doi:10.37745/ijcceo.15.
6. Momenimovahed Z, Salehiniya H. Epidemiological characteristics of and risk factors for breast cancer in the world. *Breast Cancer*. 2019; 11:151-164. doi: 10.2147/BCTT.S176070. PMID: 31040712; PMCID: PMC6462164.

7. Mandong BM, Madaki AKJ, Mannaseh AN. Malignant diseases in Jos: A follow-up. *Annals of African Medicine*. 2003; 2(2):49–53.
8. Yusuf, Atanda AT, Umar AB, Imam MI, Mohammed A, Ochicha O, et al. Cancer in Kano, Northwestern Nigeria: A 10-year update of the Kano cancer registry. *Annals of Tropical Pathology*. 2017; 8(2):87.
9. Jedy-Agba E, McCormack V, Adebamowo C, Dos-Santos-Silva I. Stage at diagnosis of breast cancer in sub-Saharan Africa: a systematic review and meta-analysis. *Lancet Global Health*. 2016; 4(12):e923-e935. doi: 10.1016/S2214-109X(16)30259-5. PMID: 27855871; PMCID: PMC5708541.
10. Sadik ABMZ, Hasan MM, Haque HEZA, Ahmed FU, Kabir MZ. Different Types of Breast Lump in Relation to Different Age Groups. *Faridpur Medical College Journal*. 2013; 8(2):56–58.
11. Adesunkanmi AR, Lawal OO, Adelusola KA, Durosimi MA. The severity, outcome and challenges of breast cancer in Nigeria. *Breast*. 2006; 15(3):399-409. doi: 10.1016/j.breast.2005.06.008. Epub 2005 Aug 8. PMID: 16085418.
12. Vorobiof DA, Sitas F, Vorobiof G. Breast cancer incidence in South Africa. *Journal of Clinical Oncology*. 2001; 19(18 Suppl):125S-127S. PMID: 11560987.
13. Raftery AT, Lim E, Andrew JK. Breast lumps. In: Churchill's Pocketbook of Differential Diagnosis. 4th ed. Philadelphia, Pa: Churchill Livingston Elsevier, 2014. pp. 21-28.
14. Amer MH. Genetic factors and breast cancer laterality. *Cancer Management Research*. 2014; 6:191-203. doi: 10.2147/CMAR.S60006. PMID: 24790468; PMCID: PMC3998851.
15. Cheng SA, Liang LZ, Liang QL, Huang ZY, Peng XX, Hong XC, Luo XB, Yuan GL, Zhang HJ, Jiang L. Breast cancer laterality and molecular subtype likely share a common risk factor. *Cancer Management Research*. 2018; 10:6549-6554. doi: 10.2147/CMAR.S182254. PMID: 30568509; PMCID: PMC6278699.
16. Lawan A, Eme O. Refractive errors in Aminu Kano Teaching Hospital, Kano, Nigeria. *Nigerian Postgraduate Medical Journal*. 2011; 18(4):276-8. PMID: 22193998.
17. Roychoudhuri R, Putcha V, Møller H. Cancer and laterality: a study of the five major paired organs (UK). *Cancer Causes Control*. 2006; 17(5):655-62. doi: 10.1007/s10552-005-0615-9. PMID: 16633912.
18. Fritschi L, Divitini M, Talbot-Smith A, Knuiman M. Left-handedness and risk of breast cancer. *British Journal of Cancer*. 2007; 97(5):686-7. doi: 10.1038/sj.bjc.6603920. Epub 2007 Aug 7. PMID: 17687338; PMCID: PMC2360366.
19. Titus EL, Newcomb PA, Egan KM, Baron JA, Greenberg ER, Trichopoulos D, Willett WC, Stampfer MJ. Left-handedness in relation to breast cancer risk in postmenopausal women. *Epidemiology*. 2000; 11(2):181-4.doi:10.1097/00001648200003000017. PMID:11021617.
20. Trichopoulos D, Lipman RD. Mammary gland mass and breast cancer risk. *Epidemiology*. 1992; 3(6):523-6. doi: 10.1097/00001648-199211000-00011. PMID: 1420519.
21. Hsieh CC, Trichopoulos D. Breast size, handedness and breast cancer risk. *European Journal of Cancer*. 1991; 27(2):131-5. doi: 10.1016/0277-5379(91)90469-t. PMID: 1827274.
22. Kim BK, Choi JE, Youn HJ, Park HS, Kim D, Oh SJ et al. Korean Breast Cancer Society. Clinicopathological features and prognosis associated with breast cancer laterality: a nationwide study from the Korean Breast Cancer Society. *Annals of Surgical Treatment Research.* 2022; 103(3):119-128. doi: 10.4174/astr.2022.103.3.119. Epub 2022 Sep 6. PMID: 36128032; PMCID: PMC9478426.
23. Senie RT, Rosen PP, Lesser ML, Snyder

RE, Schottenfeld D, Duthie K. Epidemiology of breast carcinoma II: factors related to the predominance of left-sided disease. *Cancer*. 1980; 46(7):1705-13. doi: 10.1002/1097-0142(19801001)46:7<1705::aid-cncr2820460734>3.0.co;2-q. PMID: 7417963.

24. Kramer MA, Albrecht S, Miller RA. Higher frequency of left-breast cancer: a possible explanation. *Perception and Motor Skills*. 1985; 61(2):583-8. doi: 10.2466/pms.1985.61.2.583. PMID: 4069924.
25. Abdalkarem R, Abdallah H, Abdalraziq A, Ali M, Allafi A, Ali A. Retrospective Histopathological Study of Breast Biopsies of Breast Lesions Among Patients Attending Al-Bayda Medical Centre. *Alq Journal of Medical and Applied Sciences*. 2023; 6(1):212-218. https://doi.org/10.5281/zenodo.7876462.
26. Reeder JG, Vogel VG. Breast cancer prevention. *Cancer Treatment Research*. 2008; 141:149-64. doi: 10.1007/978-0-387-73161-2_10. PMID: 18274088.
27. Memon MA, Donohue JH. Male breast cancer. *British Journal of Surgery*. 1997; 84(4):433-5. doi: 10.1002/bjs.1800840402. PMID: 9112887.
28. Miao H, Verkooijen HM, Chia KS, Bouchardy C, Pukkala E, Larønningen S, Mellemkjær L, Czene K, Hartman M. Incidence and outcome of male breast cancer: an international population-based study. *Journal of Clinical Oncology*. 2011; 29(33):4381-6. doi: 10.1200/JCO.2011.36.8902. Epub 2011 Oct 3. PMID: 21969512.
29. Oguntunde PE, Adejumo AO, Okagbue HI. Breast cancer patients in Nigeria: Data exploration approach. *Data Brief*. 2017; 15:47-57. doi: 10.1016/j.dib.2017.08.038. PMID: 28971122; PMCID: PMC5612794.
30. Hotko YS. Male breast cancer: clinical presentation, diagnosis, treatment. *Experimental Oncology*. 2013; 35(4):303-10. PMID: 24382442.
31. Henderson BE, Ross R, Bernstein L. Estrogens as a cause of human cancer: the Richard and Hinda Rosenthal Foundation award lecture. *Cancer Research*. 1988; 48(2):246-53. PMID: 2825969.
32. Fregene A, Newman LA. Breast cancer in sub-Saharan Africa: how does it relate to breast cancer in African-American women? *Cancer*. 2005; 103(8):1540-50. doi: 10.1002/cncr.20978. PMID: 15768434.
33. Abimiku BA & Mukhtar AU. The pattern of cancer in federal capital territory of Nigeria. *Sahel Journal of Medicine*. 2002; 5(3), 152-155.
34. Eheman CR, Shaw KM, Ryerson AB, Miller JW, Ajani UA, White MC. The changing incidence of in situ and invasive ductal and lobular breast carcinomas: United States, 1999-2004. *Cancer Epidemiology Biomarkers Preview*. 2009; 18(6):1763-9. doi: 10.1158/1055-9965.EPI-08-1082. Epub 2009 May 19. PMID: 19454615.
35. Bauer TW, Rostock RA, Eggleston JC, Baral E. Spindle cell carcinoma of the breast: four cases and review of the literature. *Human Pathology*. 1984; 15(2):147-52. doi: 10.1016/s0046-8177(84)80055-6. PMID: 6199282.
36. Shousha S, James AH, Fernandez MD, Bull TB. Squamous cell carcinoma of the breast. *Archives of Pathology Laboratory and Medicine*. 1984; 108(11):893-6. PMID: 6548366.
37. Gilles R, Lesnik A, Guinebretière JM, Tardivon A, Masselot J, Contesso G, Vanel D. Apocrine carcinoma: clinical and mammographic features. *Radiology*. 1994; 190(2):495-7. doi: 10.1148/radiology.190.2.8284405. PMID: 8284405.
38. Ashikari H, Jun MY, Farrow JH, Rosen PP, Johnston SF. Breast carcinoma in children and adolescents. *Clinical Bulletin*. 1977; 7(2):55-62. PMID: 198162.
39. Njeze GE. Breast lumps: a 21-year single-centre clinical and histological analysis. *Nigerian Journal of Surgery*. 2014; 20(1):38-41. doi: 10.4103/1117-6806.127111. PMID: 24665202; PMCID: PMC3953633.

Effect of Selenium Yeast Supplementation on Mechanical and Thermal Pain Threshold in Streptozotocin-Induced Diabetic Neuropathy in Wistar Rats

*H. S. Abubakar[1], A. Mohammed[2], Y. Tanko[2], A. S. Isa[2], Z. Muhammad[3]

[1]*Department of Physiology, Yusuf Maitama Sule University, Kano*
[2]*Department of Human Physiology Ahmadu Bello University, Zaria*
[3]*Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria*
Corresponding Author: H. S. Abubakar
Corresponding Email: hajab21@gmail.com

Abstract

Diabetic neuropathy is a long-term complication of diabetes which affects half of the diabetic population. There is no prophylactic therapy against painful diabetic neuropathy. Current approaches are restricted to alleviating established pain by using drugs that are effective in other pain conditions and hoping to find one with enough efficacy and minimal side effects to be useful. The study investigated the effect of selenium yeast supplementation, an antioxidant and anti- inflammatory compound, on some pain responses using Randal Selitto and tail flick tests in streptozotocin-induced diabetic neuropathy in Wistar rats. Thirty (30) adult Wistar rats with an average weight of 150 g were randomly divided into groups of five rats each (n=5). Diabetic neuropathy was induced by a single intraperitoneal injection of 60 mg/kg streptozotocin dissolved in 0.1ml citrate buffer (pH 4.5). Groups I, II and III received 1mg/kg distilled water and aspirin (300mg/kg) while groups IV and V received 0.2 mg/kg and 0.3 mg/kg selenium yeast, respectively. Development of neuropathy was assessed in control and diabetic animals from all groups by evaluation of blood glucose concentration and pain thresholds on the 0, 2nd, 4th and 6th weeks of respective treatment by assessment of thermal/mechanical hyperalgesia and allodynia. Results showed that 0.2mg/kg selenium yeast reduces hyperglycemia-induced thermal hyperalgesia (6.30±0.76 Vs 2.53±0.56) with an improvement in neuropathic pain in the diabetic rats. This suggests that selenium yeast (0.2mg/kg) has potential for the prevention of diabetic neuropathic pain.

Keywords: Diabetic Neuropathy, Neuropathic Pain, Selenium Yeast, Mechanical, Thermal, Nociception.

Introduction

Diabetes mellitus is a chronic metabolic disorder related to insulin deficiency and can involve many organs. Peripheral diabetic neuropathy (PDN), a significant microvascular complication of diabetes, is characterised by the prickling, tingling, burning, electric shock-like, freezing pain with allodynia and hyperalgesia in the legs, feet, and hands affecting 50% of diabetic patients[1]. Diabetic neuropathy is one of the most prevalent chronic complications that affects adults with type 1 or type 2 diabetes, while also affecting individuals with prediabetes and young people with diabetes[2]It is estimated that half of the diabetic patients develop neuropathy, and the prevalence of PDN ranges from 10 to 20 % in diabetic patients and from 40 to 50% in those with diabetic neuropathy.PDN, even though common and often severe, is frequently unreported (12.5 %) and more frequently untreated (39.3 %)[4]Patients with PDN experience reduced mobility, physical activity, and enjoyment of life, fatigue, limitations in social activity, diabetic foot infections, sleep impairment, anxiety, and depression[5]

Diabetic neuropathy is a common complication of both type 1 diabetes and type 2 diabetes. Nerve damage or diabetic neuropathy resulting from chronically high blood glucose can be one of the most frustrating and debilitating complications of diabetes, because of the pain, discomfort and disability it can cause, and because available treatments are not uniformly successful.

Neuropathy associated with type 2 diabetes may be present at the time of diagnosing diabetes.

Neuropathy associated with type 1 diabetes usually develops more than 10 years after the diagnosis of diabetes. Diabetes is the most common cause of peripheral neuropathy in the world[645esrdfxcsv bn]

Current treatment of peripheral diabetic neuropathy (PDN) involves the use of tricyclic antidepressants, selective serotonin reuptake inhibitors[7]anticonvulsants, opioids and antioxidant protein kinase C inhibitors, COX-2 inhibitors[8]non

steroidal anti-inflammatory drugs as mild analgesics. However, these therapies provide relief only to a fraction of patients, and their side effect profiles limit their use. Thus, there is a need for new therapeutic interventions targeting primary mechanisms resulting in nerve damage in PDN. The study aims to investigate the ameliorative effect of Selenium yeast supplementation in diabetic neuropathic pain by assessing mechanical pain responses, using the Randall-Selitto and thermal pain responses using the tail flick test.

Materials and Methods

A total of twenty-five (25) apparently healthy rats of both sexes, weighing 100-150g, were used for this study. The animals were purchased from the Animal house in the Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria. The animals were fed with pelletized grower's mash and water *ad libitum*. All procedures, including the euthanasia procedure, were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research (NIH Publications No. 80-23; 1996).

Animal Groupings

The animals were divided into five groups of five animals each and treated as follows:

Group 1- Control group: 2ml/kg distilled water orally

Group 2- Diabetic Neuropathic group (STZ)

Group 3- Diabetic Neuropathic group + Aspirin orally.

Group 4- Diabetic Neuropathic group + 0.2 mg/kg Selenium yeast orally.

Group 5- Diabetic Neuropathic group + 0.3 mg/kg Selenium yeast orally.

Experimental Induction of diabetes

Diabetes was induced in 20 rats by single intraperitoneal injection of Streptozotocin (STZ) at a dose of 60 mg/kg body weight dissolved in citrate buffer (pH 4.5, 0.1 M)[10]. Seventy-two hours after the injection, diabetes was confirmed by collecting blood samples through the tail vein. Plasma glucose levels were estimated with a commercial blood glucose analyser (Accusoft, Roche Diagnostics, Laval, QC, Canada). Rats with fasting plasma glucose levels higher than 200 mg/dL were deemed to be diabetic and included in the study[11]. Body weight and plasma glucose levels were recorded once weekly for the duration of the study.

Vehicle (distilled water) and drug treatment were started six weeks after the diabetes induction and continued for two consecutive weeks. Pain assessments were undertaken before and after treatments for the six-week duration of the experiment.

Assessment of Pain Responses

Development of neuropathy was assessed in control and diabetic animals from all groups by evaluation of pain thresholds on the 0, 2nd, 4th and 6th weeks of respective treatment by assessment of thermal/mechanical hyperalgesia and thermal allodynia[11]

Randall and Selitto method

Mechanical hyperalgesia was assessed by the method of Randal and Selitto (1957). Paw pressure thresholds were recorded with the paw pressure analgesia meter (MK-20D Analgesy meter). Pressure increasing at a linear rate of 10 g/s, with a cut-off at 250 g to avoid tissue injury, was applied to the centre of the hindpaw. The animals display pain by withdrawal of the paw, and the applied paw pressure was recorded by an analgesia meter and expressed in grams. Three tests separated by at least 10 min were performed for each rat, and mean values recorded[12].

In all experimental sessions, obtained thresholds were compared to the baseline. Changes in pain threshold were calculated as a percentage of the baseline value according to the following formula:
Hyperalgesia (%)= (A/B × 100%) - 100%
Where, A - Pressure (in g), baseline pain threshold;
B - Pressure (in g) in consecutive measurements.

Tail flick test

The tail-flick test was performed according to the method described by Ugwah-Oguejiofor *et al.* (2013). The terminal (2cm) of each rat's tail was immersed in hot water contained in 500ml beaker and maintained at 51 ±1°C using a thermoregulated hot bath, and the time between the onset of stimulation and tail withdrawal was measured as the tail-flick latency. The reaction time was recorded at 30 and 60 min[14] Cut-off time was 20s for tail flick measurements in order to minimise tissue injury[15]. For each animal, 2 to 3 recordings were made at an interval of 15 min, and the mean value was used for statistical analysis.

Blood Glucose Level Determination

Blood glucose level determination was done by the collection of blood samples from the rat's tail vein every two weeks for a period of six weeks. Blood glucose level was determined by the glucose-oxidase principle of Beach and Turner (1958), using a commercial blood glucose analyser (Accusoft, Roche Diagnostics, Laval, QC, Canada), and the results were recorded in mg/dl.

Results

Blood Glucose Levels

Blood glucose levels of the different doses of selenium yeast administered on STZ-induced diabetic neuropathic Wistar rats are shown in Figure. 1 below. Plasma glucose levels in the diabetic neuropathic groups were significantly ($p \leq 0.05$) increased when compared with the normal control group from the 2nd week up to the 6th week of the experiment. But a significant decrease in the glucose levels was seen from the 4th week of the treatment with selenium yeastSelenium yeast group (0.2mg) was seen to have a more significant effect than the 0.3mg selenium yeast and aspirin groups, with between-group analyses.

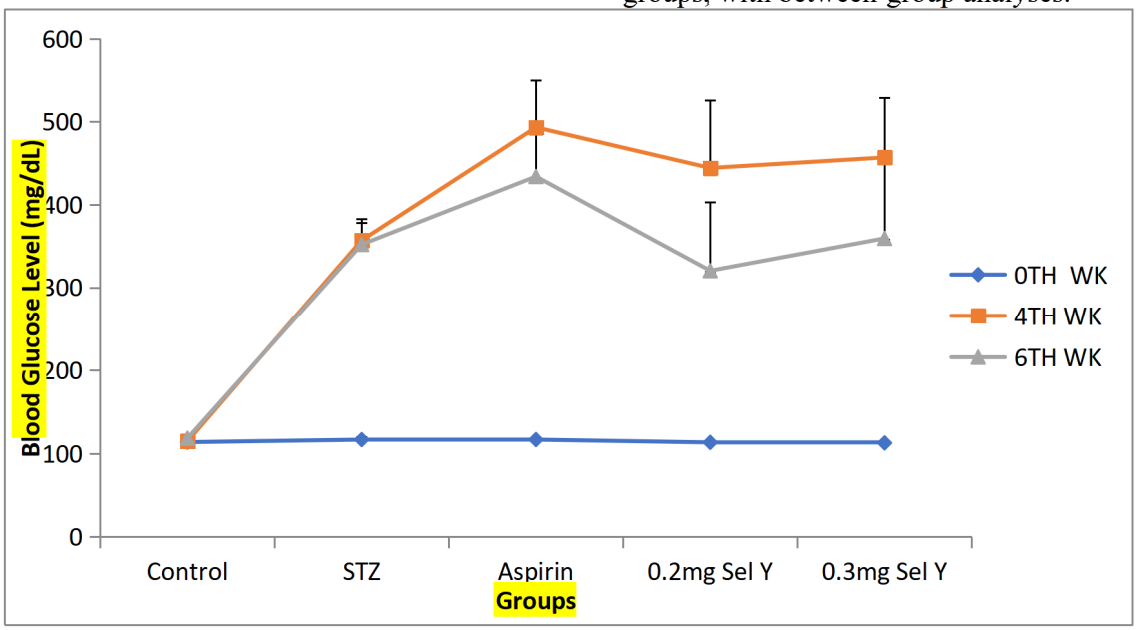


Figure 1: *Blood Glucose Levels, STZ -Streptozotocin, Sel Y- Selenium Yeast*

Pain Assessment

Mechanical Hyperalgesia

In the Paw withdrawal analgesia test, a significant ($p \leq 0.05$) decrease in the paw withdrawal was seen from the 4th week of the experiment in the STZ-induced diabetic neuropathic groups when

compared to the two control groups (Table 1). There was a significant increase in the group treated with 0.2mg selenium yeast by the 6th week of the experiment, that is 2 weeks after treatment with selenium yeast and aspirin, in the diabetic neuropathic groups, but no significant changes in the group treated with 0.3mg selenium yeast and aspirin groups, though a slight increase was observed when compared with the diabetic control group

Table 1: Changes in Paw Withdrawal

GROUP	0 WEEK	2 WEEK	4 WEEK	6 WEEK
CONTROL	8.13 ± 1.2s	6.93 ± 1.59s	8.60 ± 2.06s	7.80 ± 2.21s
STZ	4.7 ± 0.87s	3.90 ± 0.26s*	3.50 ± 0.21s*	2.87 ± 0.20s*
ASPIRIN	4.45 ± 0.95s	3.70 ± 0.50s*	2.95 ± 0.85s*	3.25 ± 0.45s*
0.2mg Sel Y	5.02 ± 0.62s	3.80 ± 0.84s*	2.40 ± 0.42s*	2.95 ± 0.49s
0.3mg Sel Y	6.22 ± 2.37s	5.50 ± 0.95s*	3.33 ± 1.07s*	3.38 ± 1.17s*

Table 1: Results presented as mean ± SEM using one-way ANOVA. ∗= statistically significant ($p \leq 0.05$) between control and other groups. STZ Streptozotocin, Sel Y- Selenium Yeast

Tail flick

The tail flick latency was also significantly ($p \leq 0.05$) decreased in the diabetic neuropathic groups when compared to the control group from the 2nd week up to the 4th week before treatment. Two weeks after treatment, there was a significant ($p \leq 0.05$) increase in the tail flick latency in all groups with the exception of the diabetic control group. In between group comparisons, 0.2mg selenium yeast was more effective than the 0.3mg Selenium yeast.

Table 2: Changes in Tail Flick

GROUP	0 WEEK	2 WEEK	4 WEEK	6 WEEK
CONTROL	6.10 ± 0.65s	6.46 ± 0.81s	6.30 ± 0.76s	6. 33 ± 0.64s
STZ	4.60 ± 0.83s	3.70 ± 0.26s*	2.67 ± 0.37s*	2.23 ± 0.30s*
ASPIRIN	4.40 ± 1.05s	3.73 ± 0.29s*	2.53 ± 0.56s*	2.97 ± 0.22s*
0.2mg Sel Y	5.37 ± 0.73s	3.07 ± 0.59s*	2.53 ± 0.56s	3.37 ± 0.37s
0.3mg Sel Y	2.60 ± 0.35s	3.67 ± 0.44s*	1.83 ± 0.88s	2.33 ± 0.69s

Table 2: Results presented as mean ± SEM using one-way ANOVA. ∗= statistically significant ($p \leq 0.05$) between control and other groups. STZ Streptozotocin, Sel Y- Selenium Yeast

Discussion

Diabetic neuropathy is a debilitating consequence of diabetes that may be present in as many as one in five patients with diabetes[17]The objective assessment of diabetic neuropathy is difficult, making it challenging to diagnose and assess in both clinical practice and clinical trials. No single treatment exists to prevent or reverse neuropathic changes or to provide total pain relief.

Selenium is one of the most interesting of all nutrients. It was first known for its toxicity and was then discovered to be an essential nutrient[18]. The power of its antioxidant activity has been widely acclaimed. The importance of selenium in immune response and as an anticarcinogen has recently received a great amount of attention. In fact, selenium has been claimed to have a protective role in at least 50 diseases of humans[18.]

Streptozotocin (STZ) -treated rats are the preclinical efficacy model of Diabetic Neuropathy. STZ is a cytotoxic agent derived from the gram-positive *Streptomyces acromogenes* bacterium that selectively kills pancreatic β- cells. Administering a single, low-dose STZ injection to the healthy adult rats resulted in their rapid development of a

condition much like type 1 diabetes in humans, where lack of functional β-cells in pancreatic islets impairs normal glycemic control. The treated animals became hyperglycemic within 72 hours of injection and developed significant neuropathic pain symptoms after approximately 4 weeks[19].

The increase in serum glucose concentration of STZ treated group was in agreement with Akbarzadeh et al. (2007), who reported high blood sugar three times in diabetic animals as compared to normal. They also showed a high blood sugar level, insulin deficiency blood, polyphagia, polyuria and polydipsia with weight loss in adult rats three days after streptozotocin treatment due to irreversible destruction of pancreatic islet cells.

Hyperglycemic nerve ending damage occurs through either the inflammatory process or interference with the blood supply (ischemia of the microvessels). In addition to the mechanisms related to the hyperglycemic state, studies also suggest a mechanism of neuronal damage that occurs following STZ that is unrelated to the hyperglycemic state.

From the results obtained from this study, blood plasma levels were significantly higher in all the groups that were treated with STZ, when compared to the control group, at the 2nd week after the treatment with STZ. This, therefore, indicates the potency of the STZ used for this study. The diabetogenic action of STZ was accompanied by the development of persistent hyperalgesia. There was a considerable lowering of paw withdrawal threshold to mechanical stimuli, which started manifesting around the 4th week of treatment with STZ, which became more pronounced as the days increased; hyperalgesia remained on similar level. These results are similar to those reported by Aley and Levine (2002), who demonstrated the appearance of hyperalgesia in response to mechanical stimulus after administration of a single intravenous dose (60 mg/kg) of STZ.

Results obtained from this study indicated that blood glucose level in the diabetic control group was significantly higher when compared with the normal control group in the 6 weeks of the study. The developed hyperglycemia has been attributed to the specific toxic effects of STZ uptake through glucose transport-2 (GLUT-2); these effects lead to an end to toxic damage by activating the aldose-reductase pathway that leads to the toxic accumulation of sorbitol in the nervous system[21].

The effect of selenium yeast on the blood glucose level was shown when the values obtained from the diabetic control group were compared with those of the 0.2mg selenium yeast and 0.3mg sel yeast. From the results, there was also a significant change in the blood glucose level when the aspirin group was compared to the diabetic control group.

When all the groups treated with selenium yeast and aspirin were compared, 0.2mg sel yeast seems to be more potent than the other two groups, from the 4th to the 6th week of the study.

There was also a slight improvement that is significant from the 4th week to the 6th week, when the glucose level reduced in the groups treated with selenium yeast, when compared with the diabetic control group. The 0.2mg sel yeast seemed to have more effect when between-group comparisons were done.

In this study, in the STZ-treated groups, the tail flick latency was significantly shorter than that observed in the normal control group, indicating the development of thermal hyperalgesia. The present results are in good agreement with the literature, which frequently reports on hypersensitivity to mechanical stimulation[23].

Results obtained from the present study showed that there was a decrease in paw withdrawal threshold that was observed from the 4th week of treatment with Selenium yeast as compared with the diabetic control group. A decrease in the pressure withdrawal threshold after 3 weeks of STZ-diabetes is reported by Romanovsky *et al.* (2004). Increased nociceptor activity and sensitivity during hyperglycemic hypoxia could be a mechanism, e.g. burning pain attacks in painful neuropathy[25].

The treatment of the diabetic rats for 2 consecutive weeks showed significant improvement in tail flick

latency rate as compared to untreated diabetic animals in this study. This may be due to the anti-inflammatory effect of selenium yeast.

Conclusion

- Results obtained from this study showed that selenium yeast reduces hyperglycemia-induced thermal and mechanical hyperalgesia, with an improvement in neuropathic pain.
- The results also suggest that selenium yeast treatment might be beneficial in chronic diabetics exhibiting neuropathy.

Reference

1. Galiero R, Caturano A, Vetrano E, Beccia D, Brin C, Alfano M, et al. Peripheral neuropathy in diabetes mellitus: pathogenetic mechanisms and diagnostic options. Int J Mol Sci. 2023 Feb 10;24(4):3554.
2. Pop-Busui R, Ang L, Boulton AJM, Feldman EL, Marcus RL, Mizokami-Stout K, et al. Diagnosis and treatment of painful diabetic peripheral neuropathy. ADA Clin Compend. 2022;1(1):1–32.
3. Veves A, Backonja M, Malik RA. Painful diabetic neuropathy: epidemiology, natural history, early diagnosis, and treatment options. Pain Med. 2008;9(6):660-74.
4. Daousi C, MacFarlane IA, Woodward A, Nurmikko TJ, Bundred PE, Benbow SJ. Chronic painful peripheral neuropathy in an urban community: a controlled comparison of people with and without diabetes. Diabet Med. 2004;21(9):976-82.
5. van Laake-Geelen CCM. Living with painful diabetic neuropathy: physical activity and quality of life. *Nederlands tijdschrift voor pijn en pijnbestrijding.* 2022;41(83
6. National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Diabetic neuropathies: the nerve damage of diabetes. Bethesda, MD: National Diabetes Information Clearinghouse; 2009. NIH Publication No.: 09-3185.
7. McKeage K. Treatment options for the management of diabetic painful neuropathy: best current evidence. Curr Opin Neurol. 2007;20(5):553-7.
8. Kellogg AP, Cheng HT, Pop-Busui R. Cyclooxygenase-2 pathway as a potential therapeutic target in diabetic peripheral neuropathy. Curr Drug Targets. 2008;9(1):68-76.
9. O'Connor AB. Neuropathic pain: quality-of-life impact, costs and cost effectiveness of therapy. Pharmacoeconomics. 2009;27(2):95-112.
10. Wang S, Li Y, Zhao J, Zhang J, Huang Y. Mesenchymal stem cells ameliorate podocyte injury and proteinuria in a type 1 diabetic nephropathy rat model. Biol Cell. 2013;105(12):538-46.
11. Randall LO, Selitto JJ. A method for measurement of analgesic activity on inflamed tissue. Arch Int Pharmacodyn Ther. 1957;111(3):231-42.
12. Al-Enazi MM. Ameliorative potential of rutin on streptozotocin induced neuropathic pain in rats. Afr J Pharm Pharmacol. 2013;7(41):2743-54.
13. Ugwah-Oguejiofor CJ, Abubakar K, Ugwah MO, Njan AA. Evaluation of the antinociceptive and anti-inflammatory effects of Caralluma dalzielii. J Ethnopharmacol. 2013;150(3):967-72.
14. Cha DS, Eun JS, Jeon H. Anti-inflammatory and antinociceptive properties of the leaves of Eriobotrya japonica. J Ethnopharmacol. 2011;134(2):305-12.
15. Sánchez-Mateo CC, Bonkanka CX, Hernández-Pérez M, Rabanal RM. Evaluation of the analgesic and topical anti-inflammatory effects of Hypericum reflexum L. fil. J Ethnopharmacol. 2006;107(1):1-6.
16. Akbarzadeh A, Norouzian D, Mehrabi MR, Jamshidi S, Farhangi A. Induction of diabetes by streptozotocin in rats. Indian J Clin Biochem. 2007;22(2):60-4.
17. Javed S, Petropoulos IN, Alam U, Malik RA. Treatment of painful diabetic neuropathy.

The Advanced Chronic Diseases. 2015;6(1):15-28.
18. Nazıroğlu M, Öz A, Yıldızhan K. Selenium and Neurological Diseases: Focus on Peripheral Pain and TRP Channels. *Current Neuropharmacology*. 2020;18(6):501-517.
19. Aley KO, Levine JD. Different peripheral mechanisms mediate enhanced nociception in metabolic/toxic and traumatic painful peripheral neuropathies in the rat. Neuroscience. 2002;111(2):389-97.
20. Green DA, Lathimer SA, Sima AA. Sorbitol, phosphoinositides and sodium-potassium-ATPase in the pathogenesis of diabetic complications. N Engl J Med. 1987;316(10):599-606.
19. Yilmaz HR, Uz E, Yucel N, Altuntas I, Ozcelik N. Protective effect of caffeic acid phenethyl ester (CAPE) on lipid peroxidation and antioxidant enzymes in diabetic rat liver. J Biochem Mol Toxicol. 2004;18(4):234-8.
20. Romanovsky D, Hastings SL, Stimers JR, Dobretsov M. Relevance of hyperglycemia to early mechanical hyperalgesia in streptozotocin-induced diabetes. J Peripher Nerv Syst. 2004;9(2):62-9.
21. Fuchs D, Birklein F, Reeh PW, Sauer SK. Sensitised peripheral nociception in experimental diabetes of the rat. Pain. 2010;151(2):496-505.

A Comparative Study of the Effects of Aqueous Extracts of *Tamarindus Indica* Fruit Pulp and *Zingiber Officinale* Rhizomes on CCL_4 –Induced Oxidative Stress in Rats

Sadi, N.S. *[1], Ibrahim, A.[1], Umar, A.M.[2], & Kurfi, B.G. [1]

[1]Department of Biochemistry, Faculty of Basic Medical Science, Bayero University, Kano
[2]Yusuf Maitama Sule Federal University of Education

Corresponding Author: Nasir Sirajo Sadi
Corresponding Email:dr.nas2020@gmail.com

Abstract

The tamarind tree is a multipurpose tree of which almost every part finds at least some use, either nutritional or medicinal. Due to its pleasant acidic taste and rich aroma, the pulp is widely used for domestic and industrial purposes. Ginger is known to ease oxidative stress due to stimulation of superoxide dismutase, catalase, glutathione peroxidase and reduced glutathione activities. They are believed to protect against cancer, atherosclerosis, heart diseases and several other diseases. A comparative study was carried out to evaluate the effect of Tamarind and Ginger extracts intake in CCl_4 induced oxidative stress in Wistar albino rats. The Proximate, antinutrient, and phytochemicals of both ginger and tamarind extracts were analysed using standard AOAC methods, while mineral contents were determined using atomic absorption spectrometry. Oxidative stress markers were also analysed using a colourimetric assay kit. Serum oxidative stress markers were compared between the normal and test groups. Experimental rats were divided into seven groups: Normal control group, negative control (CCl_4) group, standard drug (Vitamin C) group, tamarind low and high dose group, and ginger low and high dose group. At the end of the experiment, significant increase in malondialdehyde level (18.52 ±1.69) and decrease in superoxide dismutase (2.77±0.69), catalase (3.840±4.36), reduced glutathione (14.06±3.03) and glutathione peroxidase (8.95±5.23) activities were recorded in CCl_4 exposed rats as compared to control group which had (10.57±4.36), (11.96±1.62), (12.20±4.23), (34.86±9.19) and (36.23±7.07) respectively. In the tamarind and ginger supplemented groups, the level of MDA, along with the activities of SOD, CAT, GSH and GPx were comparable with the apparently healthy control rats ($p>0.05$). Thus, it appears that ginger and tamarind extracts ameliorate the effect of CCl_4-induced oxidative stress, suggesting that consumption of natural compounds with an antioxidant profile may be a preventive measure against those diseases associated with oxidative stress.

Keywords: Tamarind, Ginger, Carbon tetrachloride, Oxidative stress, antioxidant

Introduction

Tamarind or *Tamarindus indica* L. of the Fabaceae, subfamily Caesalpinioideae, is an important food in the tropics. It is a multipurpose tree of which almost every part finds at least some use (Kumar and Bhattacharya, 2008), either nutritional or medicinal. Tamarind is scientifically known as *Tamarindus indica,* and widely known as "*Tsamiya*" in Hausa. *Tamarindus indica* (*T. indica*) is an evergreen tree that can reach 24 m high and 7 m in girth, that has pale yellow and pink flowers (Momodu &Idemudia 2023). It thrives in arid climates, and its distribution in Africa extends from Senegal in the west to Sudan and Ethiopia in the east, and as far south as Mozambique and Madagascar (Havinga et al., 2010).

Every part of the *Tamarindus indica* plant (root, body, fruit, and leaves) not only has rich nutritional value and a broad usage area in medicine, but also has industrial and economic importance. According to the World Health Organisation report, tamarind fruit is an ideal source of all essential amino acids except tryptophan (Momodu &Idemudia, 2023).

Mieanwhile Ginger (*Zingiber officinale Roscoe)* is one of the essential dietary products that have been used for the treatment of numerous ailments such as hypertension, migraines, arthritis, nausea and

Date submitted-21/08/2022 Date Accepted-27/11/2022

colds. Recently, interest has increased in ginger and its active components as therapeutic agents. Other than its anti-oxidative, anti-inflammatory, and anti-carcinogenic activities, it also has lifespan-extending property (Ippoushi *et al.,* 2003; Lee *et al.,* 2018). *Zingiber officinale Roscoe*, Zingiberaceae, is one of the regularly eaten spices and also used as traditional herbal medicine for thousands of years (Han *et al.,* 2013).

Oxidative stress has been implicated in the natural ageing process as well as a variety of disease states: Neoplastic- Hematological and Solid Tumor, Metabolic- Obesity and Diabetes, and Neurological- Alzheimer's and Parkinson's diseases. The present study aimed at evaluating the effects of aqueous extracts of *Tamarindus indica* fruit pulp and *Zingiber officinale* rhizomes on some oxidative stress markers of CCl_4 induced rats.

Materials and Method

Chemicals

All chemicals and reagents used for the research were of analytical grade and purchased from reputable chemical manufacturers. The laboratory equipment used was also of standard quality.

Plant Materials

The plant materials (Ginger and tamarind) were obtained from a farm in Kabuga satellite town, Gwale L.G.A, Kano, Nigeria. They were authenticated and assigned voucher number -BUKHAN00074 at the herbarium of Botany Unit, Department of Biological Sciences, Faculty of Life Sciences, Bayero University Kano, Nigeria.

Ethical approval

All animal studies conducted were approved by the Animal Ethics Committee of the College of Health Science, Bayero University, Kano.

Preparation of Tamarind Extract

Fresh Tamarind Fruits were allowed to dry in the shade. The skins were peeled, and the pulp was separated from the seeds manually by scrubbing. The pulp was ground into powder. 100g of the powder was weighed and soaked in 500ml of distilled water for 24 hours. The extract was filtered using Whatman No.1 filter paper, and the residue was dried and reweighed. It was stored in an air-tight container until required.

Preparation of Ginger Extract

Ginger rhizomes were peeled and thoroughly washed in clean water. The cleaned ginger rhizomes were cut into small pieces by using a clean stainless steel knife and allowed to shade dry. The rhizomes were ground to powder. 100g of the powder was weighed and soaked in 500ml of distilled water for 24 hours. The extract was filtered using Whatman No.1 filter paper and kept in a small bottle, ready for administration. The concentration of extract was calculated as follows;
Concentration of Extract = Weight of Extract / Volume of distilled water Vitamin C, as a standard drug, was also prepared using the above relation and was administered using the relation in "**Extract Administration"**.

Extract Administration

The following formula was used in calculating the volume of extract to be administered.
Volume (ml) = Weight (kg) of rat × Dosage / Concentration of extract (mg/ml)

Experimental Animals

Wistar albino rats of either sex weighing between 150 and 200g were obtained from the Animal House, Department of Biological Sciences, Bayero University, Kano. They were maintained under standard conditions and were given standard feeds with water available. They were acclimatised for one week before commencement of the study.

Acute Toxicity Study

Acute toxicity studies were performed for both Ginger and Tamarind Extracts according to the toxicity classification method as per guidelines 423 prescribed by OECD (2001) using Wistar albino rats. Thirteen experimental animals were used for the test. In the investigation, three groups containing three rats each were administered 10, 100, and 1000mg/kg, respectively, of the aqueous extracts intraperitoneally. They were observed closely for 24 hours for lethality or any other behavioural response. Based on the result, further increased doses of 1500, 2000, 3000 and 5000 mg/kg were administered intraperitoneally to four

other rats, respectively. They were also observed for 24 hours for any death or behavioural changes.

Experimental Design

A total of forty two rats were randomly distributed into seven groups, with six per group. Carbon tetrachloride (150mg/kg) and extracts (1500 and 4000 mg/kg, respectively) were administered to the animals orally by gastric intubation. Group one received distilled water as a control. Group two received a single dose of CCl_4 in mineral oil (1:1 v/v). Group three in addition to CCl_4 received 48h later, a daily dose of the standard drug (Vitamin C) 250mg/kg, for four weeks. Groups four and five were administered with CCl_4 and treated with 1500 and 4000 mg/kg of Tamarind extract for four weeks. Groups six and seven were also administered with CCl_4 but treated with 1500 and 4000 mg/kg of Ginger extract for four weeks.

Sample Collection and Preparation

Rats from the various groups were sacrificed by decapitation 24h after the respective treatment period. The blood was collected into a plain container and allowed to stand for 30 minutes to clot before being centrifuged at 2000rpm for 10 minutes to separate the serum. Immediately, the serum was used to estimate the levels of oxidative stress markers (catalase, GSH, GPx, SOD) and MDA. After blood sampling, the liver of each animal was dissected out, and a part of the liver was fixed in 10% formalin for histopathological studies.

Preliminary Phytochemical Screening

Both extracts (Tamarind and ginger) were subjected to preliminary phytochemical tests to detect the presence or absence of plant phytochemical constituents such as alkaloids, saponins, tannins, flavonoids, carbohydrates, proteins, protein and amino acids. All screening procedures were carried out using the method of Tiwari *et al.* (2011).

Quantitative Determination of Some Phytochemicals

Total phenolic compound was determined according to Ganapaty *et al.* (2013). Total flavonoids, total alkaloids and glycosides were all determined according to Soladoye and Chukwuma (2012).

Anti-nutrients Determination

Phytic acid and oxalate were determined using the AOAC method (2005).

Proximate Analysis

The proximate compositions of the extracts were determined using conventional standard methods of analysis of the Association of Official Analytical Chemists, AOAC (1995).

Mineral Analysis

Ca, Mg, Fe, and Zn were determined using an Atomic Absorption Spectrophotometer (AA6300 Shimadzu Model, England). Flame photometer (Model 400, Corning U.K.) was used for K and Na determination, while phosphorus was determined by the vanodo-molybdate method using a spectrophotometer (Optima SP-300 model) at 660 nm according to the method described by AOAC (2005).

Estimation of Oxidative Stress Markers

Lipid peroxidation was determined by measuring the levels of malondialdehyde produced during lipid peroxidation according to the method described by Varshney and Kale (1990), catalase activity was determined according to the method of Claiborne (1985), SOD activity was determined by the method of Misra and Fridovich (1972), the method of Beutler *et al*. (1963) was used in estimating the level of reduced glutathione, while GPx activity was determined by the method of Albrecht Wendel (1981).

Statistical Analysis

All values were expressed as mean +/standard deviation (SD). To evaluate differences between the studied groups, statistical analysis was carried out using the one-way analysis of variance (ANOVA), with the least significant difference post hoc test to compare group mean and $P<0.05$ was considered statistically significant.

Results

The result for the qualitative phytochemical analysis of tamarind and Ginger extracts in Table 1 revealed the presence of alkaloids, carbohydrates,

glycosides, saponins, phenols, flavonoids, tannins, protein and amino acids.

The results for the concentrations of some phytochemicals, such as total flavonoids, total alkaloids, total phenols, glycosides and total saponins of tamarind and ginger extracts were presented in Table 2.

Table 3 shows the results of anti-nutrients and mineral contents of tamarind and ginger extracts, respectively. The mineral analysis of tamarind and ginger extracts indicated richness in Calcium, Magnesium, Potassium, Phosphorous and Manganese. The trace elements Copper and Zinc varied in content among the extracts within much narrower limits.

Table 4 shows the proximate analysis results of ginger and tamarind extracts, respectively. Tamarind and ginger extract both have low moisture content with high protein content, and both extracts contain appreciable amounts of carbohydrates.

The results of the effect of treatment with graded concentrations (4000 and 1500 mg/Kg) of ginger and tamarind extracts on some antioxidant indices (Catalase activity, Reduced Glutathione, superoxide dismutase, glutathione peroxidase and malondialdehyde levels) are presented in Table 5. Significant increase in malondialdehyde level (18.52 ±1.69) and decrease in superoxide dismutase (2.77±0.69), catalase (3.840±4.36), reduced glutathione (14.06±3.03) and glutathione peroxidase (8.95±5.23) activities were recorded in CCl_4 exposed rats as compared to the control group which had (10.57±4.36), (11.96±1.62), (12.20±4.23), (34.86±9.19) and (36.23±7.07) respectively. In the tamarind and ginger supplemented groups, the level of MDA, along with the activities of SOD, CAT, GSH and GPx, were comparable with the apparently healthy control rats ($p>0.05$). **Table 1:** Some Phytochemicals Detected in Tamarind and Ginger Extracts

Table 1: Some Phytochemicals Detected in Tamarind and Ginger Extracts

Phytochemicals	**Qualitative**	
	Tamarind Extract	**Ginger Extract**
Alkaloids	+	+
Carbohydrates	+	+
Glycosides	+	+
Saponins	+	+
Phenols	+	+
Tannins	+	+
Flavonoids	+	+
Protein and amino acids	+	+

Key: (+) = present (-) = absent

Table 2: Concentrations of Some Phytochemicals in Tamarind and Ginger Extracts

Phytochemicals	**Quantitative (%)**		X^2
	Tamarind Extract	**Ginger Extract**	
Phenols	13.39±0.25	20.11±0.47	0.052
Flavonoids	37.15±0.49	45.04±0.46	0.071
Alkaloids	38.18±0.54	52.07±0.45	0.032
Saponins	18.04±0.48	32.11±0.48	0.024
Glycosides	21.02±0.47	27.13±0.47	0.251

All values are means of triplet determinations ± standard deviation (SD)

Table 3: Anti-nutrient and Mineral Contents of Tamarind and Ginger Extracts (mg/100g)

Parameters	Tamarind Extract	Ginger Extract	X^2
Phytic acid	79.20±1.54	72.61±1.23	0.713
Oxalate	37.41±0.98	33.00±0.77	0.421
Calcium (Ca)	94.25±20.55	187.55±29.45	0.040
Magnesium (Mg)	201.84±6.59	195.20±4.52	0.072
Sodium (Na)	5.32±0.20	4.85±0.16	0.130
Potassium (K)	144.54±2.81	142.77±2.81	0.221
Iron (Fe)	3.56±0.77	3.23±1.24	0.172
Zinc (Zn)	2.98±0.59	3.76±0.50	0.052
Phosphorous (P)	5.68±0.15	5.24±0.10	0.231
Manganese (Mn)	6.76±0.72	4.57±0.59	0.094
Copper (Cu)	1.58±0.25	1.26±0.15	0.127

All values are means of triplet determinations ± standard deviation (SD)

Table 4: Proximate Contents of Tamarind and Ginger Extract (%)

Proximate Composition	Tamarind Extract	Ginger Extract	X^2
Moisture content	9.15±1.37	9.98±2.94	0.770
Crude Fat	7.38±0.73	8.22±0.58	0.391
Ash content	9.57±2.75	6.67±0.60	0.272
Crude fibre	12.78±0.80	10.33±0.26	0.130
Crude protein	8.81±0.19	10.85±0.79	0.430
Carbohydrates	48.78±0.55	35.66±0.55	0.040

All values are means of triplet determinations ± standard deviation (SD)

Table 5: Serum Catalase Activity, Reduced Glutathione (GSH), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx) and MDA Levels in Rats Induced With Oxidative Stress Using CCl_4 and Treated With Ginger and Tamarind Extracts for Four week

Group	MDA(nmol/mL)	SOD (µ/mL)	GSH(µmol/L)	CAT(µ/mL)	GPx(µ/mL)
Normal Control	10.57±4.36[a]	11.96±1.62[a]	34.86±9.19[b]	12.20±4.23[a]	36.23±7.07[b]
Negative Control	18.52±1.69[f]	2.77±0.69[c]	14.06±3.03[a]	3.840±4.36[c]	8.95±5.23[d]
Standard Drug (Vitamin C) (250mg/kg)	9.917±5.42[d]	11.13±2.25[a]	23.61±2.95[e]	9.883±6.51[d]	29.77±19.84[e]
Ginger Extract (1500mg/kg)	14.04±0.15[a]	9.233±5.10[d]	17.55±8.54[f]	7.687±6.30[d]	21.38±2.44[e]
Ginger Extract (4000mg/kg)	11.00±3.86[a]	10.30±3.67[a]	18.47±8.68[f]	8.587±4.41[d]	24.20±5.48[e]
Tamarind Extract (1500mg/kg)	16.03±0.16[f]	4.167±3.88[c]	14.67±2.70[a]	4.293±2.18[c]	12.34±0.87[a]
Tamarind Extract (4000mg/kg)	12.12±6.05[a]	4.787±4.25[c]	16.82±2.27[f]	4.970±2.74[c]	20.25±4.48[e]

Values are expressed as mean ± SD. Mean values having different superscript letters in the same column are significantly different (p<0.05).

Discussion

Antioxidants (inhibitors of lipid peroxidation) are important not only for the preservation of food but also for the defence of living cells against oxidative damage (Sadi *et al*., 2024). Plants naturally serve as major sources of antioxidant biomolecules. Several natural products are reported to contain high amounts of antioxidant compounds apart from traditional vitamin C (ascorbic acid) and vitamin E.

The qualitative phytochemical screening of tamarind extract revealed the presence of alkaloids, carbohydrates, glycosides, saponins, phenols, flavonoids, proteins, amino acids and tannins. A comparative study on phytochemicals screening conducted by Sadiq *et al*. (2016) using the pulp and seed of *Tamarindus indica* shows the presence of tannins, volatile oils, saponins and steroids with the absence of alkaloids and flavonoids, respectively, which findings agree with the present study on saponins and disagree on alkaloids, flavonoids and tannins. A study conducted by Gomathi *et al*. (2017) reported the presence of Quinines in addition to the phytochemicals detected in this study. The pharmacological significance of these secondary metabolites ranges from antibacterial to fungal effects against micro-organisms (Sadi *et al*., 2021).

Quantitative determination indicated that *Tamarindus indica* have high level of flavonoids and alkaloids compared to other phytochemicals quantified. Flavonoids are found to be beneficial to Humans due to their biological properties. It is found in several parts of plants which include seeds, fruit, flower, herbs, and stem; they are classified as plant secondary metabolites and are known for their antioxidant property (Chong *et al*., 2012). Saponins and phenols were also found in appreciable quantities.

The phytic acid content of tamarind pulp is similar to that of commonly consumed legumes like *Psophocarpus tetragonolubus* and Lima Bean (Egbe and Akinyele 1990; Sadi *et al*., 2021). Ishola *et al*. (1990) reported that tamarind pulps do not contain any detectable amount of phytic acid. Phytic acid is known to decrease the bioavailability of certain minerals and may interfere with the utilisation of proteins due to the formation of phytate-protein and phytate-mineral-protein complexes, and also inhibits the digestive enzymes. The present study findings on anti-nutrients are similar to those of Adeola and Aworh (2012).

A similar phytochemical study reported by Osabor *et al*. (2015) conducted on Ginger revealed the presence of alkaloids, saponins, flavonoids,

polyphenols and reducing sugars in the aqueous extracts, which were all found in the present study. A study conducted by Ajayi *et al*. (2013) on two varieties of ginger (Yellow and White) indicated that the two ginger varieties are rich in phytonutrients. The anti-nutrients such as oxalate and phytic acid in this study were found to be similar to those obtained by Olubunmi Ajayi *et al*. (2013). The levels at which they occur in the two ginger varieties are safe for consumption by humans and animals.

The Proximate compositions of tamarind and ginger extract were presented in Table 4.4. Tamarind and ginger extract both have low moisture content, which is similar to the findings of Olununmi Ajayi *et al.* (2013). However, Krithika and Radhail Sri (2007) reported higher moisture content for tamarind pulp. The difference in moisture content could be explained by the time interval between harvest and analysis, the method of drying and storage. The protein content determination is one of the most important and widely used analytical measurements in processing and testing the quality of food samples. The results obtained showed that ginger extract has higher protein content than tamarind extract, but these values were higher when compared with those reported by Okoye *et al*. (2023). In this study, both tamarind and ginger extracts contained appreciable amounts of carbohydrates. However, the carbohydrate content of tamarind extract was higher than that of ginger extract, and these values are higher than what was reported by Okoye *et al*. (2023). The protein and carbohydrate contents of tamarind extract confirm the report of BAIF (2002) that tamarind fruits contain one of the highest levels of protein and carbohydrate of any fruit. Tamarind and ginger extracts showed high fat and crude fibre contents, respectively. The ash content of tamarind extract was higher than that of ginger extract.

The mineral analysis of tamarind and ginger extracts indicated richness in Calcium, Magnesium, Potassium, Phosphorous and Manganese. The trace elements Copper and Zinc varied in content among the extracts within much narrower limits. Potassium was found to be higher in both tamarind and ginger juice. A high amount of potassium in the body was reported to increase iron utilisation (Gropper *et al*., 2018), and it is beneficial to people taking diuretics to control hypertension and suffering from excessive excretion of potassium through the body fluids (Gropper *et al*., 2018). The levels of Manganese, Potassium and magnesium were higher than those of ginger extract, while ginger extract had a higher Calcium content, which is similar to the findings of (Olunbur *et al*., 2013). Most of these mineral elements are essential activators for enzyme-catalysing reactions. Calcium, like Phosphorous plays a major role in teeth and development, and its deficiency causes osteomalacia, poor fertility and subnormal growth (Ezeagu *et al*., 1997). Iron plays a major role in the synthesis of amino acids and protein and it is an essential activator for enzyme catalyzing reactions. Iron and Copper exist as Iron-Copper proteins (Ayaz *et al*., 2006).

A measure of the organism's redox status is the balance between oxidative factors – namely products of lipid peroxidation (peroxide radicals, malondialdehyde), endogenous and exogenous antioxidant substances (antioxidative enzymes, low-molecular antioxidants or cations of divalent metals (Bartosz, 2004). In the current study, it was observed that there was a decrease in the level of oxidative stress markers in the CCl_4-induced oxidative stress control rats. The results indicated that the CCl_4-induced oxidative stress control group have lower levels of serum reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx) and Catalase activity but havehigher level of serum MDA (a marker of lipid peroxidation).

The increase in lipid peroxidation, as revealed by the high level of MDA formed in the CCl_4-induced oxidative rats compared to the normal control rats, suggests that the natural antioxidant defence mechanism to scavenge excessive free radicals has been compromised in rats induced with oxidative stress. This finding agrees with a previous study of Babandi *et al*. (2017), who suggested that the increased levels of lipid peroxidation products (MDA) observed generally induce compensatory changes expressed by enhanced production and activity of serum antioxidative vitamins and serum redox metals.

The increased MDA observed may also support the hypothesis of Sengupta *et al*. (2001) who suggested that a decrease in the levels of antioxidant accelerate the lipid peroxidation, thereby generating more MDA. It also causes inactivation of enzymes and receptors in membranes and thus changes the membrane molecular properties. Increased levels of MDA were observed in this study, which represents an important finding to support the documented hypothesis that depression of the antioxidant defence potential in the liver of experimental rats as a result of different doses of CCl_4 induction. Results of the effect of treatments with different doses (1500 and 4000mg/Kg) of ginger and tamarind extracts, respectively, after four weeks of treatment showed elevation in the serum levels of reduced GSH, superoxide dismutase, glutathione peroxidase and CAT activity in comparison with that of the negative control (CCl_4) group. Singh *et al*. (2010) reported that the activities of antioxidant enzymes such as SOD, CAT, glutathione peroxidase, and GST were found to increase significantly in the erythrocytes, accompanied by a decrease in the activity of the glucose-6-phosphate dehydrogenase, following atrazine exposure.

Similarly, Siddaraju and Dharmesh (2007) reported that ginger–free phenolic and ginger hydrolysed phenolic fractions exhibited free radical scavenging, inhibition of lipid peroxidation, DNA protection and reducing power abilities, indicating strong antioxidant properties. Ansari *et al*. (2006) showed that the ethanolic *Z. officinale* extract pre-treatment for 20 days in isoproterenol-treated rats induced oxidative myocardial necrosis in rats, enhanced the antioxidant defence (CAT, SOD and GSH) and exhibited cardioprotection property. Bhandari *et al*. (1998) studied the effect of an ethanol extract of ginger on country-made liquor (CML)-induced liver injury in rats. Ajith *et al*. (2007) reported that ginger ameliorated cisplatin-induced nerphrotoxicity and this protection is mediated either by preventing the cisplatin – induced decline of renal antioxidant defence system or by their direct free radical scavenging activity. Amin and Hamza (2006) demonstrated that *Z. officinale* increased the activities of testicular antioxidant enzymes (SOD and CAT) and reduced the level of malondialdehyde. These results indicate the possible involvement of free radicals in CCl_4-induced toxicity and highlight the protective action of ginger, an indigenous medicinal plant product. This makes it a very effective agent for preventing reactive oxygen species production. Oxidation of biological molecules induces a variety of pathological diseases, including atherosclerosis or cancer. These damages are caused by the presence of free radicals. For that reason, the concept of pharmacological supplements to defend against free radicals with antioxidants has become an intense area of research (Gounder and Lingmallu, 2012). According to Atashak (2014), [6]-gingerol, [6]-shogaol have displayed strong antioxidant activity *in vitro.* It is known that the antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals. Traditionally, fruits, leaves and seeds of tamarind are used for the treatment of a wide variety of ailments and diseases. Pods (fruits) are rich in ascorbic acid and sugars and have been used in drinks and many food preparations. Medicinally, the fruit pulp is used as a cathartic, astringent, febrifuge, antiseptic and refrigerant. The extract of tamarind fruit is reported to contain a high concentration of zinc, a nutritional antioxidant (Glew *et al*., 2005); fat, carbohydrates, fibre, ash, calcium, phosphorus, iron, magnesium, sodium, thiamine, riboflavin, niacin, vitamin C and tannin (Feungchan *et al*., 1996).

It also contain pectin, organic acids mainly potassium–hydrogen tartarates as well as free acids such as malic, tartaric and citric acids, phenolic compounds (+) catechin, (-) epicatechin, taxifolin, apigenin, eriodictyol, luteolin and naringenin (Sudjaroen *et al*., 2005). These flavonoids participate in the cellular antioxidant network due to their ability to regenerate ascorbyl radicals and to protect endogenous vitamin E and glutathione from oxidative depletion (Rohdewald, 2002). *In vitro* tamarind fruit pulp extract (70% ethanolic) exhibited significant radical scavenging activity and decreased lipid peroxidation in serum with improved antioxidant defence in terms of SOD, CAT and glutathione peroxidase activities (Martinello *et al*., 2006). The efficacy of tamarind fruit pulp extract in alleviating CCl_4- induced

oxidative stress in rats may therefore be associated with the presence of these phyto-constituents.

Conclusion

In conclusion, results of the study demonstrated that CCl_4 induced oxidative stress in rats, in terms of an increase in malondialdehyde level and a decrease in the activities of GSH, SOD, CAT and GPx. However, ginger and tamarind administration ameliorated the effects of CCl_4 as level of MDA, GSH, SOD, CAT and GPx were comparable with those of normal, apparently healthy rats, suggesting that both ginger and tamarind have potential antioxidants against CCl_4-induced oxidative stress and may be of benefit to those diseases associated with oxidative stress**.**

Conflicts of Interest

The authors have no conflicts of interest to declare.

References

Adeola, A.A. and Aworh, O.C. (2012). A Comparative Evaluation of the Chemical Properties of Wild Tamarind (Tamarindus indica L.) Fruits in Nigeria. *Food,* 6(1): 49-57.

Ajith T.A., Nivitha V. and Usha, S. (2007). *Zingiber officinale* Roscoe alone and in combination with alpha-tocopherol protects the kidney against cisplatininduced acute renal failure. *Food Chemical Toxicology,* 45: 921-927.

Albrecht Wendel, (1981). Glutathione Peroxidase, Methods in Enzymology, Academic Press, 77: 325-333.

Amin, A. and Hamza, A.A. (2006). Effects of roselle and ginger on cisplatin-induced reproductive toxicity in rats. *Asian Journal of Andrology*, 8: 607-612.

Ansari, M.N., Bhandari, U. and Pillai, K.K. (2006). Ethanolic *Zingiber officinale* R. Extract pretreatment alleviates isoproterenol-induced oxidative myocardial necrosis in rats. *Indian Journal of Experimental Biology,* 44: 892-897.

Association of Official Analytical Chemist AOAC. (1995). Association of Official Analytical Chemists. Official Methods of Analysis, 16th ed. Washington DC, USA.

Association of Official Analytical Chemists (2005).Official Method of Analysis of the Association of Officiating Analytical Chemists. I& II, (18th edn). Maryland, USA: 122-135.

Atashak, S., Maghsoud, A., Mohammad, A., and Stannard, S. (2014). Effects of ginger (Zingiber officinale Roscoe) supplementation and resistance training on some blood oxidative stress markers in obese men. *Journal of Exercise Science & Fitness*. 12. 10.1016/j.jesf.2014.01.002.

Ayaz, F.A., Glew, R.H., Millson, M., Huang, H.S., Chuang, L.T., Sanz, C. and Ayaz, S. (2006). Nutrient Contents of Kale (Brassica Oleracea L. Varacephala DC). *Food Chemistry*, 96: 572-579.

Babandi, A., Muhammad, I.Y., Murtala, Y., Ibrahim, A.A., Madugu, A.U. and Babagana, K. (2017). Oxidative stress indices and Calcium level among hypertensive patients in Kano, Nigeria. *Bayero Journal of Medical Laboratory Science,* 2(1).

Baif (2002). Fruit for the Future: Tamarind. Messages programme and Technologies on sustainability newsletter no.25. Available online: www.baif.com/mpts6.htm

Bartosz, G. (2004). *The second face of oxygen*. Free radical in nature editor 2004, PWN, Warsaw, Poland, pp.31.

Beutler, E., Duron, O. and Kelly, B.M. (1963). Improved method for the determination of blood glutathione. *Journal of Laboratory and Clinical Medicine.* 61: 882.

Bhandari, U., Sharma, J.N. and Zafar, R. (1998). The protective action of ethanolic ginger (*Zingiber officinale*) extract in cholesterol-fed rabbits. *Journal of Ethnopharmacology*, 61: 67-171.

Chong, U.R.W., Abdul-Rahman, P.S., Abdul-Aziz, A.A., Hashim, O.H. and Junit, S.M. (2012). Tamarindus indica extract alters the release of alpha enolase, apolipoprotein A-I, transthyretin and rab GDP dissociation inhibitor beta from HepG2 cells. *PloS One*.

Claiborne, A. (1985). Catalase activity. In R. A. Greenwald (Ed.), CRC handbook of methods for oxygen radical research,

vitamin E on the antioxidant status of the muscle of the turkey. Journal Vol. 1 (pp. 283-284). Boca Raton, Florida, USA: CRC Press

Egbe, I.A and Akinyele, I.O. (1990). Effect of cooking on the antinutritional factors of lima beans (Phaseolus lunatus). *Food Chemistry*, 35(2): 81-87.

Ezeagu, I.E., Metges, C.C., Proll, J., Petze, K.J. and Akinyosinu, A.O. (1997). Chemical composition and nutritive value of some wild-gathered tropical plant seeds. *Plants Foods for Human Nutrition*, 50: 151-162.

Feungchan, S., Yimsawat, T., Chindaprasert, S. and Kitpowsong, P. (1996). Tamarind (*Tamarindus indica* L.) plant genetic resources in Thailand. *Thai Journal of Agricultural Science* (Special issue), 1: 1-11.

Ganapaty, S., Ramaiah, M., Yasaswini, K., Nuthakki, V. K. and Harikrishnareddy D. (2013)."Quantitative Phytochemical Estimation and Evaluation of Hepatoprotective Activity of Methanolic Extract of *Dendrobium ovatum* (L.)Kraenzl. Whole Plant against CCl4 Induced Hepatotoxicity". *Journal of Pharmacognosy and Phytochemistry,* 2(3): 113-118.

Glew, R.S., Vanderjagt, D.J., Chuang, L.T., Huang, Y.S., Milson, M. and Glew, R.H. (2005).Nutrient content of four edible wild plants from West Africa. *Plant Foods for Human Nutrition*, 60(4): 187-193.

Gomathi, M., Rajkumar, P.V., Prakasan, A. and Ravichandran, K. (2017). Green synthesis of silver nanoparticles using *Datura stramonium* leaf extract and assessment of their antibacterial activity. *Resource - Efficient Technologies,* 3(3): 280-284

Guo, L., Chen, Z., Amarnath, V. and Davies, S. S. (2012).Identification of novel bioactive aldehyde-modified phosphatidylethanolamines formed by lipid peroxidation. *Free Radical Biology and Medicine*; 53(6): 1226–1238.

Han, Y.A., Song, C.W., Koh, W.S., Yon, G.H., Kim, Y.S., Ryu, S.Y., Kwon, H.J. and Lee, K.H. (2013).Anti-inflammatory effects of the Zingiber officinale Roscoe constituent 12-dehydrogingerdione in lipopolysaccharide-stimulated cells, *Phytotherapy Research*, 27: 1200-1205.

Havinga, R.M., Hartl, A., Putscher, J., Prehsler, S., Buchmann, C. and Vogl, C.R. (2010). *Tamarindus indica* L. (Fabaceae): patterns of use in traditional African medicine. *Journal of Ethnopharmacology*, 127(3): 573-588.

Ippoushi, K., Azuma, K., Ito, H., Horie, H. and Higashio, H. (2003). [6]-Gingerol inhibits nitric oxide synthesis in activated J774.1 mouse macrophages and prevents peroxynitrite-induced oxidation and nitration reactions. *Life Science*, 73: 3427-3437.

Ishola, M.M., Agbaji, E.B., Agbaji, A.S. (1990). A Chemical Study of Tamarindus indica (Tsamiya) Fruits Grown in Nigeria. *Journal of the Science of Food and Agriculture*, 51: 141-143.

Krithika, V. and Radhai Sri S. (2007). Value-added products from tamarind, Available online:www.technopreneur.net/information_desk/sciencetechmagazine/2007/nov07/value.pdf

Kumar, C.S. and Bhattacharya, S. (2008). Tamarind Seed: Properties, Processing and Utilisation. *Critical Reviews in Food Science and Nutrition*, 48: 1-20.

Lee, E.B., Kim, J.H., Kim, Y.J., Noh, Y.J., Kim, S.J., Hwang, I.H. and Kim, D.K. (2018).Lifespan-extending property of 6-shogaol from Zingiber officinale Roscoe in Caenorhabditis elegans. *Archives of Pharmacal Research*, 41: 743-752.

Martinello, F., Soares, S.M., Franco, J.J., Santos. A.C Sugohara, A. Garcia, S.B. Curti, C. Uyemura, S.A. (2006). Hypolipemic and antioxidant activities from *Tamarindus indica* L. pulp fruit extract in hypercholesterolemic hamsters. *Food and Chemical Toxicology*, 44(6): 810-818.

Misrah, H.P. and Fridovich, I. (1972). The univalent reduction of oxygen by reduced flavins and quinones. *Journal of Biological Chemistry,* 247(1): 188-192.

OECD, Organisation for Economic Co-operation and Development Guidelines for the Testing of Chemicals, Test no. 423. Acute

Oral Toxicity-Acute Tociz Class Method, 2001.

Olubunmi, B.A., Seun, F.A. and Funmilayo, T.A. (2013). Food Value of Two Varieties of Ginger (Zingiber officinale) Commonly Consumed in Nigeria. *ISRN Nutrition,* pp. 5.

Osabor, V.N., Bassey, F.I. and Ibe, K.A. (2015).Chemical profile of the endocarp and exocarp of yellow Monkey cola (cola lepidota). *Global Journal of Pure and Applied Sciences,* 21(1):1-10.

Rohdewald, P. (2002). A review of the French maritime pine bark extract (Pycnogenol), a herbal medication with diverse clinical pharmacology. *International Journal of Clinical Pharmacology and Therapeutics*, 40: 158-168.

Sadi, N.S., Kurfi, B.G., Ibrahim, A., Umar, A.M., Gadanya, A.M., Abubakar, S.M. (2021). Effect of *Tamarindus indica* Juice Intake on Some Oxidative Stress Markers in Carbon Tetrachloride induced Rats. *Bayero Journal of Pure and Applied Sciences*, 14(1): 31-37

Sadi, N.S., Ali, M.G., Ibrahim, A., Umar, A.M., Kurfi, B.G. (2024). Effect of Aqueous Extract Of *Zingiber Officinale* Rhizomes On CCl_4 –Induced Oxidative Stress In Rats. *Watari Multidisciplinary Journal of |Science and Technology*.

Sadiq A., Ullah, F., Junaid, M., Ayaz, M., Subhan F., Ahmad W., Ali G., Imran M., and Ahmad, S. (2016). Molecularly Characterised Solvent Extracts and Saponins from Polygonum hydropiper L. Show High Anti-Angiogenic, Anti-Tumour, Brine Shrimp, and Fibroblast NIH/3T3 Cell Line Cytotoxicity. *Frontiers in Pharmacology*, 7: 74.

Sengupta, S., Wehbe, C., Majors, A. K., Ketterer, M. E., DiBello, P. M. and Jacobsen, D. W. (2001). Relative roles of albumin and ceruloplasmin in the formation of homocystine, homocysteine-cysteine-mixed disulfide, and cystine in circulation. *Journal of Biological Chemistry*, *276*(50): 46896-46904.

Siddaraju, M.N. and Dharmesh, S.M. (2007). Inhibition of gastric H(+), K(+)- ATPase and Helicobacter pylori growth by phenolic antioxidants of *Zingiber officinale*. *Molecular Nutrition and Food Research*, 51: 324-332.

Singh, M., Sandhir, R. and Kiran, R. (2010). Oxidative stress induced by atrazine in rat erythrocytes: Mitigating effect of vitamin E. *Toxicology Mechanisms and Methods*, 20(3): 119-126.

Soladoye, M. O. and Chukwuma, E. C. (2012)."Quantitative phytochemical profile of the leaves of *Cissus populnea* Guill.& Perr. (Vitaceae) – an important medicinal plant in central Nigeria". *Archives of Applied Science Research*, 4(1): 200-206.

Sudjaroen, Y., Haubner, R., Würtele, G., Hull, W.E., Erben, G., Spiegelhalder, B., Changbumrung, S., Bartsch, H. and Owen, R.W. (2005). I*solation and structure elucidation of phenolic antioxidants from Tamarind (Tamarindus indica* L.*) seeds and pericarp.Food and Chemical Toxicology*, 43: 1673-1682.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G. and Kaur, H. (2011). "Phytochemical screening and Extraction: A Review". *Internationale Pharmaceutica Sciencia,* 1(1): 98-106.

Varshney, R. and Kale, R.K. (1990). Effects of Calmodulin Antagonists on Radiation-induced Lipid Peroxidation in Microsomes. *International Journal of Radiation Biology*, 58(5): 733-743.

Momodu, O.I. and Idemudia, O.U. Investigating the Effects of Aqueous Zingiber officinale Rhizome Extract on CCL4-induced Liver Alterations in Wistar Rats. J Exp Clin Anat 2023; 20(2):15- 19. doi.org/10.4314/jeca.v20i2.3.

Okoye Obiageli I., Nwobi Adaobi C., Chukwuka Oluebube F., Agbo Chinyere E, Ezeja Enechojo P. (2023). Comparative Analysis of Antioxidant Vitamins, Minerals and Bioactive Compounds of Black Pepper (Piper guineense) Seed, Turmeric (Curcuma longa) and Ginger (Zingiber officinale)Rhizomes Spices. JHER Vol. 30(2): 81– 93.

Gropper, S. A., Smith, J. L., & Carr, T. P. (2018). Advance Nutrition and Human Metabolism, Seventh Edition. Boston, MA: Cengage Learning.

Antidiabetic and Antioxidant Potentials of Aqueous Leaf Extracts of *Crateva adansonii* (Sacred Garlic Pear Plant) on Alloxan-Induced Diabetic Rabbits

Gimba, M. Y.[1] & *Bello, A. M.[1]

[1]Department of Biochemistry, Faculty of Basic Medical Science, Bayero University, Kano

***Corresponding Author:** Aliyu Maje Bello,

***Corresponding Email:** ambello.bch@buk.edu.ng

Abstract

Diabetes mellitus is a chronic metabolic disorder, and according to the World Health Organisation (WHO), more than 180 million people worldwide have diabetes. This metabolic disorder is characterised by hyperglycemia and disturbances of carbohydrate, protein, and fat metabolism, secondary to an absolute or relative lack of the hormone insulin and by the year 2030, this number (180 million) may be more than double. The present study evaluated the antidiabetic and antioxidant properties of aqueous leaf extracts of *Crateva adansonii* (sacred garlic pear plant) in alloxan-induced diabetic rabbits. Dried powdered leaves of C. adansonii were extracted using the maceration method for 14 days. The antidiabetic and antioxidant potential of C. adansonii was evaluated *in vivo* in alloxan-induced diabetic rabbits. Diabetes was induced in the rabbits by injecting alloxan monohydrate at a dose of 150mg/kg body weight. Six groups of three (3) Rabbits per group were used for the experimental procedure. Groups I and II serve as normal and diabetic control groups, and group III was treated with Glibenclamide (an antidiabetic drug). Aqueous extracts of *C. adansonii* at doses of 300mg/kg, 600mg/kg and 900mg/kg body weight were administered to treatment groups IV, V and VI orally for 14 days, respectively. The treatment group showed a significant decrease in fasting blood glucose, lipid peroxides, superoxide dismutase and an increase in the levels of vitamin C, catalase, and reduced glutathione. In conclusion, aqueous extract of *C. adansonii* exhibited significant antidiabetic and antioxidant activities in alloxan -induced diabetic rabbits.

Keywords: Antidiabetics, Antioxidants, *Crateva adansonii*, Aqueous Extracts

Introduction

Diabetes mellitus is a serious, complex, chronic condition affecting millions of people worldwide.[1] This metabolic disorder is characterised by hyperglycemia and disturbances of carbohydrate, protein, and fat metabolism, secondary to an absolute or relative lack of the hormone insulin. Besides hyperglycemia, several other factors, including dyslipidemia or hyperlipidemia, are involved in the development of micro and macrovascular complications of diabetes that are the major causes of morbidity and death.[1] Globally, the prevalence of diabetes was estimated to be 2.8 % in 2000 and projected to be 4.4 % in 2030. Worldwide, the total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030.[2]

Oxidative stress is associated with the development and progression of diabetes-associated complications. In hyperglycemic conditions, continuous generation of reactive oxygen species (ROS) occurs, and evidence showed diabetes induced changes in the activities of antioxidant enzymes in various tissues.[3] Antioxidants play an important role in scavenging the free radicals and protect the human body from oxidative stress. [3, 4] Hence, a drug with both antioxidant and antidiabetic properties would be useful for the treatment of diabetes mellitus. In recent times, many medicinal plants have been reported to cure diabetes worldwide and have been used widely used as antidiabetic remedies. WHO has suggested the evaluation of traditional plant treatments for diabetes as they are effective, nontoxic, with less or no side effects, and are considered to be excellent candidates for oral therapy. [5]

Crateva adansonii DC, belonging to the family *Capparidaceae,* is commonly called "Varun" or "garlic pear" in English. [6, 7] *C. adansonii* is a moderately sized deciduous tree found throughout the tropics, especially along the riverbanks. The leaves are trifoliate and ovate to oblong in shape. The flowers are white or creamy and occur at the terminal corymbs. The barks are gray, smooth, and sometimes horizontally wrinkled. Different parts of the plant are extensively used in folkloric medicine for the cure of many disease conditions. The powdered bark is used in the treatment of urinary, renal tubular, gastrointestinal, and uterine conditions. [6] In ethnomedicine, the plant is used in inflammatory conditions, asthma, snakebites, and as an astringent. [8] In Senegal, the roots are used in the treatment of syphilis, jaundice, and yellow fever. The scientific evaluation of the antimicrobial, antigout, antitrypanosomal and antiplasmmodial activities has been reported. [6, 9, 10-11] Abdullahi *et al*. [10] and Tsado *et al.* [11] also report the presence of phytochemicals such as phenolics, alkaloids, flavonoids, anthraquinones, saponins, cardiac glycosides and steroids in the leaves of *C. adansonii*.

2.0 Materials and Methods

2.1 Collection and Identification of Plant Material

The leaves of *Crateva adansonii* were collected in May 2017 from Emi-Ndagunji opposite River Gbako in Katcha Local Government of Niger State, Nigeria. It was identified and authenticated at the herbarium section of the Department of Biological Science, Bayero University Kano, Kano State, where an Identification Number BUKHAN 0484 was assigned to it for reference purposes.

2.2 Preparation of the Plant Material

The leaves of the plant were dried at room temperature on a laboratory bench for 16 days and then grounded to powder using a mortar and pestle. The powder was macerated in distilled water for 48h with intermittent shaking at 3h intervals. The extract was filtered using Whatman No. 1 filter papers. The aqueous filtrate was kept in a water bath and allowed to evaporate for 18 days, and the residue was weighed.

2.3 Experimental Animals

Totally 18 rabbits of both sexes, weighing 0.5-1kg, obtained from a local breeder at Sabon Gari in Kano State, were used for the study. The animals were housed in aluminium cages at room temperature under natural light/dark cycles. The rabbits were supplied with clean drinking water and fed *ad libitum* with standard commercial pelleted grower feed (Vital Feed Nigeria). The rabbits were allowed to acclimatise for two weeks prior to the study. They were maintained in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals.[12]

2.4 Experimental Design

Aqueous leaf extract, standard drug glibenclamide (10 mg/kg) and saline were administered with the help of a feeding cannula. Group I serve as the negative control, which received saline for 14 days. Group II are diabetic control rabbits treated with alloxan. Group III are administered with the standard drug glibenclamide (10 mg/kg). Group IV to VI are administered with different doses of aqueous leaf extract of *C adonsonii* (300 mg/kg), (600 mg/kg), (900 mg/kg) for 14 consecutive days.

Six groups of 3 rabbits each received the following treatment schedule.

Group I: Negative Control (saline).
Group II: Positive Control (Alloxan-treated group (150 mg/kg, ip).
Group III: Alloxan (150 mg/kg, ip) + Standard drug, Glibenclamide (10 mg/kg, p.o).
Group IV: Alloxan (150 mg/kg.ip) + *Crateva adansonii*
Aqueous leaf extract (300 mg/kg, p.o)
Group V: Alloxan (150 mg/kg.ip) + *Crateva adansonii.*
Aqueous leaf extract (600mg/kg, p.o)
Group VI: Alloxan (150 mg/kg.ip) + *Crateva adansonii.*
Aqueous leaf extract (900mg/kg, p.o)

2.5 Induction of Diabetes in Experimental Animals

Rabbits were induced with diabetes by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg). Alloxan was first weighed individually for each animal according to the body weight and then solubilised with 0.5 ml saline (154 mM NaCl) just prior to injection. Two days after alloxan injection, rabbits with plasma glucose levels of >180 mg/dl (>10 mmol/L) were included in the study. Treatment with plant extracts started 48 h after alloxan injection.

2.6 Collection of Blood Sample and Blood Glucose Determination

Blood samples were drawn from the ear tip of the rabbit at weekly intervals till the end of the study (i.e., 2 weeks). Fasting blood glucose estimation and body weight measurement were done on days 1, 7, and 14 of the study. Blood glucose estimation was determined by one touch Accu-check electronic glucometer using glucose test strips.

2.7 *In Vivo Antioxidant Activity*

The serum activities of the antioxidant enzymes Catalase (CAT), Superoxide dismutase (SOD), Reduced glutathione (GSH), Serum Lipid peroxides, concentration of vitamin C were estimated. Catalase (CAT) was assayed by the modified method described by Aebi. [13] Superoxide dismutase (SOD) was assayed by the method of Sun *et al.* [14]. Reduced glutathione (GSH) was estimated by the method of Ellman. [15] Serum Lipid peroxides were estimated using thiobarbituric acid reactive substances (TBARS) by the method of Jain *et al*. [16]. The concentration of vitamin C in the serum was estimated by the modified phenyl-hydrazine spectrophotometer method of Roe and Knether. [17]

2.8 Data Analysis

Data obtained were analysed using one-way analysis of variance (ANOVA), and the variant mean was separated by least significant difference (LSD) of the different groups. Significance was accepted at the level of $P<0.05$.

3.0 Results

3.1 Change in Body Weight of Experimental Animals

Figure 1 presents the changes in body weight in normal and experimental diabetic rabbits. Alloxan produced a significant loss in body weight as compared to normal rabbits during the study. Positive control rabbits continued to lose weight till the end of the study, while *Crateva adansonii*-treated rabbits at a dose 300 mg/kg, 600mg/kg, and 900mg/kg body weight. showed a significant improvement in body weight compared to the positive control group. There was a significant difference between the *C. adansonii* and glibenclamide-treated groups.

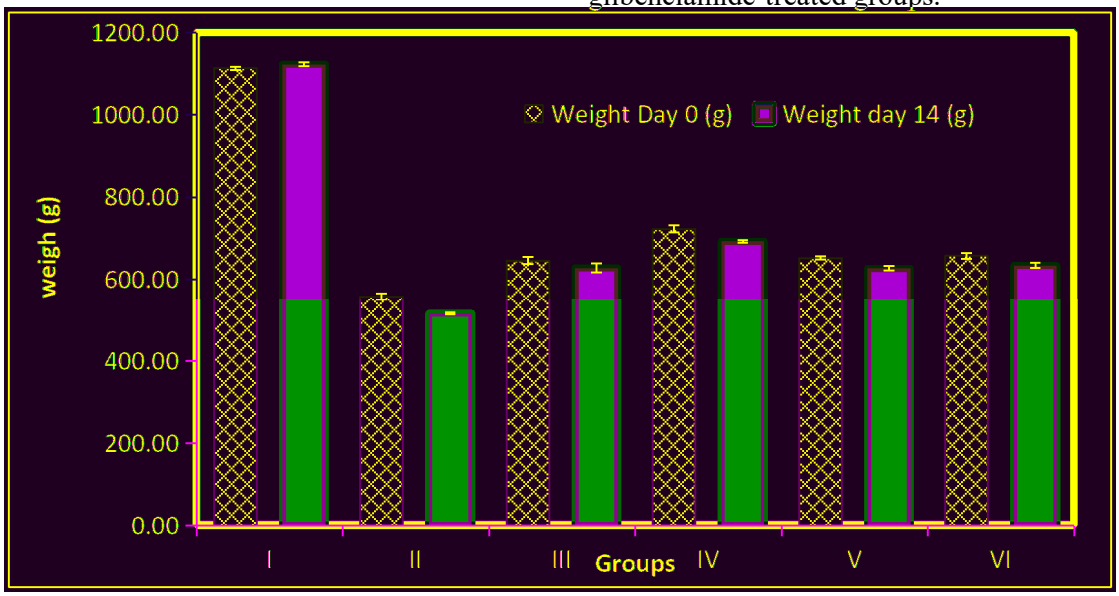


Figure 1: Weight (g) of the experimental animals (rabbits) before induction of diabetes with alloxan and after 14-day treatment. Values are Mean ± SD for three animals in each group.

Key:
Group I: Negative control Group
Group III: Standard (Glibenclamide) Group
Group V: 600mg/kg C.A.E
II: Positive control Group
Group IV: 300mg/kg C.A.E
Group IV: 900mg/kg C.A.E

3.2 Serum Blood Glucose Levels of the Experimental Animals

Results of the effect of *C. adansonii* on the blood glucose level of alloxan -induced diabetic rabbits are presented in **Figure 2**. Alloxan caused a significant increase in blood glucose level. However, serum blood glucose levels significantly decrease during the first week of treatment with the plant extract. Moreover, the reduction in blood glucose was maximum in the second week for the group of animals receiving 300mg/kg, 600mg/kg and 900 mg/kg doses of *C. adansonii*. The hypoglycemic effect of C. *adansonii* leaf extract was comparable to that of glibenclamide standard drug. Thus, the results of the present study clearly indicated that the aqueous extract of C. *adansonii* leaves exhibited significant hypoglycemic activity in alloxan-induced diabetic rabbits.

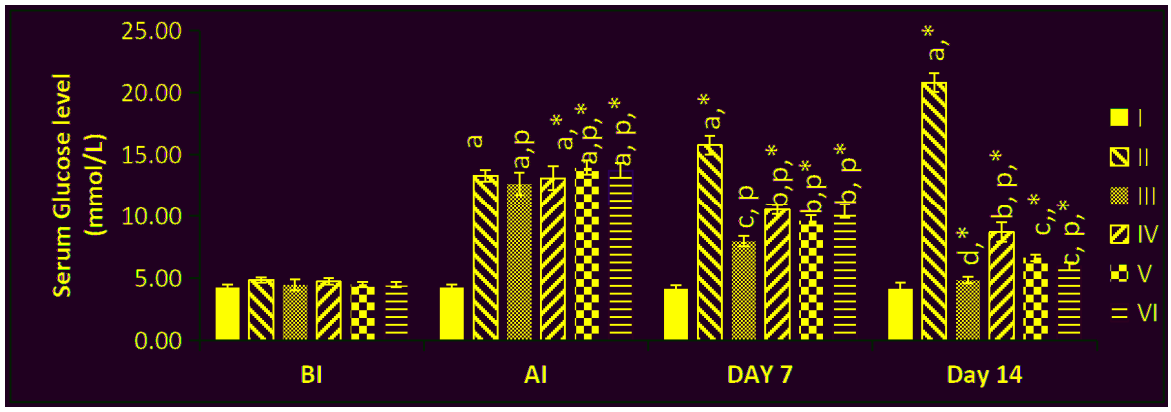


Figure 2: Effect of *C. adansonii* extract on serum glucose level in alloxan induced diabetic rabbits.
Values are expressed as Mean ± SD at P<0.05 (n=3)
(a,b,c,d)= Values with same superscript show no significant difference
p= Values with similar superscript are significantly different as compared to the group negative control.
*= Values with the same superscript show a significant difference as compared to group Glibenclamide.

Key: **Group I: Negative** Control Group,
Group II: Positive control Group
Group III: Glibenclamide
Group IV: 300mg/kg C.A.E
Group V: 600mg/kg C.A.E
Group IV: 900mg/kg C.A.E
BI: Before induction
AI: After induction

3.3 Antioxidant Potential of the *C. adansonii* leaf extract

After 14 days treatment period, it was observed that the animal groups treated with *C. adansonii* aqueous extract and glibenclamide showed significant *in-vivo* antioxidants activities as shown in **Figure 3**. There was a significant ($p < 0.05$) elevation in MDA, and SODlevels and reduction in GSH, CAT, and vitamin C levels in the serum of diabetic rabbits treated with plant leaf extract compared to control rabbits. The administration of *C. adansonii* 600 mg/kg and glibenclamide significantly ($p < 0.05$) reversed these changes to near-normal levels.

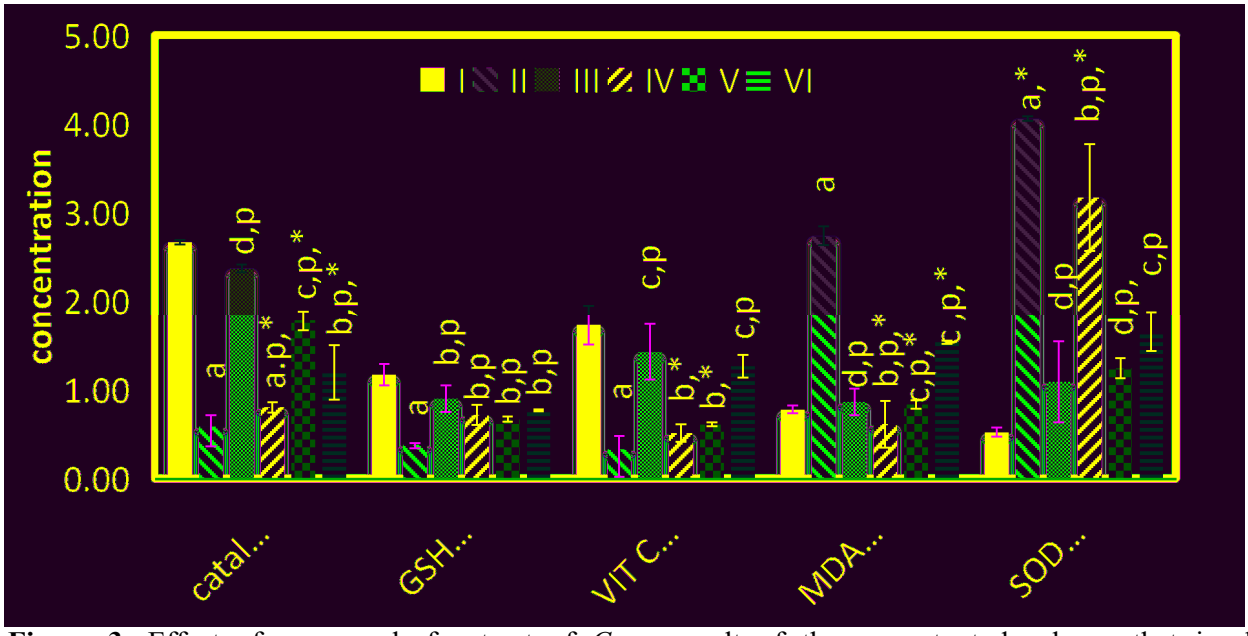


Figure 3: Effect of aqueous leaf extract of *C. adansonii* on the serum antioxidant markers in alloxan - Induced diabetic Rabbits superscript.
Values are expressed as Mean ± SD at P<0.05 (n=3)

(a,b,c,d)= Values with the same superscript show no significant difference
. p = Values with the same are significantly different as compared to group negative control.
*= Values with similar superscript show a significant difference as compared to group Glibenclamide.

Key:
Group I: Negative Control Group
Group II: Positive Control Group
GSH: Reduced glutathione
Group III: Glibenclamide
Group IV: 300mg/kg C.A.E
SOD: superoxide dismutase
Group V: 600mg/kg C.A.E
Group IV: 900mg/kg C.A.E
VIT C: vitamin CMDA: malondialdehyde
CATALASE: catalase

4.2 Discussion

The present study assessed the antioxidant and anti-diabetic effects of the aqueous extract of *C. adansonii* on alloxan -induced diabetic rabbits. The result of the present study shows that insulin-producing cells are still functioning, and the stimulation of insulin release could be responsible for most of the metabolic effects. The medicinal plant compounds may have mechanisms having an insulin-like effect, improving insulin sensitivity, augmenting glucose-dependent insulin secretion, and stimulating the regeneration of islets of Langerhans in the pancreas of alloxan -induced diabetic rabbits. At present, the only option to achieve permanent normoglycemia in diabetic patients is renewal of the β-cells or stimulating insulin secretion by medicinal plants. [18-19]

Presence of phenolic compounds such as alkaloids, flavonoids, and tannins in *C. adansonii* was detected in preliminary phytochemical analysis as reported by Tsado *et al*. [11]. *C. adansonii* may be responsible for the insulin-trophic effect that enables the reduction of blood glucose levels due to its antidiabetic effect. The possible mechanism of action of *C. adansonii* extract-treated groups could be potentiating the pancreatic secretion of insulin from β-cells of islets, as evidenced by significantly lowering the level of glucose. Induction of diabetes by alloxan causes loss of body weight due to increased muscle wasting and loss of tissue proteins. [20] Due to the destruction of β-cells, the decrease in body weight and increase in

food and water intake were commonly observed in diabetes, and this may be due to metabolic changes caused by a lack or deficiency of insulin. [21]

In diabetic rabbits, a reduction in body weight changes observed might be the result of the degradation of structural proteins due to a deficiency of carbohydrate for energy metabolism. [22] A significant increase in body weight of diabetic rabbits treated with *C. adansonii* leaf extract showed the blood glucose stabilisation effect, which in turn prevents the loss of body weight. Administration of sacred garlic plant leaf extract at 600 and 900mg/kg body weight significantly lowers blood glucose in alloxan -induced diabetic rabbits, and its effect was not equal to that of glibenclamide. The reference drug glibenclamide is an ATP-sensitive K^+ channel blocker used to treat noninsulin-dependent diabetes. It appears to be a useful addition to the range of oral hypoglycemic sulphonylureas, but because of its potency, it is probably best used as an antidiabetic drug. [23-24] Diabetic rabbits treated with the sacred garlic pear plant leaf extract showed an improvement in body weight in comparison to the Positive control and standard glibenclamide-treated groups, which signifies the reversal of gluconeogenesis. Further, this antidiabetic activity indicates that sacred garlic leaf extract may stimulate insulin secretion from the remaining β-cells or regenerated β-cells.

Diabetes is marked by increased production of free radicals or impaired antioxidant defences. The generation of superoxide anion radicals by glucose oxidation and its dismutation to hydrogen peroxide leads to the formation of reactive hydroxyl radicals. [25] In the present study, elevated MDA and SOD levels and reduced GSH, CAT, and vitamin C levels were observed in alloxan -induced diabetic rabbits compared to control rabbits. These changes may be due to the glucose oxidation, the formation of free radicals, and the nitric oxide donor property of alloxan. [26] Administration of *C. adansonii* leaf extract significantly reduced Lipid peroxides, SOD levels and increased CAT, vitamin C, and GSH levels in diabetic rabbits. The action of the plant to restore the altered antioxidant enzymes in alloxan -induced diabetic rabbits indicates its free radical scavenging potential.

The activities of phenolics having antioxidant activity further confirm this view. [27] Sterols can decrease blood sugar in experimental animal models. [28] The antioxidant activity of the phenolic, tannins, and flavonoid compounds is attributed to their redox properties, which can act as reducing agents, hydrogen donators, and singlet oxygen quenchers. [29] Polyphenolics having hydroxyl groups are very important plant constituents which can protect the body from oxidative stress. [30] Flavone glycosides have also been reported as strong antioxidants and potent hypoglycemic agents.

Conclusion

The findings of the present study demonstrate that the aqueous extract of the *C. adansonii* leaves exerts significant hypoglycemic effects in diabetic rabbits. Moreover, its pronounced antidiabetic and free radical scavenging activities highlight its potential in mitigating diabetes-related complications. Collectively, these results lend scientific credence to the traditional use of the sacred garlic plant as a therapeutic agent in the management of diabetes.

References

1. Kameswararao B, Kesavulu MM, Apparao C. (2012). Evaluation of antidiabetic effect of *Momordica cymbalaria* fruit in alloxan-diabetic rats. *Fitoterapia*. ; **74**:7–13. [PubMed]
2. Badran M, and Laher I (2012) Type II Diabetes Mellitus in Arabic-Speaking Countries. *International Journal of Endocrinology*: 902873.
3. Eurich, D. T., McAlister, D F., Blackburn, A. F. (2007). "Benefits and harms of antidiabetic agents in patients with diabetes and heart failure: systematic review," *British Medical Journal*, vol. **335**, no. 7618, pp. 497–501, 2007.
4. Ahmed, R.G. (2005) "Thephysiological and biochemical effects of diabetes on the balance between oxidative stress and antioxidant defence system," *Medical*

Journal of the Islamic World Academy of Sciences, vol. **15**, pp. 31–42.
5. Maritim A. C., Sanders R. A., and Watkins J. B. III (2003). "Diabetes, oxidative stress, and antioxidants: a review," *Journal of Biochemical and Molecular Toxicology*, vol. **17**, no. 1, pp. 24–38. (Maritim *et al.,* 2003)
6. Igoli N. P, Grey A. I, Clements C. J, Igoli J. O, Nzekwe U, Singla R. K (2012). Scientific investigation of antitrypanosomal activity of *Crateva Adansonii* DC leaves extracts*. Indo Global J Pharm Sci*. ;**2**:226–9.
7. Gitte T.A, Kare M. A, Deshmukh A. M. (2012). Ethnomedicinal Studies on barks of some medicinal plants in Marathwada (M. S.), India –I. *Recent Res Sci Technol*. ;**4**:08–10.
8. Akanji M. A, Salau A. K, Yakubu M. T. (2013). Safety evaluation of aqueous extract of *Crateva adansonii* leaves on selected tissues of rats. *Fount J Nat Appl Sci.* ;**2**:17–28.
9. Agboke AA, Attama AA, Momoh MA. (2011). Evaluation of the antimicrobial activities of crude extract of *Cryptolepis sanguinolenta* and *Crateva adansonii* leaves and their interactions. *J Appl Pharm Sci*. ;**1**:85–9.
10. Abdullahi A, Hamzah RU, Jigam AA, Yahya A, Kabiru AY, Muhammad H, (2012). Inhibitory activity of xanthine oxidase by fractions *Crateva adansonii*. *J Acute Dis*. ;**1**:126–9.
11. Tsado A N, Bashir L, Mohammed S S, Famous I O, Yahaya A M, Aibu M Shu, Caleb T. Phytochemical Composition and Antimalarial Activity of Methanol Leaf Extract of *Crateva adansonii* in Plasmodium berghei Infected Mice. *British Biotechnology Journal.* 2015 **6**(4): 165-173.
12. National Research Council (2011). Guide for the care and use of laboratory animals. (8th Edition). Washington, DC: *The National Academies Press*.
13. Aebi, H. (1974). Catalase. In *Methods of enzymatic analysis* (pp. 673-684). Academic Press.
14. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988; **34**: 497–500.
15. Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of biochemistry and biophysics*, *82*(1), 70-77.
16. Jain SK, McVie R, Duett J, Herbst JJ. Erythrocyte membrane lipid peroxidation and glycocylated haemoglobin in diabetes. Diabetes 1989; **38**: 1539–1543.
17. Roe H. Joseph and Carl A. Kuether (1943). The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. *Journal of Biological. Chemistry*. **147**: 399
18. Robertson RP, Kahn CR, Kahn B, Moller D, Abrahamson M. (1992); Seminars in medicine of the Beth Israel Hospital, Boston: pancreatic and islet transplantation for diabetes—cures or curiosities? *New England Journal of Medicine*. **327**(26):1861–1868.
19. Yogesha Mohan, Grace Nirmala Jesuthankaraj, and Narendhirakannan Ramasamy Thangavelu. (2013); Antidiabetic and Antioxidant Properties of *Triticum aestivum* in Streptozotocin-Induced Diabetic Rabbits. Adv Pharmacol Sci.; 2013: 716073
20. Shirwaikar A, Rajendran K, Barik R. (2006); Effect of aqueous bark extract of Garuga pinnata Roxb. in streptozotocin-nicotinamide induced type-II diabetes mellitus*. Journal of Ethnopharmacology*. **107**(2):285–290.
21. Rodríguez T, Alvarez B, Busquets S, Carbó N, López-Soriano FJ, Argilés JM. (1997); The increased skeletal muscle protein turnover of the alloxan-diabetic rat is associated with high concentrations of branched-chain amino acids. *Biochemical and Molecular Medicine;* **61**(1):87–94.
22. Pepato MT, Migliorini RH, Goldberg AL, Kettelhut IC. (1996); Role of different proteolytic pathways in degradation of

muscle protein from streptozotocin and alloxan-diabetic rats. American Journal of Physiology*: Endocrinology and Metabolism*; **27**1(2): E340–E347.
23. Campbell RK. (2009); Type 2 diabetes: where we are today: an overview of disease burden, current treatments, and treatment strategies. *Journal of the American Pharmacists Association;* **49**:S3–S9.
24. Guastella V, Mick G. (2009); Strategies for the diagnosis and treatment of neuropathic pain secondary to diabetic peripheral sensory polyneuropathy. *Diabetes and Metabolism*. **35**(1):12–19.
25. Jiang Z-Y, Woollard ACS, Wolff SP. (1990); Hydrogen peroxide production during experimental protein glycation. *FEBS Letters*; **268**(1):69–71.
26. Mohamed AK, Bierhaus A, Schiekofer S, Tritschler H, Ziegler R, Nawroth PP. (1999); The role of oxidative stress and NF-κB activation in late diabetic complications. *BioFactors*; **10**(2-3):157–167.
27. Imran M, Khan H, Shah M, Khan R, Khan F. (2010); Chemical composition and antioxidant activity of certain Morus species. *Journal of Zhejiang University*. **11**(12):973–980.
28. Suba V, Murugesan T, Bhaskara Rao R, et al. (2004); Antidiabetic potential of Barleria lupulina extract in rats. *Fitoterapia*; **75**(1):1–4.
29. Gülçin I, Elmastaş M, Aboul-Enein HY. (2007); Determination of antioxidant and radical scavenging activity of basil (Ocimum basilicum L. Family Lamiaceae) assayed by different methodologies. *Phytotherapy Research*. **21**(4):354–361.
30. Jing LJ, Mohamed M, Rahmat A, Bakar MFA. (2010); Phytochemicals, antioxidant properties and anticancer investigations of the different parts of several gingers species (Boesenbergia rotunda, Boesenbergia pulchella var attenuata and Boesenbergia armeniaca) *Journal of Medicinal Plant Research*.; **4**(1):27–32.

Assessment of Acute Toxicity of New Psychoactive Substance: Focus on Akuskura (VIP-AK47) Via Oral Administration and by Gargling in Mice

Hafsat H. Muhammad[1] & Abbas B. El-ta'alu[1]

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, Bayero University, Kano, Nigeria

The Nigerian National Drugs Law Enforcement Agency (NDLEA) named Akuskura as a group of new psychoactive substances (NPSs) nicknamed *VIP AK-47, DanMilla* (Plate 2B)*, Hajia Ayisha; Hajia Salma* (Plate 3) by its users and are mostly used in Northern and some parts of Southern Nigeria; and as claimed, for the treatment of catarrh, malaria, typhoid, headache, body pains, energy boosting for efficient work without being subjected to physical fatigue, enhancement of sexual arousal in males. The name Akuskura, sometimes known as *Kuskura* or *Akurkura*, is derived from the Hausa word «Kuskura», a noun which can be used interchangeably to mean *gargling* and *rinsing*. The study aimed to investigate sign of toxicity of VIP AK-47 Akuskura via oral administration and by gargling. Thirteen albino mice were used using Lorke's method. Acute lethal effect of low doses of VIP-AK47 Akuskura extract at 10- and 100/kg via oral route of administration did not show any mortality, but those that received 1,000mg/kg of the substance via the same route died within 24 hours. No death was recorded at doses of 10, 100 and 1,000 mg of the Akuskura substance via gargling. The acute lethal effect of high doses did not show any mortality in phase-I (at 100-, 225-, 370-, and 600mg/kg). In phase-II, however, death was recorded at 1,600- and 2,900mg/kg of the substance. It was found that the acute lethal effect of high doses of VIP-AK47 by gargling was higher than via oral administration.

Keywords: VIP AK-47 Akuskura, Toxicity, oral administration, gargling

Introduction

New Psychoactive Substances (NPS) are a broad and complicated group of chemical substances frequently referred to as either '*created' or 'synthetic'* drugs or by the more common but deceptive colloquial term of «*legal highs*» (Pates 1, 2). According to the United Nations Office on Drugs and Crime (UNODC),[1] NPSs are substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychoactive Substances but which may pose a public health threat. The UNODC stated that the use of NPSs is often linked to health problems and that their side effects range from seizures to agitation, aggression, acute psychosis, as well as potential development of dependence.[1]

According to Mahmud [2], Akuskura is of different varieties, and is used in both liquid and powdered forms by people who are mostly seeking to raise their levels of psychological or nervous activity or as users say, to '*get high'* (Pates 1, 2). Akuskura consists of herbs laced with tobacco and cannabis; the substance has very limited safety data on its toxicity and carcinogenic potential, and information on long-term adverse effects or risks is still largely unknown.[2] The majority of NPSs are solids (Snuffs), while some are in liquid forms, which are used for gargling and rinsing (Pates 1, 2). The different varieties of NPSs include: VIP AK-47, DanMilla, Hajia Ayisha, and Hajia Salma. The VIP AK-47 Akuskura was the object of our investigations (Pates 1, 2).

Date submitted-16/04/2022 Date Accepted-13/10/2022

Plate 1: A-*VIP AK-47* **B-***DanMilla.*

A B

Plate 2: New Psychoactive Substances – **A:** AK-47, **B:** DanMilla

The AK-47 is famous because of its unmatched reliability, simple design, low cost, and global proliferation, making it a durable and accessible weapon for virtually any environment or user.

A broad range of negative health outcomes has been associated with the use of VIP AK-47.[1,5] These can be physical or psychological, including cardiovascular problems, seizures, renal failure, myocardial infarction, sudden violence, irregular movements of the body and increased muscle contractions, vomiting, diarrhea and sudden collapse, anxiety, agitation, memory loss, depression, and psychosis.[2] Raised psychological or nervous activity of the body causes compulsive desire to commit crime, aggression and mood swings; these substances are very affordable and

accessible, peddled mostly by herbalists and religious herbal chemists.[6,7]

There has been an increased concern about the effects of the deadly Akuskura in Nigeria and some West African countries; the NPSs represent a serious threat to public healthcare systems and pose a challenge for researchers, forensic toxicologists, healthcare systems and drug control policy globally – and have been described as a 'growing global epidemic'.[6,7] As the negative health impacts and social harms of Akuskura are largely unexplored, prevention and counselling are extremely difficult. It is therefore expedient to study the mean lethal doses of the substance, as well as compare the mean lethal doses when orally administered and when gargled.

Acute Toxicity Test

Lethal mean dose (LD_{50}) is the amount of a substance (given all at once), which causes the death of 50% (one half) of a group of test animals; it is one way to measure the short-term poisoning potential (acute toxicity) of a substance. Researchers use many kinds of animals, but most often testing is done with rats and mice. The LD_{50} is usually expressed as the amount of chemical substance administered (e.g., milligrams) per 100 grams (for smaller animals) or per kilogram (for bigger test subjects) of body weight.[3]

When an organism is subjected to a variety of doses of a test chemical through the duration of 24 hours by a known route, acute systemic toxicity assesses the adverse effects that follow.[12] Before the test chemical produces a systemic adverse effect, it is absorbed and dispersed throughout the body after administration.[13] To ascertain the harmful effects that can result from inadvertent or intentional short-term exposure, it is necessary to assess the acute toxic potential of drugs or other substances.[14,15] In general terms, an active ingredient is more dangerous based on an LD_{50} value.[3]

Calculation of LD_{50}

Lorke's method was used to calculate the LD_{50}.[13]

The study aimed to investigate and compare the acute toxicity of VIP AK-47 via oral administration and gargling in male mice. To achieve this, the following tasks were set as objectives:

i. To determine the lethal mean dose (LD_{50}) of VIP AK-47 via oral administration using cotton swabs.
ii. To determine the lethal mean dose (LD_{50}) of VIP AK-47 via gargling using cotton swabs.
iii. To compare the lethal mean dose (LD_{50}) of VIP AK-47 via oral administration and via gargling.

Materials and Methods

Experimental Animals

A total of twenty-six (26) adult male mice (7-8 weeks old, 18-25g by weight) were used in this study. The Animals were obtained from the animal house of the Department of Human Physiology, Bayero University, Kano (BUK), Kano, Nigeria. Mice were housed in groups in clean cages (five per cage) under standard laboratory conditions, including a good aerated room with suitable temperature, relative humidity, and dark/light cycle and free access to feed and water *ad libitum*. Animals were grouped into:

- **Group One/Phases I (n=9) and II (n=4):** Mice were divided into three groups of three mice each and were orally administered lower doses of the extract at 10-, 100- and 1000mg/kg of VIP AK-47 extract using cotton swabs, respectively.
- **Group Two/Phases I (n=9) and II (n=4):** Animals were administered higher doses of the extract at 140-, 225-, 370-, and 600mg/kg of VIP AK-47 via oral administration and gargling using cotton swabs, respectively.

Determination of Median Lethal Dose (LD_{50}): This was carried out using the method of Lorke (1983) via the oral route of administration and by gargling in two different phases; thirteen (13). mice in each of the four phases. In phase-I, nine (9) mice were divided into three groups of three and were orally administered the extract at different doses of 10-, 100- and 1000mg/kg of VIP AK-47 using cotton swabs. The mice were observed for mortality for 24 hours.[3] In phase-II, four (4) mice

were administered the extract, respectively, via gargling using cotton swabs and were also observed for mortality and other signs of toxicity for 24 hours.

Ethical Consideration: Ethical approval (№ BUK/ACUREC/CAP/PG3) was obtained from the Animal Care and Use in Research Ethics Committee (ACUREC) of Bayero University, Kano, Nigeria.

Results and Discussion

Results of the present study are presented in Tables 1 and 2.

Table 1: Acute Lethal Effect of Low Doses of VIP-AK47

Phase-I (Oral Route)		**Phase-I (Gargling)**	
Dose (mg/Kg)	№ of Dead Mice after 24 hours	Dose (mg/Kg)	№ of Dead Mice after 24 hours
10	0/3	10	0/3
100	0/3	100	0/3
1000	3/3	1000	0/3

Table 2: Acute Lethal Effect of High Doses of VIP-AK47

Phase-II (Oral Route)		**Phase-II (Gargling)**	
Dose (mg/Kg)	№ of Dead Mice after 24 hours	Dose (mg/Kg)	№ of Dead Mice after 24 hours
100	0/1	600	0/1
225	0/1	1000	0/1
370	0/1	1600	1/1
600	0/1	2900	1/1

Results and Discussion

According to Lorke's postulation, lethal doses of substances can be classified according to their LD_{50} values: for $LD_{50} \geq$ 1mg/kg, the substance is highly toxic; for $LD_{50} \geq$ 5mg/kg, the substance is toxic; for $LD_{50} \geq$ 100 mg/kg, the substance is moderately toxic; for $LD_{50} \geq$ 1000 mg/kg, the substance is slightly toxic; and for $LD_{50} \geq$ 5000 mg/kg, the substance was non-toxic.[3]

Assessment of Mean Oral Lethal Dose:

Using the formula $LD_{50} = \sqrt{(D_0 + D_{100}}$, where:

D_0 = Highest dose that gave no mortality, and

D_{100} = Lowest dose that produced mortality.

By simple computation, this result gave rise to an LD_{50} of 774 mg/kg by oral administration and 1265 mg/kg by gargling.

In this study, Table 1 shows the results of the acute lethal effect of low doses of VIP-AK47 extract at 10-, 100 and 1000/kg via oral route of administration (phase-I) and gargling (phase-II) in mice. Animals in phases I and II that orally received 10- and 100/kg of the VIP-AK47 extract survived; this agrees with findings reported by Tijjani *et al.*[3] who reported that oral administration of a low dose of Akuskura mixture did not cause mortality after 24 hours of treatment, but other signs of toxicity, such as tremors, fits, and loss of balance, were visible.[3] However, those that received 1000mg/kg of the substance via the same route died within 24 hours after treatment. This also agrees with findings reported by Tijjani *et al.*[3] who reported that at a higher dose of VIP-AK47, death was observed.[3] This indicated that doses of 10- and 100mg/kg of the VIP-AK47 substance were safe to be administered orally, while 1000 mg/kg was a lethal dose.

In phase-II (Table 1), no mortality was recorded, indicating that doses of 10, 100 and 1000 mg/kg of VIP-AK47 were safe if used by gargling. This agrees with findings reported by Tijjani *et al.*[3] who reported that oral administration of a low dose of Akuskura mixture did not cause mortality after 24 hours of treatment.

Table 2 shows results of acute lethal effect of the VIP-AK47 extract [via oral administration (phase-I) and by gargling (phase-II)] in mice, using different but higher doses of the substance; no mortality was recorded at in phase-I (at 100-, 225-, 370-, and 600mg/kg), indicating that the new psychoactive substance VIP-AK47 had no lethal effect and was safe to be administered orally. This also agrees

with findings reported by Tijjani *et al.*[3] who reported that oral administration of a low dose of Akuskura mixture did not cause mortality after 24 hours of treatment. In phase-II of the same table (Table 2 - gargling), however, death was recorded at 1,600- and 2,900mg/kg of the Akuskura substance, indicating that these concentrations of VIP-AK47 were lethal doses. This agrees with findings reported by Tijjani *et al.*[3] who reported that oral administration of a high dose of Akuskura mixture caused mortality after 24 hours of treatment.

The most common mechanism of absorption for drugs is passive diffusion, which can be explained through the Fick law of diffusion, in which the drug molecule moves according to the concentration gradient from a higher drug concentration to a lower concentration until equilibrium is reached.[16] Unlike buccal administration, which often leads to slower effects and less drug absorption because of digestion and liver metabolism, sublingual delivery method avoids liver metabolism, resulting in faster absorption into the bloodstream. The high vascularity and thin mucosa under the tongue allow for rapid absorption with sublingual delivery.[18-21]

Bioavailability is the extent to which a substance or drug becomes completely available to its intended biological destination(s); it is a measure of the rate and fraction of the initial dose of a drug that successfully reaches either the site of action or the bodily fluid domain from which the drug's intended targets have unimpeded access.[22-25]

The bioavailability of a 5-mg sublingually administered dose is around 35%; therefore, the dose to reach saliva saturation is about 5.4 mg.[26] The observation that higher bioavailability is not observed at doses below the saturation solubility and that bioavailability is constant across this dose range is consistent with a rapid mass transport equilibrium being established between the drug in solution in the mouth and the drug in the mucosal membranes.[26] Bioavailability is essential in maintaining drug plasma concentration within the therapeutic range.[16]

From the aforementioned, it can perhaps be concluded that the mortalities observed in phases I and II (Tables 1 and 2) can be explained by the rate and fraction of the initial dose of AK-47 that successfully avoided liver metabolism, resulting in faster absorption into the bloodstream.

Conclusion

The acute lethal effect of high doses of VIP-AK-47 by gargling was higher than via oral administration.

Limitation of the Study

There is a need to determine the time taken by VIP-AK-47 to be absorbed into the bloodstream.

Acknowledgement - We acknowledge the support given by the technical staff of the laboratory, Department of Human Physiology, Faculty of Basic Medical Sciences, Bayero University, Kano, while carrying out most of the experiments in this study.

References

1. United Nations Office on Drugs and Crime (UNODC). Vienna: United Nations Office on Drugs and Crime. (World Drug Report). Available from: https://www.unodc.org/unodc/secured/wdr/wdr2013/World_Drug_Report_2013.pdf.
2. Mahmud IY. Akuskura: The New Deadly Psychoactive Substance in Town – NDLEA. Available from: https://www.channelstv.com/2022/09/02/akuskura-the-new-deadly-psychoactive-substance-in-town-ndlea/ (Accessed 20th April, 2024).
3. Tijjani RG, Suleiman SA, Mansur MS, Umar I, Adamu I, Sheidu ML and Umaru AR. Toxicological Evaluation of 'Akuskura', a Herbal Mixture Widely Abused by Youths in Northern Nigeria. *International Research Journal of Medical and Pharmaceutical Sciences.* 2025; 10(2): 1-11. DOI: 10.5281/zenodo.15274514.
4. Okoyo C, Njambi E, Were V, Araka S, Kanyi H, Ongeri L, Echoka E, Mwandawiro C, Njomo D. Prevalence, types, patterns and risk factors associated

with drugs and substances of use and abuse: A cross-sectional study of selected counties in Kenya. *Plos one*. 2022; 17(9): e0273470.
5. Soonest N. Akuskura, the new deadly psychoactive substance in town. Channels. Available from https://www.channelstv.com/2022/09/02/akuskura-the-new-deadlypsychoactive-substance-in-town-ndlea/ (Retrieved 8 October 2023).
6. Soussan C, Kjellgren A. The Users of Novel Psychoactive Substances: Online Survey about their Characteristics, Attitudes and Motivations. *International Journal of Drug Policy*. 2016; 32: 77–84. https://doi.org/10.1016/j.drugpo.2016.03.007.
7. Luethi D, Liechti ME. Designer Drugs: Mechanism of Action and Adverse Effects. *Arch Toxicol*. 2020; 94:1085–1133. doi: 10.1007/s00204-020-02693-7.
8. Saganuwan SA. Toxicity study of drugs and chemicals in animals*: An overview. BJVM.* 2016. *Online first*. ISSN 1311-477. DOI: https://doi.org/10.15547/bjvm.983.
9. EURL-ECVAM. European Union Reference Laboratory for Alternatives to Animal Testing. Available from: *https: //eurl ecvam.jrc.ec.europa.eu/glossary/glossary/invitro-methods* (2017).
10. Clemedson C *et al*. MEIC evaluation of acute systemic toxicity. Part VII. Prediction of human toxicity by results from testing of the first 30 reference chemicals with 27 further *in vitro* assays*. ATLA* .2000; 28: 159–200831.
11. Sadasivan KP, Thomson M, Katsumi K, Bhavana S, Mathews M, Parvathy S. John William Trevan's concept of Median Lethal Dose (LD50 /LC_{50}) – more misused than used. *Journal of Pre-Clinical and Clinical Research.* Available from: www.jpccr.eu (2021).
12. EURL-ECVAM. European Union Reference Laboratory for Alternatives to Animal Testing. Available from: *https: //eurl ecvam.jrc.ec.europa.eu/glossary/glossary/invitro-methods* (2017).
13. Lorke D. A new approach to practical acute toxicity testing. *Archives of Toxicology.* 1983. 54:275–87. DOI: 10.1007/BF01234480.
14. Maheshwari DG, Shaikh NK. An overview on toxicity testing method. *Int J Pharm Technol*. 2016/ 8(2): 3834–49.
15. Senin R. Acute toxicity study. 2006. *(http://www.ccohs.ca/oshanswers/chemicals/Id50.html) on 27/3/2010*.
16. Alagga AA, Mark V, Pellegrini MV, Gupta V. Drug Absorption Treasure Island (FL): StatPearls Publishing. (2024).
17. Chinna RP, Chaitanya K*S*, Madhusudan R*Y*. A review on bioadhesive buccal drug delivery systems: current status of formulation and evaluation methods*. Daru.* 2011, *19 (6): 385–403.*
18. *Sattar* M, Sayed OM, Lane ME. *Oral transmucosal drug delivery--curr*ent status and future prospects*. Int J Pharm. 2014*; *471*(1–*2*): 498–*506.* Doi*:*10.1016/j.ijpharm.2014.05.043. PMID 24879936.
19. *Fonseca-Santos, B*, Chorilli, M. *An overview of polymeric dosage forms in buccal drug delivery: State of art, design of formulations and their in vivo* performance evaluation*. Materials Science & Engineering. C, Materials for Biological Applications. 2018*; *86:* 129–*143.* Doi*:*10.1016/j.msec.2017.12.022.
20. Wanasathop A, Patel PB, Choi H*A*, Li S*K*. *Permeability of Buccal Mucosa. Pharmaceutics. 2021*, *13 (11): 1814.* Doi*:*10.3390/pharmaceutics13111814.
21. Bahraminejad S, Almoazen H. Sublingual and Buccal Delivery: A Historical and Scientific Prescriptive. *Pharmaceutics.* 12025, 7(8):1073.
22. Herkenne C, Alberti I, Naik A, Kalia YN, Mathy FX, Préat V, Guy RH. *In vivo* methods for the assessment of topical drug bioavailability. *Pharm Res*. 2008, 25(1):87-103.

23. Chow SC. Bioavailability and Bioequivalence in Drug Development. *Wiley Interdiscip Rev Comput Stat.* 2014, 6(4):304-312. Doi: https://doi.org/10.3390/pharmaceutics 17081073.
24. Currie GM. Pharmacology, Part 2: Introduction to Pharmacokinetics. *J Nucl Med Technol*. 2018, 46(3):221-230.
25. Jennifer Le, Evam MV. Drug Bioavailability. Available from: *https://www.msdmanuals.com/professional/clinical-pharmacology/pharmacokinetics/drug-bioavailability* (2024).
26. Bartlett JA, van der Voort Maarschalk K. Understanding the oral mucosal absorption and resulting clinical pharmacokinetics of asenapine. *AAPS PharmSciTech.* 2012, 13(4):1110-5. doi: 10.1208/s12249-012-9839-7.

Fluorescence Study of Antimalarial Drugs Interactions with Bovine Serum Albumin and Haemoglobin under Different Physiological Conditions

*[1]Lawal Almustapha, [1]Abubakar Mu'azu Gusau, [1]Umar Shamsuddeen Dandare., [1]Abubakar Aminu Lailaba & [2] Wali Usman

[1]Department of Biochemistry and Molecular Biology, Faculty of Chemical and Life Sciences, Usmanu Danfodiyo University, Sokoto - Nigeria.
[2]Department of Chemical Pathology, School of Medical Lab Science, Usmanu Danfodiyo University, Sokoto State,
Department of Biochemistry and Molecular Biology, Faculty of Chemical and Life Sciences, Usmanu Danfodiyo University, Sokoto State

***Corresponding Author:** Almustapha Lawal
***Corresponding Email:** lawal.almustapha@udusok.edu.ng
ORCHID ID: https://orcid.org/0000-0001-5261-7211

Abstract

Drug-protein interactions significantly impact the pharmacology of drug distribution, free drug concentration, and metabolism. Haemoglobin and albumin are associated with drug interactions in systemic blood circulation. The research aimed to study the in vitro interactions between chloroquine (CQ) and amodiaquine (AQ) with haemoglobin (Hb) and bovine serum albumin (BSA) under different physiological conditions. Changes in spectral shift and fluorescence emission were monitored using a fluorescence spectrophotometer. The effects of increased drug concentration, pH, and temperature at constant Hb and BSA concentrations were studied using excitation at 350 nm and emission wavelength between 300 and 900 nm. The drugs were prepared in concentrations between 5 - 25µM using methanol. The Hb and BSA were prepared in 0.05M phosphate buffer (pH 7.2) and Tris-HCl buffer (pH 10.2). The fluorescence of Hb and BSA was attributed to the presence of amino acid residues, especially tryptophan (Trp). However, the results show that the concentration of CQ, AQ, pH, and temperature of the reaction affect the denaturation of Hb and BSA via positive interactions and structural shift expressed by emission intensity after binding of antimalarial drugs to BSA and Hb, which signifies denaturation, and the denaturation takes place in the absence of other cellular matter. In conclusion, the results suggested that different mechanisms of interactions may have occurred between the CQ and AQ in the two major proteins, Hb and bovine serum albumin.

Keywords: Antimalarial Drugs, Haemoglobin (Hb), Bovine Serum Albumin (BSA), Fluorescence Intensity and Denaturation.

Introduction

Drug-protein interactions affect the pharmacokinetics and pharmacodynamics of the drug, such as distribution, free drug concentration, and metabolism [1, 2]. These interactions can affect drug stability and toxicity during the chemotherapeutic process [1]. Haemoglobin (Hb), a tetramer that contains four globin chains, two α-chains and two β-chains, is the most important functional protein in blood that carries oxygen to the lungs, transports carbon dioxide, and regulates the blood pH [3]. The four globin chains carry a haem group as a prosthetic group as oxygen-binding sites. The drug that enters the systemic circulation has to be transported through the blood and has the possibility of interacting with Hb. Albumin, the most abundant protein in the blood, plays a dominant role in drug disposition and efficiency [4]. Many drugs and other small bioactive molecules are reversibly bound to albumin and other serum components as carriers. Serum albumin (SA) often increases the apparent solubility of hydrophobic drugs in plasma and modulates their delivery to cells [6]. The primary sequence of SA suggests 583 amino acids in three homologous domains (classified as I, II, and III

domains) connected by disulfide bonds, and two tryptophan residues at positions Trp-134 and Trp-212, which influence its intrinsic fluorescence [7].

Chloroquine (CQ), a 9-aminoquinoline derivative, is believed to have been discovered and named by a scientist, Hans Andersag, and his co-workers in the year 1934 [8]. It was initially limited in its prescription due to its potential toxicity, but reinstated in 1948 due to its proven chemotherapeutic ability against extra-intestinal amebiasis [9]. It was also deployed by the World Health Organisation (WHO) after World War II to combat malaria, and since then, CQ has remained on the list of WHO's essential medicines. It is effective against malarial parasites of the Plasmodium genus, such as Plasmodium malaria, *Plasmodium ovale*, and *Plasmodium vivax*, but not against *Plasmodium falciparum* owing to the resistance developed [10]. The mechanism used by CQ to attack the malaria parasite in the red blood cell is by interaction and intercalation of the DNA, thereby inhibiting haem polymerase and also acts via calcium-binding protein Ca-calmodulin mediated mechanisms and can also form complexes with hepatic porphyrins, which chelate iron, thereby depriving the parasite of acquiring those amino acids for constructing its proteins and energy metabolism. CQ is a lysomotrophic agent and crosses plasma membrane and organelle membranes by simple diffusion and efflux of the cells via a protein called multi-drug resistance protein or MRP-1, an ATP-binding protein [10, 11].

In addition, amodiaquine hydrochloride (AQ) is structurally a 4-aminoquinoline, and it is more effective against *P. falciparum* than chloroquine and is used in combination with Artemisinin-based combination therapy (ACT). The drug interacts with haemoglobin and inhibit prevent its broken down, via glutathione-dependent interactions, stoping the destruction of ferriprotoporphyrin IX in the malaria parasite, which becomes more accumulated, thereby becoming toxic (amodiaquine reactive quinone-imine metabolite) to the parasite, and degrading haemoglobin in vacuoles of the parasitic cell and prevents it from acquiring those amino acids for constructing its proteins and energy metabolism. Although the CQ is no longer being prescribed in the malaria medicine in most African countries, with the recent outbreak of COVID-19, many researchers speculate on the effectiveness of hydroxyl CQ (HCQ) against the coronavirus, retroviruses, and flaviviruses and have also demonstrated its anti-viral potency [12]. For example, Savarino et al. [13] reported its ability to inhibit viruses at the entry point of the host cell in the case of the Borna disease virus and the avian leucosis virus in the hepatitis (A), chloroquine has also shown to inhibit the coating formation in viruses and, thereby, denying the virus to replicate leading to the destruction of the virus [14, 15].

Therefore, a study on CQ, AQ interactions with Hb and BSA will provide information on the structural features of the drugs and help to elucidate the properties of the drug-protein interaction [16, 17]. Studies on drug-protein interactions have been reported; only a few reports are available on the *in vitro* interactions between antimalarial CQ and AQ with Hb and BSA using fluorescence under different physiological conditions. Therefore, the work aimed to study the *in vitro* interactions between chloroquine (CQ) and amodiaquine (AQ) on haemoglobin (Hb) and bovine serum albumin (BSA) under different physiological conditions.

Materials and Methods

Chemicals and Reagents

Horse haemoglobin Lyophilised powder (molecular weight 64,500 D), Bovine Serum Albumin, Chloroquine phosphate, and Amodiaquine hydrochloride are purchased from Sigma (Sigma Chemicals USA). And the Tris-HCl buffer (pH 10.2) was purchased from Hopkin and Williams (Hopkin and Williams, UK).

Assay Procedure

Preparation of Chloroquine Phosphate and Amodiaquine Hydrochloride Stock Solutions

The stock solutions of Chloroquine and Amodiaquine were prepared by dissolving 25 mg and 20 mg, respectively, in 10 mL of deionised water and diluted with phosphate and Tris-HCl buffers to 100 mL, respectively.

Preparation of Haemoglobin and BSA working solutions

A 10 mg of haemoglobin was dissolved in 100ml of phosphate buffer (0.05M, pH 7.2), giving a (10 mg%) solution of haemoglobin, and 10 mg of BSA in 100 ml of Tris-HCl buffer (pH = 10.2) (10 mg%) was prepared as a stock solution in Tris-HCl buffer (pH = 10.2).

Fluorescence Spectra Measurement of Pure Hb and BSA

The fluorescence spectral analysis of pure bovine serum albumin and haemoglobin was carried out using an RF-5301PC Spectrofluorimeter (Shimadzu- Japan). The excitation wavelength was set at 370 nm, and the emission wavelength was between 220 and 900 nm. The fluorescence spectrophotometer was first allowed to warm up for 30 minutes and zeroed using phosphate buffer free of Hb and Tris-HCl buffer free of BSA, respectively, in two different experiments. And 2 mL of freshly prepared pure BSA in Tri-HCl buffer and Hb in phosphate buffer were transferred into a quartz cuvette and scanned for 10 minutes, the spectrum of pure Hb and BSA were obtained.

Effect of increased Concentrations of CQ and AQ on Hb and BSA in phosphate and Tris-HCl buffers

The spectrofluorimeter was first set up, with the emissions wavelength from 220-900nm and excitation at 370nm, and left for 30 minutes to warm up. Before sample scanning, the machine was zeroed using Tris-HCl buffer for BSA and phosphate buffer for Hb, respectively. In separate experiments, using a serial dilution, the concentration of CQ was diluted using freshly prepared buffer into 5, 10, 15, 20, and 25 mg, and AQ into 2.5, 5, 10, 15, and 20 mg, and a constant concentration of (10 mg%) of BSA and Hb was used. One millilitre (1 ml) of each of the prepared concentrations of the drugs and 1 ml of BSA and Hb were mixed separately in the cuvette. The mixture was vortexed and allowed to stand for 30 minutes before a different spectrum was obtained.

Effect of Temperature on BSA with AQ in Tris-HCl buffers

In the second experiment, the effect of a change in temperature in the mixture of BSA and AQ was monitored. The temperature of the reaction mixture was set at 25° C, 35° C, and 40° C using an in-built thermostat to adjust the temperature. The reaction mixture was allowed to stand in the thermostat setting for 30 minutes before scanning. The emission wavelength was taken from 220 to 900nm, and excitation at 370nm.

Effect of Change in pH of the Reaction Medium for AQ-BSA Interaction

The effect of a change in pH in the AQ-BSA complex was investigated. The spectrofluorimeter was first set up, with the emissions wavelength from 220-900nm and excitation at 370nm, and left for 30 minutes to warm up. Before sample scanning, the machine was zeroed using Tris-HCl buffer for BSA and phosphate buffer for Hb, respectively. The pH was adjusted to 10.2, 8.2, and 6.2 using 0.1M NaOH. The reaction mixture was incubated at room temperature (25^0 °C) for 30 minutes. The sample was scanned for 10 minutes, and the spectrum was obtained at an emission wavelength of between 220 and 900 nm and an excitation wavelength of 370nm.

Results

Spectra of Pure Haemoglobin and Bovine Serum Albumin in phosphate and Tris-HCl buffers

The fluorescence intensity (FI) of the pure haemoglobin showed that Hb was stable in phosphate buffer under pH condition 7.2 (**Figure 1**) and BSA in TrisHCl buffer (pH 7.2) presented in Fig. 2, the spectrum was recorded with excitation at 350 nm and emission range of 220 nm-900 nm with one prominent peak was observed at 400 nm for haemoglobin, which shows the characteristic absorption of porphyrin at a soret region. Two peaks at 374 nm and 698 nm were observed for BSA (**Figure 2**), respectively. These may represent two sub-domains in BSA, which are predominantly helical and contain amino-acid sequences linked by disulfide bridges, one free thiol (Cys 34), a single tryptophan (Trp-214) and a prominent peak at 380 nm and a lighter groove at 700 nm.

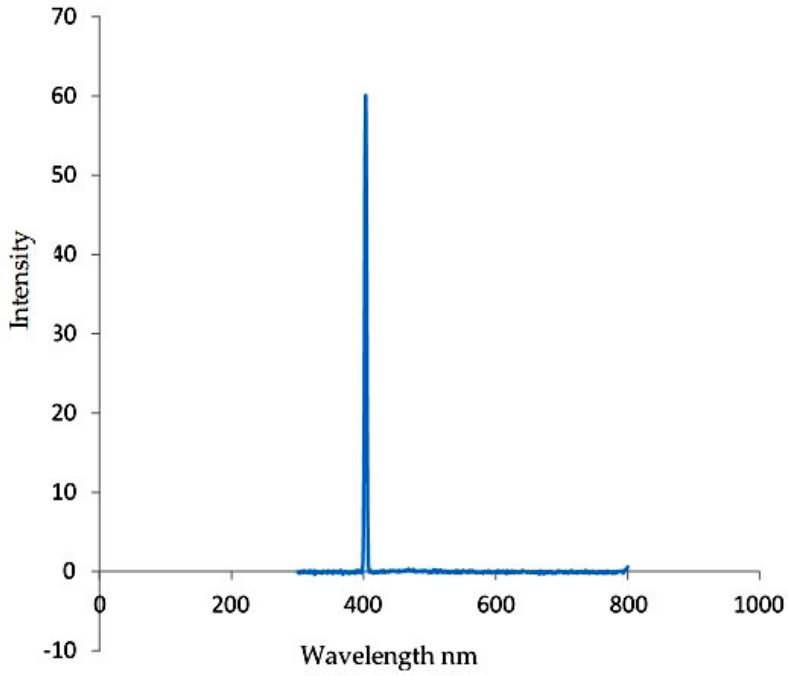


Figure 1: *Spectrum of pure haemoglobin in 0.05M phosphate buffer at pH 7.2, λ = 350 nm*

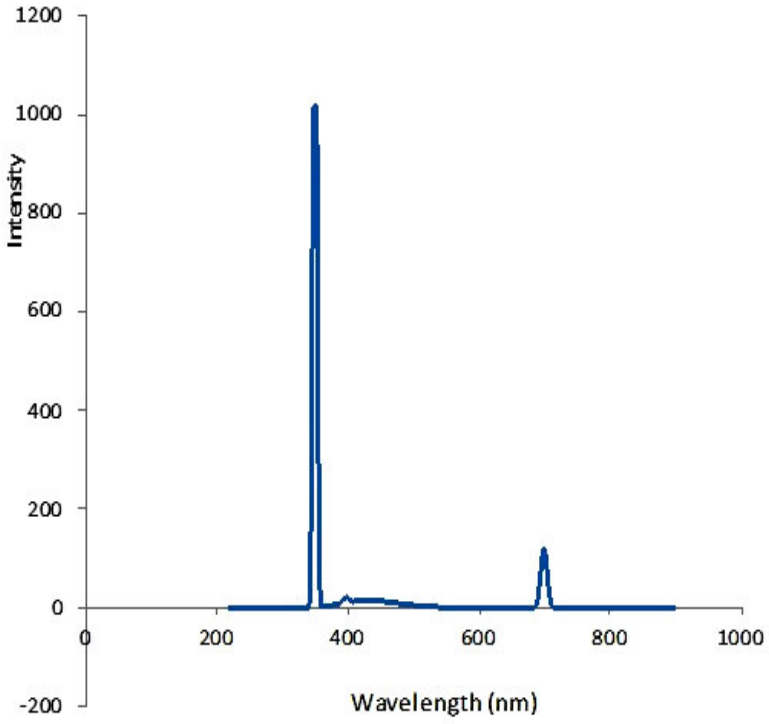


Figure2: *Spectrum of pure BSA in Tris-HCL buffer (pH = 7.2) at room temperature, λ_{ex} = 350 nm*

Effect of Increased Concentrations of CQ and AQ on Hb and BSA

In determining the effect of increased concentrations on drug-protein complex structure and structure perturbation, different concentrations of CQ and AQ were titrated against a constant concentration of Hb and BSA. The results in Figure 3 showed a non-quenching effect in a concentration-dependent pattern, whereby an increase in chloroquine concentration increases the Hb fluorescence intensity (FI). The amodiaquine-Hb complex showed structural modification and increased FI (**Figure 4**). This was attributed to the binding mode of the amodiaquine, which interacts with amino acid residues due to protein unfolding, attributed to the thermodynamic state of the reaction medium. It showed a non-quenching effect via drug-protein interaction, causing an increase in fluorescence intensity. Structurally, amodiaquine has an additional -OH group that can influence its chemical properties. This observation has been due to interactions between amino acid residues that led to structural modification via hydrogen bonding and Van der Waals forces. Protein unfolding may increase the interactions between the ligand and protein in an aqueous environment, forming a protein-ligand complex and, ultimately, changes in conformation. In addition, amodiaquine showed significant variation compared with the chloroquine mode of interactions with haemoglobin

.

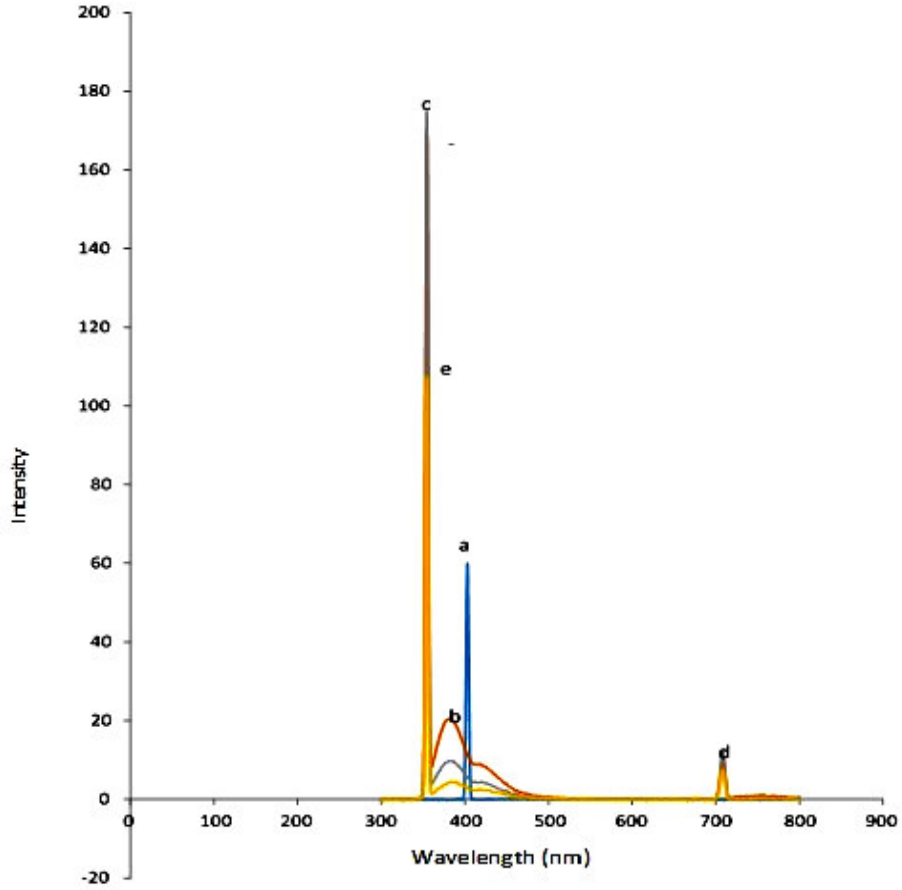


Figure 3: *Fluorescence spectra of increased chloroquine concentrations against constant haemoglobin concentration at pH 7.2.* **a = pure Hb, c = 25 mM, e = 20 mM, b = 15 mM and d = 5 mM of CQ respectively.**

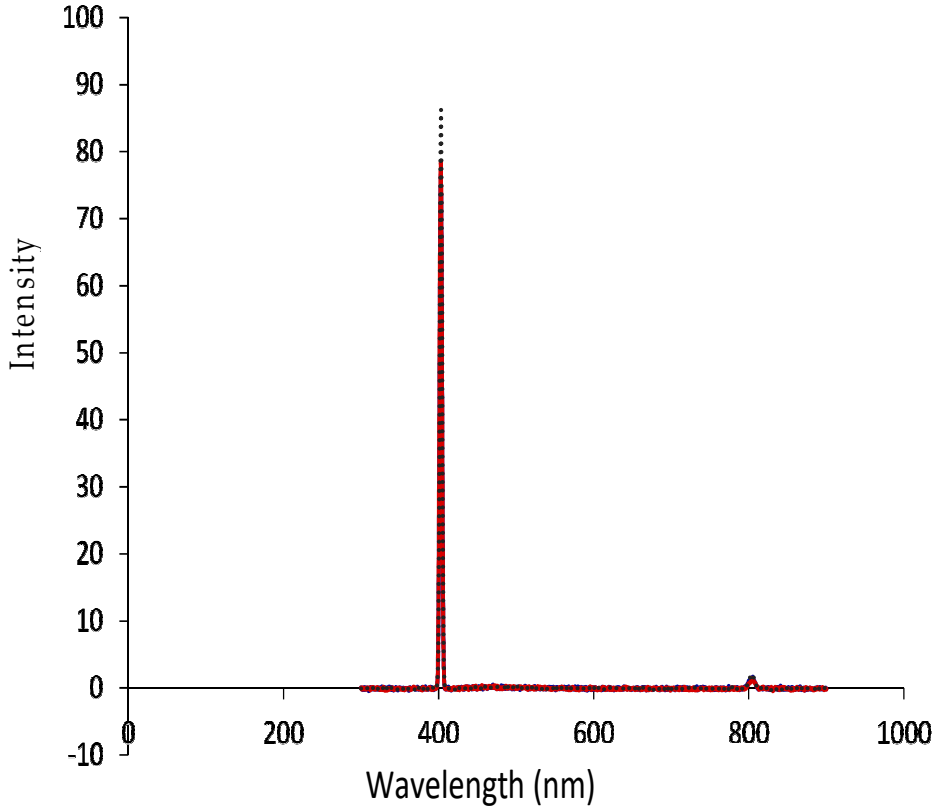


Figure 4: *Fluorescence spectra of Amodiaquine–haemoglobin complex at pH 7.2*

Different biochemical mechanisms were observed in in vitro interactions of CQ, AQ, and BSA. With increasing concentration of CQ, in a constant concentration of haemoglobin, the interaction increases in a concentration-dependent pattern (**Figure 5**). The sharp peak at 370 nm was expanded above 400 nm with a flat curve, and the second peak at 700 nm became prominent, which indicates that the CQ has influenced the spectral changes of BSA, indicating the denaturation of BSA. Similarly, AQ revealed an opposite interaction apart from CQ. Increased concentration of AQ shows that the higher the concentration, the less the fluorescence (**Figure 6**), which could indicate binding of more ligands to the amino acid residues in BSA and vice versa.

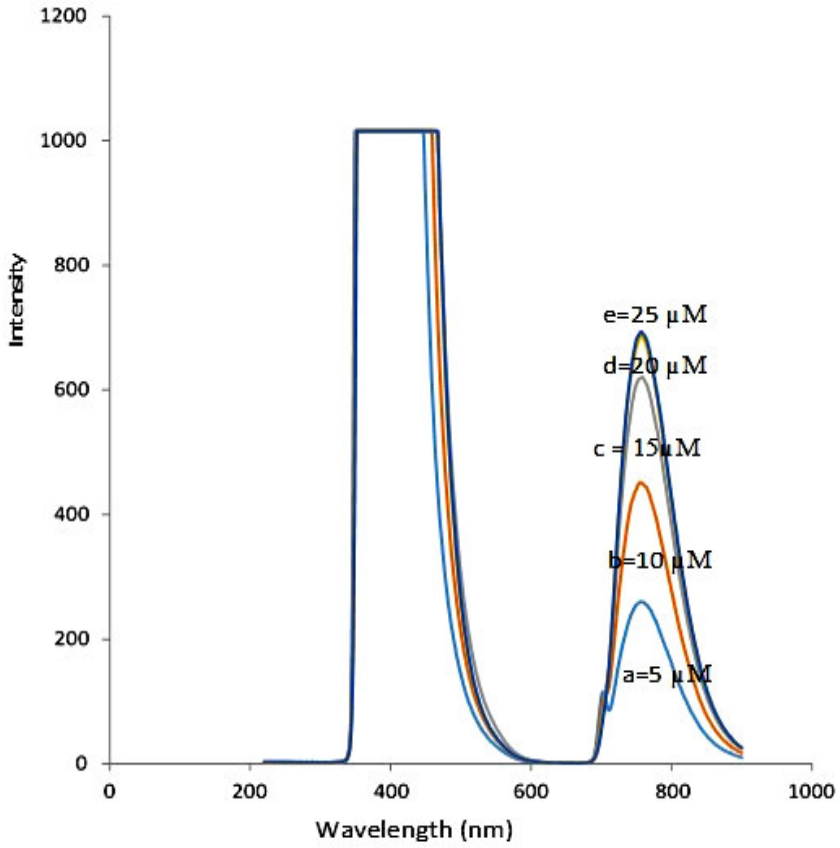


Figure 5: *BSA-drug Interactions in increasing Chloroquine concentration*

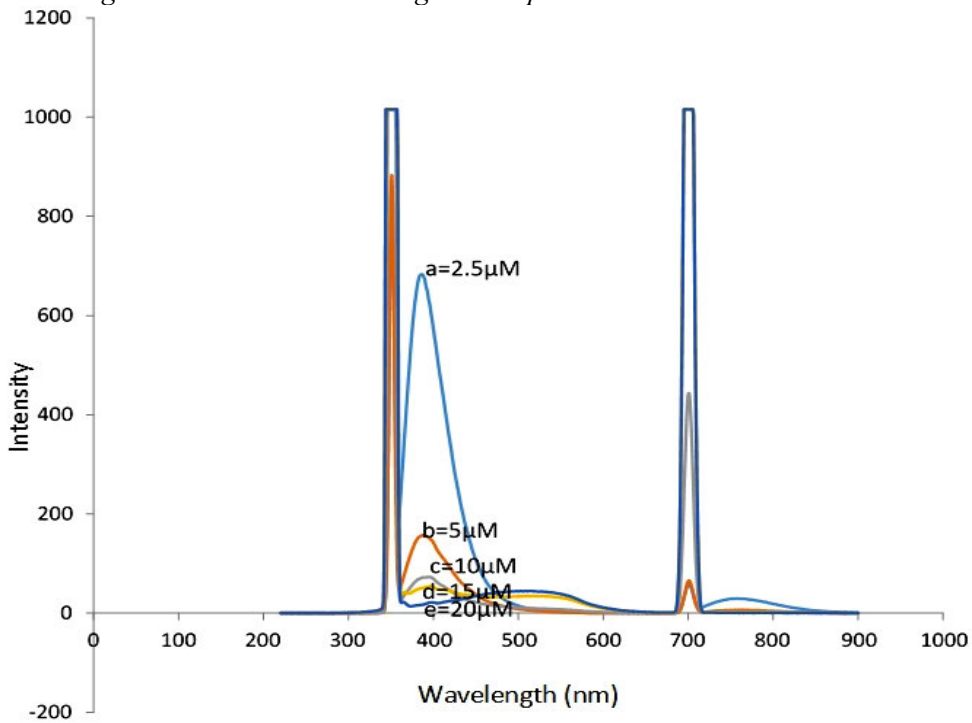


Figure 6: *BSA-drug Interactions in increasing Amodiaquine concentrations*

Effect of Temperature on BSA with AQ in Tris-HCl buffers

Temperature changes affect protein structure and functions. Thereby, thermodynamically destabilising different forces involved in structure formation. Investigating the effect of temperatures on drug-protein interaction and structural perturbation, the mixture of BSA and AQ was studied at various temperatures. The result showed an unsimilar pattern at 250 °C compared to 350 and 400 °C, which shifted the wavelength above 400 nm in the BSA-AQ interaction. This could be attributed to changes in the structure of proteins, and the number of ligand binding sites changes considerably under high temperatures (**Figure 7**). Ligand binds in more stable and thermodynamically intact proteins. The BSA-AQ binding indicated non-significant differences between 350 and 400 °C compared to 250 °C temperatures, suggesting an increase in temperature ensured structural instability, and lower temperature revealed positive cooperativity and optimum for ligand binding (**Figure 7**).

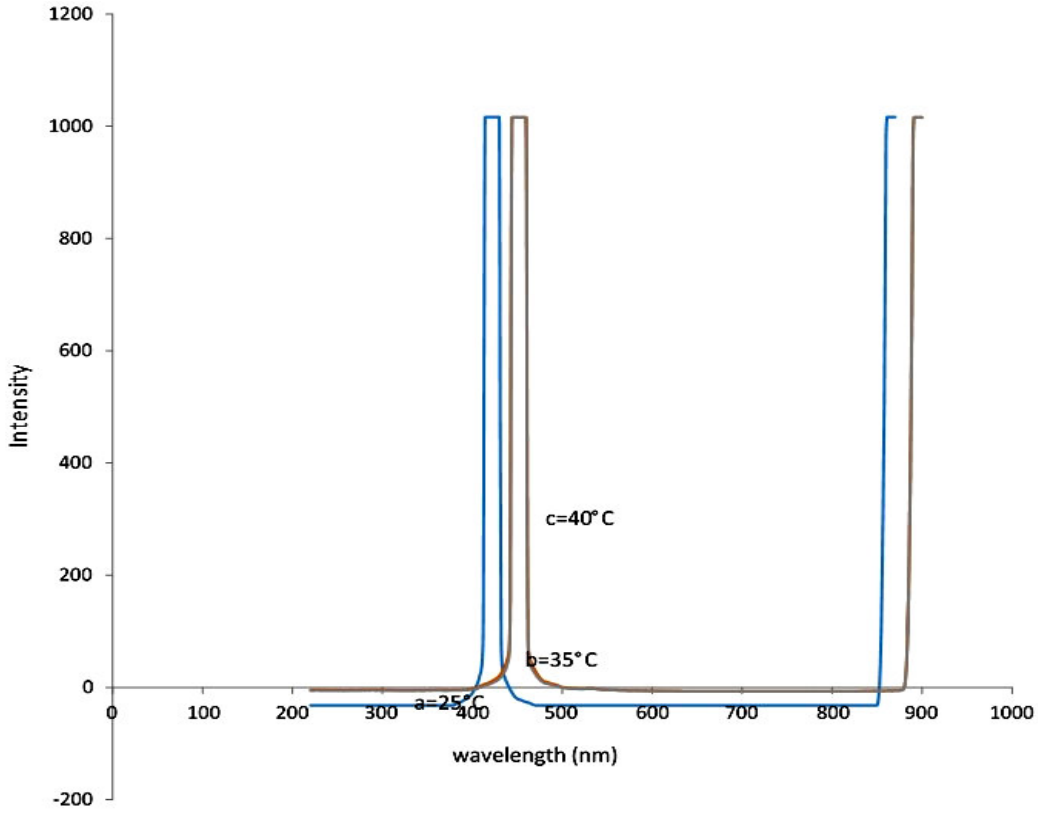


Figure 7: *Effect of Temperature (^{0}C) on the binding of AQtoBSA at λ = 350 nm.* ***Key:*** *blue curve = 25 ^{0}C, light brown curve = 35 ^{0}C and 45 °C, respectively.*

Effect of Change of pH on AQ-BSA mixture in Tris-HCl Buffer

Drug action is optimum at a given pH, and protein-drug binding is sensitive to pH variation, which affects the interaction between plasma proteins and the available drug at the site of action. A pH change induced changes in the plasma proteins and their physicochemical properties in the protein-drug interaction. The effect of changes in pH from more acidic (pH 6.2 & 8.2) to base at pH 10.2 above the arterial blood pH values between 6.7 and 7.4 indicate that the binding of the AQ to BSA caused a structural disruption and conformational changes in the structure of BSA, thereby increasing the fluorescence intensity, especially in the second subunit as presented in (**Figure 8**).

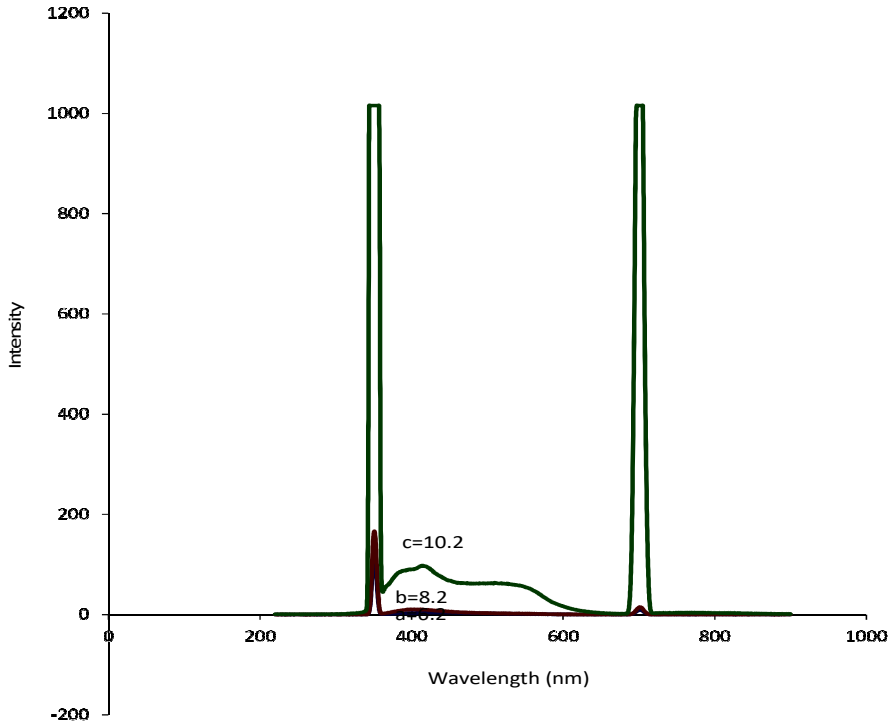


Figure 8: *Effect of increasing pH on the binding of AQtoBSA.* ***Key:*** *blue curve = pH 6.2, brown curve = pH 8.2 and green curve = pH 10.2, respectively.*

Discussion

In the present study, a modified fluorescence spectroscopic technique was used to investigate the effect of drug concentration, pH, and temperature on drug-protein interactions. The results proposed different interactions at various drug concentrations, temperatures, and pH due to denaturation, resulting in conformational changes. The interactions between biological macromolecules and drug ligands can be described in terms of the mechanisms that involve interactions and binding constants, etc. The non-quenching effect of the drug ligands causes the observed increased fluorescence intensity (**Figure 6**), which is attributed to the different molecular interactions such as excited-state reactions, complex formations, energy transfer, and molecular rearrangements [5]. The effect of pH, temperature, and serum protein concentration was reported in the in vitro studies on the binding of flutamide to human serum albumin (HAS) that show positive interactions [18]. Other positive interactions reported include codeine, morphine, and methadone binding to human serum proteins [19]. The structures of drug ligands, CQ, and AQ (**Figures 3 & 4**) may interact with amino acid residues in the binding sites of the BSA and haemoglobin, which can trigger denaturation and conformational changes of their secondary structures. The drug as a ligand may be surrounded by the α-helices structures of the proteins, which in turn form electrostatic contacts and possible hydrophobic interactions, further affecting the excitation and emission of the drug-protein complex. The increase in fluorescence intensity could indicate the positive cooperative behaviour of drug ligands at high concentrations that affect protein structure stability [18]. It might also be speculated that at low ligand concentrations, the bound proteins are relatively stable, as compared to high ligand concentrations.

The study on the effect of temperature on BSA-CQ and BSA-AQ was significant in determining the temperature-dependent binding for thermodynamic and pharmacodynamic parameters. Esmaeilzadeh et al. [18] have reported on the effects of different temperatures and determined the thermodynamic indicators and state of linearity by obtaining an average number of binding sites for flutamide-HAS at various temperatures. Also, observed that at 40^0 °C and 35^0 °C, there were significant increases in the destabilisation of the structure and hence positive interactions that led to an increase in fluorescence intensity (**Figure 8**). While at 25^0 °C, the structure of BSA was stable and showed positive cooperativity and optimum for ligand binding [19]. Positive cooperative behaviour at a high temperature of 37^0 °C on the stability of the HSA structure and a decrease at 25^0 °C were reported [18, 20].

Chloroquine and other 4-aminoquinoline drugs were reported to bind to cytoplasmic proteins at a pH of 7.4 and interfere with the parasite feeding process in the food vacuole when fully protonated [21]. Chloroquine, a weak base with pKa of 8.1 and pKa of 10.2, any change in pH would affect the drug-protein chemistry, its binding, and interactions. The interactions under different pH values were performed through fluorescence emission, as shown in **Figure 9**. The change in fluorescence intensity at various pH levels for CQ and AQ is attributed to a change in the physicochemical properties of the drug molecules. At pKa of 8.2 and 10.2, CQ and AQ may exist in a unionised form at the studied pH [20]. Therefore, it is suggested that the main factor affecting the changes in the fluorescence behaviour of drugs–BSA binding at different pH conditions has been due to allosteric changes in BSA [5]. The conformation changes in BSA structure at the pH range of 7–8.7 were known as the neutral to base or N-B transition [18]. The result suggested that at pH 10.2, the B conformation of BSA was increased compared to pH 8.2 and 6.2. Therefore, in such conditions, an affinity for the B conformation is important for increased fluorescence activity, while at pH 8.2 and 6.2fluorescence activity of CQ-BSA and AMQ-BSA bindings showed non-significant differences. At pH 7.2, which is very close to neutral pH, the N conformation of BSA may dominate. It was observed that the R state of haemoglobin may correspond to the N conformation of BSA [20]. Also, higher O2-haemoglobin interactions at the R state were described as confirmation of high cooperative interaction, and that could be the reason for high interactions between CQ, AQ, and BSA at pH 10.2 [18, 20].

In conclusion, the spectrofluorometric technique remains relevant for studying drug-protein interactions and the study of possible conformational changes in proteins. The results from the interactions of CQ and AQ with BSA and Hb suggest positive interactions and cooperative activity of CQ, AQ-BSA, and Hb binding affected by concentration, temperature, and pH. The variation of these factors would not only affect the magnitude of positive cooperation but could also interfere with the kinetic parameters, further affecting the average number of binding sites. However, no significant differences in binding of CQ and AQ-BSA at pH 8.2 and 6.2 were observed. These results could be useful for thermodynamic studies to determine the nature of interactions by establishing the binding constant Ka and n between BSA and Hb of these antimalarial drugs. To determine the effects of Hydrogen bonding and Van der Waals force, which are supposed to make a major contribution to the binding of CQ and AQ to BSA and haemoglobin for effective drug delivery into the target cells.

Conflict of Interest Statement

There is no conflict of interest among the authors.

Contribution by Authors

Lawal Almustapha designed and conducted the study, Abubakar Mu'azu Gusau supervised the work, Umar Shamsuddeen Dandare and Aminu Lailaba Abubakar reviewed the manuscript, and Wali Usman made the final draft.

Reference

[1]. Gene-Jack W, Nora D, Volkow JS, Fowler JL, Naji NA, Robert JH, Naomi SP, Kathy P. Dopamine D2 receptor availability in opiate-dependent subjects before and after naloxone-precipitated withdrawal.

Neuropsychopharmacology. 1997; 16 (2):174-182, https://doi.org/10.1016/S0893-133X(96)00184-4.

[2]. Banerjee I, Bhadury, T Self-medication practice among undergraduate medical students in a tertiary care medical college, West Bengal. *Journal of Postgraduate Medical*. 2012; 58:127–31.

[3]. Yang A, Mei-Hua M, Xiao-Hua L, Mao-Yun X. Interaction of Irbesartan with Bovine Haemoglobin Using Spectroscopic Techniques and Molecular Docking. *Spectroscopy.* 2012; 27 (2): 119–128. Doi:10.1155/2012/136287

[4]. Jahanban-Esfahlan A, Vahid P Interaction of glutathione with bovine serum albumin: Spectroscopy and molecular docking, *Food Chemistry*. 2016; 202; 426-431

[5]. Wani TA, Bakheit AH, Abounassif MA, Zargar S Study of Interactions of an Anticancer Drug Neratinib with Bovine Serum Albumin: Spectroscopic and Molecular Docking Approach. *Frontier of Chemistry*. 2018; 6: 47. doi: 10.3389/fchem.2018.00047.

[6]. Berezhkovskiy LM On the calculation of the concentration dependence of drug binding to plasma proteins with multiple binding sites of different affinities: determination of the possible variation of the unbound drug fraction and calculation of the number of binding sites of the protein. *Journal of Pharmacological Science.* 2007; 96: 249–257. DOI: 10.1002/jps.20777

[7]. Kragh-Hansen U Molecular aspects of ligand binding to serum albumin. *Pharmacological Review.* 1981; 33: 17–53.

[8]. Coatney GR Pitfalls in a discovery: the chronicle of chloroquine. *American Journal of Tropical Medicine and Hygiene*. 1963; 12**:** 121-128.

[9]. Conan NJ. The treatment of hepatic amebiasis with chloroquine, *The American Journal of Medicine.* 1949; 6(**3**): 309-320 doi.org/10.1016/0002-9343(49)90167-9.

[10]. Larrañaga N, Mejía RE, Hormaza JI *et al.* Genetic structure of *Plasmodium falciparum* populations across the Honduras-Nicaragua border. *Malaria Journal.* 2013; 12: 354. https://doi.org/10.1186/1475-2875-12-354

[11]. Mauthe M, Orhon I, Rocchi C, Zhou X, Luhr M, Hijlkema KJ, Reggiori F. Chloroquine inhibits autophagic flux by decreasing autophagosome-lysosome fusion. *Autophagy.* 2018; 14:1435–1455.

[12]. Picot S Coalition: advocacy for prospective clinical trials to test the post exposure potential of hydroxychloroquine against COVID-19. *One Health.* 2020; 9:100131.

[13]. Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R Effects of chloroquine on viral infections: an old drug against today's diseases? *Lancet Infect. Dis.* 2003; 3: 722–727.

[14]. Bishop NE, Anderson DA. Early interactions of hepatitis A virus with cultured cells: viral elution and the effect of pH and calcium ions. *Archived of Virology.* 1997; 142: 2161–2178.

[15]. Devaux CA, Rolain JM, Colson P, Raoult D New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19? *International Journal of Antimicrobial Agents.* 2020; 55(5):105938.

[16]. Jiani M, Chen L, Yiwen Z, Zhikang W, Shanshan L, Yuming G, Lin Z, Hui L, Xin G, Jiangning SM A unified multi-view graph autoencoder-based approach for identifying drug–protein interaction and drug repositioning, *Bioinformatics*. 2023; 39(9):524 DOI: /10.1093/bioinformatics/btad524

[17]. Yang B, Kirby S, Lewis J, Detloff PJ, Maeda N, Smithies OA Mouse model for beta 0-thalassemia. *Proceeding of National Academy of Science U S A*. 1995; 92(25):11608–11612.

[18]. Esmaeilzadeh S, Valizadeh H, Zakeri-Milani P The effects of pH, temperature and protein concentration on the in vitro binding of flutamide to human serum albumin. *Pharmaceutical Development Technology.* 2017; 22(8):982-991. doi: 10.3109/10837450.2016.1

[19]. Judis J Binding of codeine, morphine, and methadone to human serum proteins. *Journal of Pharmaceutical Science.* 1977; 66(6):802-6. doi: 10.1002/jps.2600660615.

[20]. Damodaran S, Kinsella JE The effects of neutral salts on the stability of macromolecules. A new approach using a protein-ligand binding system. *Journal of Biological Chemistry*. 1981;

256(7): 3394-3398, https://doi.org/10.1016/S0021-9258(19)69621-3.

[21]. Jaap W, Betsy JTH, Johan J, De G The Role of Albumin Conformation in The Binding of Diazepam to Human Serum Albumin. *Biochimica et Biophysica Acta*. 1980; 626: 291—298.

[22]. Parhizgar AR, Tahghighi A Introducing New Antimalarial Analogues of Chloroquine and Amodiaquine: A Narrative Review. *Iran Journal of Medical Science.* 2017; 42(2):115-128.

Effect of the Smoke of Natural Insecticides: Seeds of *Azadirachta Indica* and Peels of *Citrus Sinensis* on Kidneys of Albino Rats

*Bello, A. M.,[1] Adam, F. L.,[1] Babagana, K.,[1] & Wudil, A. M.[1]

[1]Department of Biochemistry, Faculty of Basic Medical Science, Bayero University, Kano
Corresponding Author: Aliyu Maje Bello,
Corresponding Email:ambello.bch@buk.edu.ng

Abstract

The increasing use of plant-derived products as natural insecticides is largely due to their affordability and perceived safety compared to synthetic chemical pesticides. However, their potential toxicological effects on vital organs remain poorly understood. This study investigated the comparative effect of smoke from *Azadirachta indica* (neem) seeds, *Citrus sinensis* (orange) peels, and their combination on the kidney of albino rats. Twenty-one rats were divided into seven groups: a control group and six treatment groups exposed daily to the smoke of neem seeds, orange peels, or their mixture for 1 or 2 hours over four weeks. Renal function was assessed using serum biochemical markers—urea, creatinine, total protein, and electrolytes (Na^+, K^+, Cl^-)—while histopathological examination of kidney tissues was conducted to evaluate structural alterations. The results revealed a significant elevation ($p < 0.05$) in serum urea, creatinine, sodium, and potassium levels in rats exposed for two hours daily, particularly in the group treated with *Azadirachta indica* seed smoke. Combined exposure to both plant materials also produced notable biochemical alterations, suggesting a possible synergistic effect. In contrast, one-hour exposure resulted in comparatively mild or non-significant changes. Histopathological analysis demonstrated vascular congestion and early renal alterations in rats subjected to prolonged exposure, while kidney architecture remained largely preserved in short-duration exposure groups. The control group displayed normal hepatic architecture. These findings demonstrate that inhalation of smoke from *Azadirachta indica* seeds and *Citrus sinensis* peels can induce dose- and duration-dependent renal dysfunction, despite their natural origin. The study highlights potential public health concerns associated with the domestic use of plant-based insecticides and underscores the need for safety awareness and regulatory consideration.

Keywords: *Azadirachta indica*, *Citrus sinensis*, natural insecticides, nephrotoxicity, albino rats, histopathology

1.0 Introduction

Insecticides are agents of chemical or biological origin that control insects. Control may result from killing the insects or otherwise preventing them from engaging in behaviour deemed destructive.[1] The ideal insecticide is a chemical which stays at the place of application through its active period; it is toxic to particular pests but harmless to other organisms, including man, is easily used; it is able to break down into harmless products in the environment within a reasonable time and must at the same time be cheap to produce. No chemical with all these properties is known at present.[2] The importance of pesticides in food production and public health cannot be underestimated, nor can some of the hazards involved in the use of pesticides as practised today be ignored. [3]

Peasant farmers have traditionally used a wide variety of local products with insecticidal properties; they are minerals, oils, and vegetable extracts from plants and trees. [4] The use of plant extract to control destructive insects or disease vectors is not new. Rotenone (*Derris SPP*), nicotine and pyrethrins have been used for a considerable time in small – scale subsistence and also commercial agriculture. Recently, considerable effort has been put into the development and promotion of plant-based methods and products for the control of pests.[5] Most modern insecticide acts on the nervous system and enter the insect body either by contact or through the gut or by having a fumigant action. Several insecticides enter the body in more than

one way. Nervous activity involves the transmission of nerve impulses along nerve fibres (axons) and across the synapse to another nerve cell by means of a transmitter substance. The major transmitter substances are adrenaline and acetylcholine, and these are normally inactivated by specific enzymes.[2] Resistance is probably the biggest single obstacle in the struggle against vector–borne diseases and is mainly responsible for preventing successful malaria eradication in many countries.[2] Resistance has been reported not only to the most recent insecticide but also to insect growth regulators, chemosterilants and even biological control agents.[2] Resistance to some insecticides is known to be associated with high levels of carboxylesterase.

Azadirachta indica (neem tree) is a fast-growing ornamental shade tree. For thousands of years, Indian farmers have been aware of the insecticidal properties of the neem tree. Its branches were hung in granaries to protect stored grain from insect attack. Historically, neem has also been used in India for both cosmetic and medicinal purposes. Although Neem extract/oil has been in use for centuries for control of insects, the major work on Neem oil and its impact on insects started in 1959.[6] A German Entomologist observed that during a plague of locusts in Sudan, the only greenery left untouched despite the devastation by billions of winged locusts was the Indian Neem tree. He noticed that although locusts had landed on the tree and its leaves, they did not feed. The antifeedant properties of the neem tree, which were well known in India, were the reason.[6] Recent research indicates that there are several active compounds, which are mostly concentrated in the seeds. Some of them inhibit larval development and reduce female fertility in various insect species by blocking insect hormones, while others act as repellents or antifeedants.[7] Azadirachtin, the active ingredient in many pesticides currently available, is extracted from the seed kernels. Azadirachtin consist of more than 25 different but closely related compounds.[8]

Oral administration of neem oil at 200mg/rat produces a severe hypoglycaemic effect. [9] Neem seed oil showed acute toxicity in rats and rabbits with LD_{50} of 14ml/Kg and 24ml/Kg respectively, the possible target organs for toxic effects being the liver, kidneys, CNS and the lungs. Neem seed oil produces toxic effects in humans in several isolated cases due to the neem oil intoxication by humans, which produces diarrhoea, nausea, vomiting, acidosis, encephalopathy, etc.[9] Mechanistic investigations indicate that neem oil uncouples mitochondrial oxidative phosphorylation, thus inhibiting the respiratory chain. It also decreases intramitochondrial levels of acetyl-CoA and acid soluble coA esters and reduces the mitochondrial ATP content.[9]

Citrus is a common term and genus of flowering plant in the family *Rutaceae*, originating in tropical and subtropical southeast regions of the world. One of the most widely favoured of the world's fruits, the orange sweet orange, or round orange, was for many years known as *citrus aurantium var. sinensis L.* and considered to be a form of the sour orange. It is still not universally agreed to be a distinct species, *C. sinenis* Osbeck, but it is usually treated as though it were. Volatile constituents and flavonoids of extracts of citrus fruits have been suggested to act as defences against fungi and insects. The essential oil from the fruit peels of different varieties of *Citrus sinensis* was collected by a water stream. And bioassays were performed in order to determine the possible use of these compounds as a wood preservative.[10] The active ingredient D-limonene destroys wax coatings of the insect's respiratory system when applied directly, and the insect suffocates. The citrus fragrance also acts as a repellent. The EPA calls D-limonene a broad-based insecticide. It can be used for aphids, ants, mealybugs, gnats, silver fish, fleas, mosquitoes, cockroaches and house flies.[11]

Persons in close proximity to orange trees in bloom may have adverse respiratory reactions. Sow dust of the wood of orange trees, formerly used for polishing jewellery, has caused asthma. Excessive contact with the volatile oils in orange peel can produce dermatitis. People who suck oranges often suffer skin irritation around the mouth.[12] Limonene is moderately toxic by ingestion, and its poisoning may affect the kidneys. The oral LD_{50} is 5g/Kg for a rat and 5.6g/Kg for a mouse. Local

effects include irritation to the eye, skin and respiratory tract. Acute exposure may cause sore throat, coughing, shortness of breath (diptheria, dizziness and nausea. Limonene is also a possible carcinogenic agent.[12]

In previous years and even presently in some villages, people are in the habit of using some local or natural plant products/parts to control insect pests without being informed about the side effects of such products on the vital organs of the body (such as liver, heart and kidney). Therefore, this research aimed at determining the effects of the seeds of *Azadirachta indica* and peels of *Citrus sinensis* on the kidneys of albino rats, and also to provide an alternative means of insect pest control due to the high cost, less availability, high toxicity and resistance associated with the synthetic chemical insecticides.

2.0 Materials and Methods

2.1 Collection and Preparation of Samples

The fruits of *Azadirachta indica* (neem) were collected from Bayero University, Kano, which is within the Gwale local government of Kano state. Whereas the peels (bark) of *Citrus sinensis* (sweet orange) were collected from "Hauren wanki" of Gwale Local Government of Kano state. Both the fruits and the peels were identified at the Botanical Garden of the Department of Biological Sciences, Bayero University, Kano. The seeds of the *Azadirachta indica* were exposed in the fleshy fruits. Both the seeds and peels were sun-dried (in order to reflect the actual traditional processing procedures) and ground to a powder form using a pestle and mortar. The powder was then stored in a well-dried and clean container at room temperature.

2.2 Experimental Design

Twenty-one healthy males and female white albino rats of an inbred Novergicus strain (*Rattus novergicus*) weighing between 120g and 240g were obtained from the Animal Holding Unit of the Department of Biological Sciences, Faculty of Sciences, Bayero University Kano and the Animal Holding Unit of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria. The rats were housed in a well-ventilated environment (Temperature: 25-30^{o}; Photoperiod: 12 Hours natural light and 12 Hours dark), kept in aluminium cages (Dimensions 39.50cm by 25.30cm by 14.80cm) with saw dust at the bottom of the cages and they were fed with Animal Pelletized Feed (Growers Feed) made in "Bukuru" Jos, Plateau State with free access to water. The rats were distributed on the basis of their weight into seven groups (1 to 7) of three rats each.

Group 1, which served as the control were given only food and water. The animals in the other groups (2 to 7) were exposed to the smoke of the local insecticides in an inhalation chamber (Dimensions 119cm by 61cm by 55cm). Groups 2 and 3 were exposed to the smoke of 50g of neem seeds for one hour and two hours, respectively. Groups 4 and 5 were exposed to the smoke of 50g of orange peels for one and two hours, respectively. Then, Groups 6 and 7 were exposed to the smoke of a combination of neem seeds and orange peels at a 50:50 ratio for the duration of one hour and two hours, respectively. The treatment was carried out daily for a period of four weeks.

All the rats were sacrificed on the 29th day. Blood samples were collected into dried and clean centrifuge tubes, allowed to clot and centrifuged, while the kidney tissue was collected and preserved in 2.5% formal saline in a sterilised container for histological studies. The sera were collected into plain serum bottles for laboratory investigations.

2.3 Determination of Serum Urea (Urease-Berthelot Methods)

Serum urea level was determined using commercial kits (Randox ltd., U.K) as described by Berthelot, [13] following the manufacturer's instructions.

2.4 Determination of Serum Creatinine (Jaffe`S Method)

Serum creatinine level was determined using commercial kits (Randox ltd., U.K.) as described by Jaffe [14], following the manufacturer's instructions.

2.5 Determination of Serum Total Protein (Biuret Method)

Serum total protein level was determined using commercial kits (Randox ltd., U.K.) as described by Flack and Wollen (1984). [15], following manufacturer's instructions.

2.6 Determination of Serum Electrolytes

SODIUM

Serum sodium level was determined using the Teco diagnostic kit as described by Maruna (1958) [16], following the manufacturer's instructions.

POTASSIUM

Serum potassium level was determined using Teco diagnostic kits as described by Terri *et al.* (1958) [17], following the manufacturer's instructions.

CHLORIDE

Serum chloride level was determined using Teco diagnostic kits as described by Skeggs and Hochstrasser (1964) [18], following the manufacturer's instructions.

2.7 Histopathological Studies of the Kidneys.

The biopsies of the kidneys of albino rats were fixed with 10% formal saline, dehydrated with ascending grades of alcohol, cleared with toluene, and infiltrated with molten paraffin wax. The microtome sections were stained with the haematoxylin and eosin staining technique.[19] The sections were examined under a light microscope.

2.8 Statistical Analysis

Statistical analysis was performed using SPSS software package version 3.05. The values were analysed by one-way analysis of variance (ANOVA). All the results were expressed as mean ± S.D in each group.

3.0 Results

The effect of exposure of experimental rats to the smoke of natural insecticide powders: seeds of *Azadirachta indica*, peels of *Citrus sinensis* and the combination of the two on the serum levels of urea, creatinine, total protein, sodium, potassium and chloride were shown in figures 1 to 6.

3.1 Effect of *A indica*, Peels of *C sinensis* smoke and their combination on serum level of urea in albino rats

The results show that only groups of the animals that are exposed to the smoke of the seeds of *A indica* have significantly higher serum levels of urea when compared to the control group at 1 hr exposure. However, all the 3 groups showed a significant increase in serum urea levels compared to the control when exposed for 2 hrs. As expected, prolonging the duration of exposure from 1 hr to 2 hrs increases the serum urea levels.

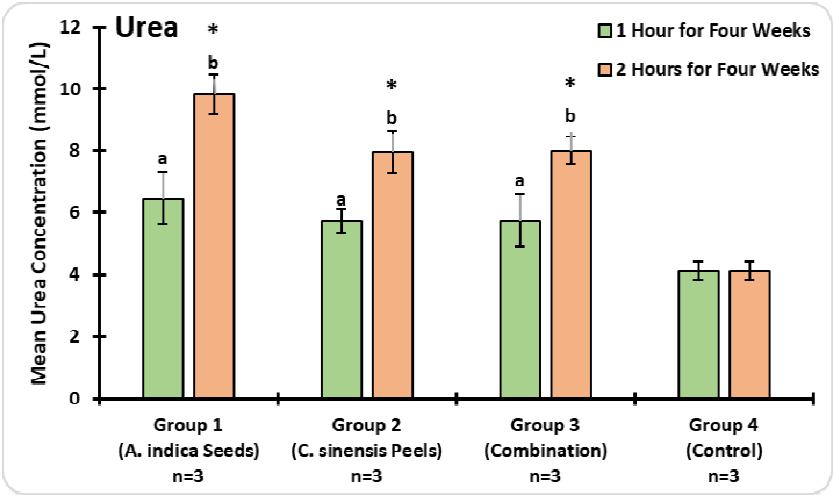


Figure 1. *Serum urea levels of albino rats exposed to the smoke of three different natural/local insecticides (Seeds of A indica, Peels of C sinensis and their combination) for 1 and 2 hours daily for four weeks*

3.2 Effect of *A indica*, Peels of *C sinensis* smoke and their combination on serum level of creatinine in albino rats.

The results showed that the serum creatinine levels of groups 1, 2 and 3 for the duration of 1 hr were not significantly different from those of the control *C sinensis* and the combination of the two insecticides. group. However, when the duration of exposure was prolonged to 2 hrs, the serum creatinine levels of the three groups were significantly higher than those of the control. Additionally, *A indica* shows the highest effect compared to

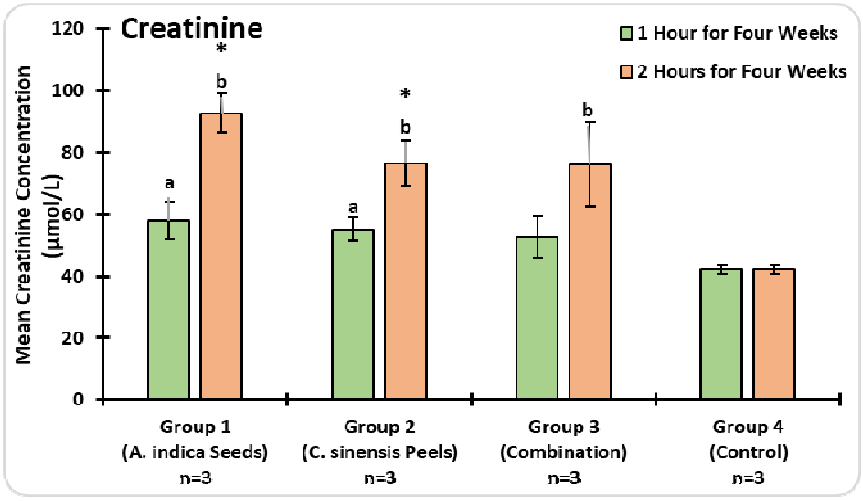


Figure 2. *Serum creatinine levels of albino rats exposed to the smoke of three different natural/local insecticides (Seeds of A indica, Peels of C sinensis and their combination) for 1 and 2 hours daily for four weeks*

3.3 Effect of *A indica*, Peels of *C sinensis* smoke and their combination on serum level of total protein in albino rats.

The results showed that the serum creatinine levels of groups 1, 2 and 3 for the duration of 1 hr were not significantly different from those of the control group. However, when the duration of exposure was prolonged to 2 hrs, the serum creatinine levels of the three groups were significantly higher than those of the control. Additionally, groups exposed to the smoke of *A indica* shows significant increase in total protein compared to groups exposed to *C sinensis* and the combination of the two insecticides

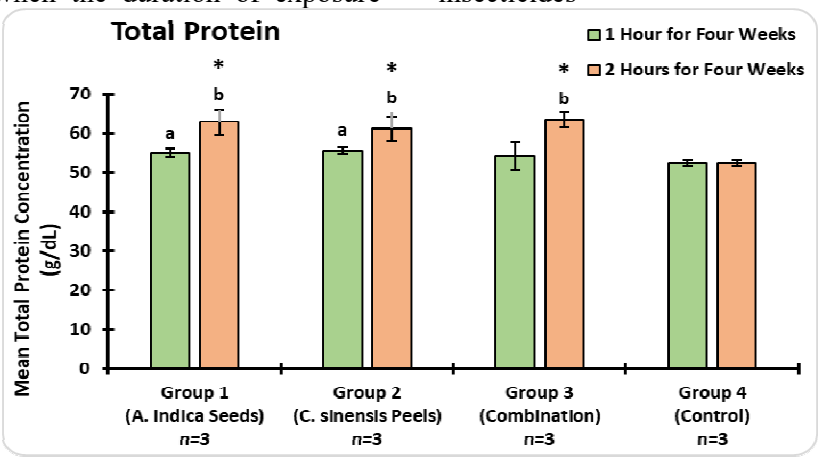


Figure 3. *Serum total protein levels of albino rats exposed to the smoke of three different natural/local insecticides (Seeds of A indica, Peels of C sinensis and their combination) for 1 and 2 hours daily for four weeks*

3.4 Effect of *A indica*, Peels of *C sinensis* smoke and their combination on serum level of sodium (Na) in albino rats.

The serum sodium levels of all three groups of rats were significantly higher than those of the control for both 1 hr and 2 hrs exposure. In addition, groups exposed to the smoke of *A indica* show the highest increase in sodium levels compared to groups exposed to *C sinensis* and the combination of the two insecticides.

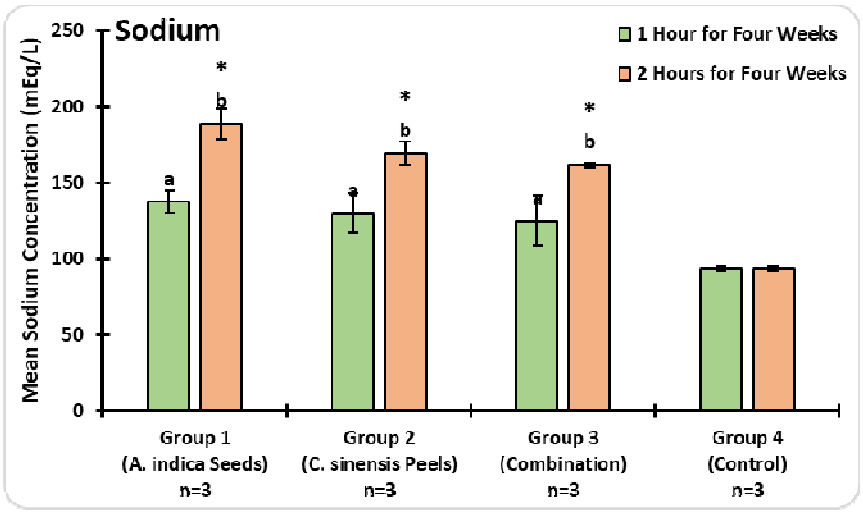


Figure 4. *Serum sodium (Na) levels of albino rats exposed to the smoke of three different natural/local insecticides (Seeds of A indica, Peels of C sinensis and their combination) for 1 and 2 hours daily for four weeks*

3.5 Effect of *A indica*, Peels of *C sinensis* smoke and their combination on serum level of potassium (K) in albino rats.

Groups 1, 2 and 3 showed a significant increase in serum potassium level when compared to the control group at 2 hrs exposure. However, there is no significant increase in potassium levels when all three groups of animals are exposed for 1 hr.

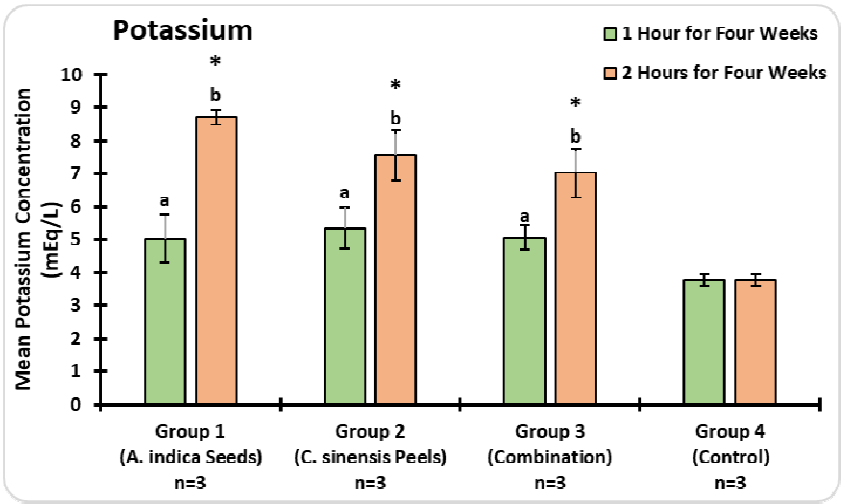


Figure 5. *Serum potassium (K) levels of albino rats exposed to the smoke of three different natural/local insecticides (Seeds of A indica, Peels of C sinensis and their combination) for 1 and 2 hours daily for four weeks*

3.6 Effect of *A indica*, Peels of *C sinensis* smoke and their combination on serum level of chloride (Cl) in albino rats.

The results showed that the serum chloride levels of groups 1, 2 and 3 for the duration of 1 hr were not significantly different from those of the control group. However, when the duration of exposure was prolonged to 2 hrs, the serum chloride levels of the three groups were significantly higher than those of the control. Additionally, groups exposed to the smoke of *A indica* show the highest increase compared to groups exposed to *C sinensis* and the combination of the two insecticides.

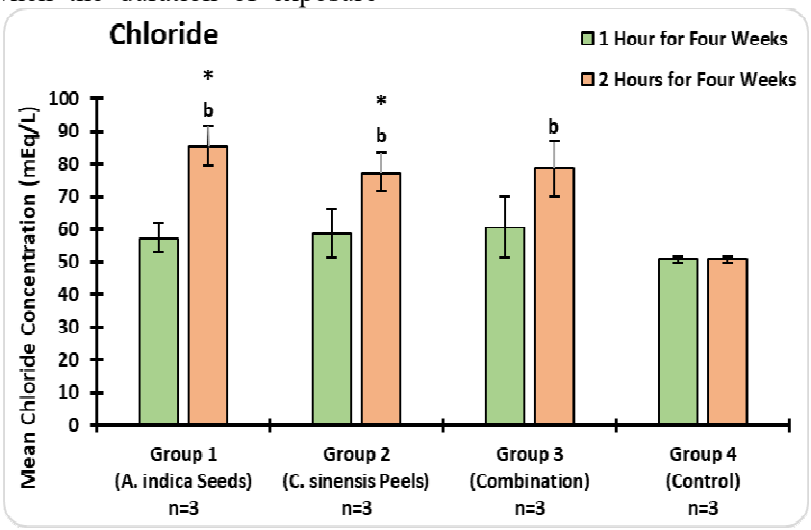


Figure 6. *Serum chloride (Cl) levels of albino rats exposed to the smoke of three different natural/local insecticides (Seeds of A indica, Peels of C sinensis and their combination) for 1 and 2 hours daily for four weeks*

3.7 Histopathological studies of the Liver

The results of histological studies on kidney tissues revealed mild hepatocyte tissue damage in only the group of rats exposed to the smoke of the seeds of *A indica* for two hours. Green arrows indicated the region of vascular congestion. Control group and groups exposed to the smoke of natural insecticides for 1 hr show normal hepatocytes arranged in cords radiating from central venules.

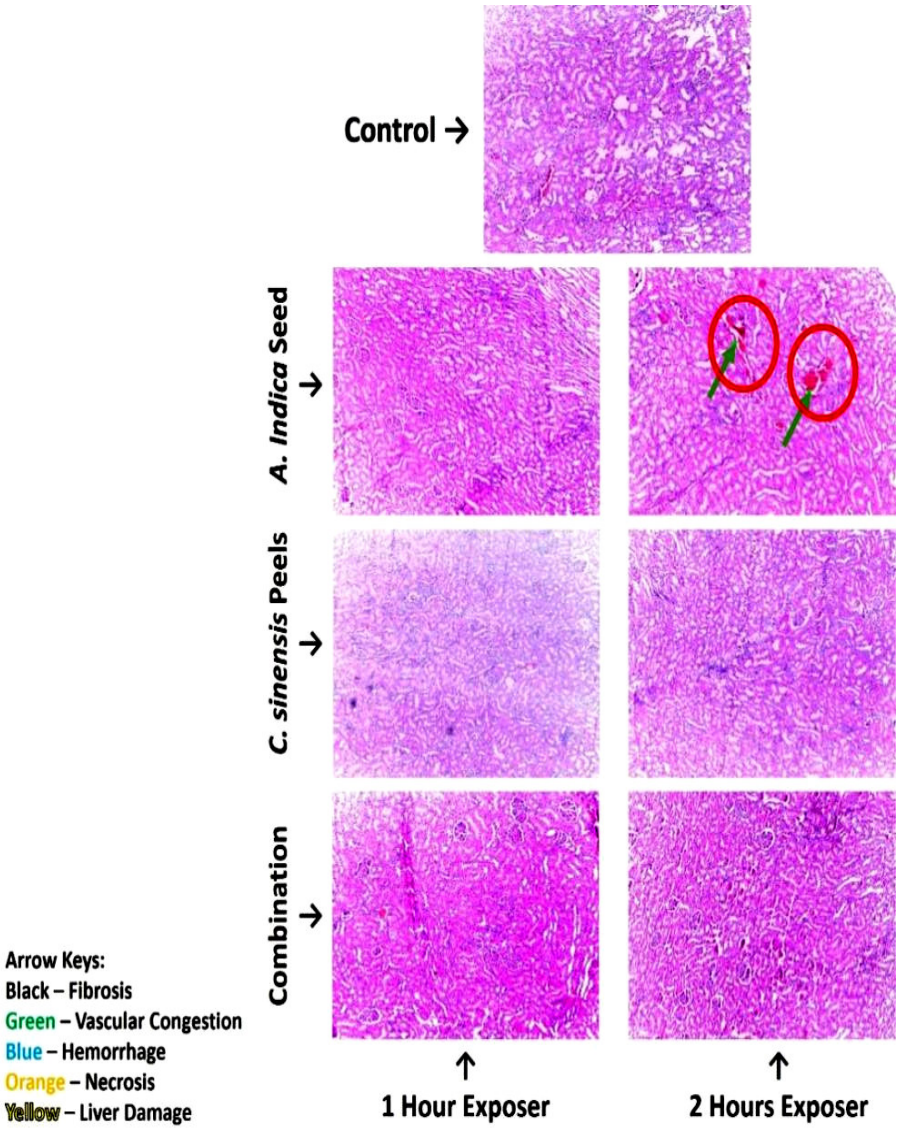


Figure 4. *Photomicrograph of a section of kidneys from rats exposed to the smoke of A indica, C sinensis and their combinations for 1 and 2 hrs duration. Note: The normal microscopic appearance of the kidney section. H & E stain x 100. Green arrows indicate vascular congestion.*

4.0 Discussion

The present study investigated the comparative nephrotoxic effects of the smoke from *Azadirachta indica* (neem) seeds, *Citrus sinensis* (orange) peels, and their combination on albino rats. The findings demonstrated that exposure to the smoke of these natural insecticides caused significant alterations in urea, creatinine, total protein and electrolytes, especially in those rats that were exposed for two hours of treatment ($P<0.05$). There are also mild histopathological changes in kidney tissue.

Serum electrolytes are used in maintaining the cellular tonicity, fluid balance, pH and regulation of neural and muscular functions. [20] The results of serum electrolytes showed an increase in the uptake of sodium ions both in one and two hours, chloride and potassium demonstrated a significant increase ($P<0.05$) in two hours only. This implies that the integrity of the kidney in boosting these cations and anions may not have been impaired severely at the macro-anatomical level. Thus, abnormal concentration of sodium in serum can

affect the osmotic pressure of the body fluid, which is related to blood pressure. [20]

The histopathological studies showed mild to no observable tissue damage in the treatment groups when compared with the control group because acute renal failure, indicated by increased creatinine and urea, occurred before the development of tubular necrosis. These parameters are markers of glomerular filtration rate. All the natural insecticides can be able to alter the biochemical parameters, but not to the extent of causing damage to the renal tissue. However, the kidney of the group treated with the smoke of seeds of *A indica* (Group 1) revealed some mild histological changes when compared with the control group, which shows a normal histological appearance.

Architectural appearance of the kidney in group 1 revealed different degrees of vascular congestion; this might be due to longer duration of exposure and presence of some toxic phytochemicals. Thus, the results highlighted that seeds of *Azadirachta indica* might be more toxic than peels of *Citrus sinensis* and the combination of the two natural insecticides. Damages to the kidney caused either by chemical agents or drugs could be manifested as vascular congestion (glomerulus). [21] Moreover, group 1 have higher value of the biochemical parameters, which could be due to the presence of active ingredients or phytochemicals in the neem seeds and peels of Citrus sinensis. The findings of a significant increase in biochemical parameters of the kidney in this study agree with the findings of [22], indicating impaired kidney function. A similar finding was reported in a study investigating the safety evaluation of neem (Azadirachta indica) derived pesticides. [23] The toxic action of the oil present in the seeds may have been due to the synergistic effects of aflatoxins and other components in the oil. Gandhi *et al*. [24] reported acute toxicity after ingestion of the oil by rats and rabbits.

5.0 Conclusion

This study has demonstrated that exposure to the smoke of *Azadirachta indica* seeds, *Citrus sinensis* peels, and their combination, especially two hours of exposure could exert deleterious effects on the kidneys of albino rats. Thus, an increase in biochemical parameters of the kidney and the appearance of vascular congestion in the histopathological studies indicate the toxic effects of these natural insecticides at the specific duration of exposure studied. The findings highlight that, although these plant products are traditionally perceived as safer and more affordable alternatives to synthetic insecticides, their prolonged use in the form of smoke carries potential health hazards. Therefore, caution should be exercised in their application for household or agricultural purposes.

References

1. Ware, W. and Whitacre, M. (2004). Introduction to Insecticides, the Pesticides Book. 6th edition. Meisterpro Information Resources. http://www.ipmworld.edu
2. Kumar, R. (1984). Insect pest control. 1st edition. Edward Arnold Publishers. Pp: 166-220.
3. Quraishi, M.S. (1977). Biochemical Insect Control (Its Impact on Economy, Environment and Natural Selection) 1st edition. a Wiley Interscience Publication. Pp: v and 3-250.
4. Ukoroije, R. B., & Otayor, R. A. (2020). Review on the bio-insecticidal properties of some plant secondary metabolites: types, formulations, modes of action, advantages and limitations. *Asian Journal of Research in Zoology*, *3*(4), 27-60.
5. Stoll, G. (2000). Natural Crop Protection in the tropics. 2nd edition. A Margraf Verlag Publication. Pp: 133-144.
6. Mahmood, T. (2009). The Neem Tree (a miracle plant), Planet Natural. http://www.planetnatural.com/site.html
7. Dreyer, M. (2009). Neem Insecticidal Uses (Some Practical Information including Oil Production and Storage of Seeds). http://wwwrunetwork.de/html/en/articles/document.html?action=displayDocument&id=1570
8. IPM (2009). Neem-Based Insecticides. http://www.hort.uconn.edu/ipm/style/menu.htm

9. Biswas, K., Ishita, C., Ranajit, K.B. and Uday. B. (2002). Biological Activities and Medicinal Properties of Neem (Azadirachta indica), Department of Physiology, Indian Institute of Chemical Biology. Kolkata, India, Pp 1336-1345.
10. Macias, F.A., Ascension, T., Maya, C.C and Fernandez, B. (2002). Natural Biocides from Citrus Waste as New Wood Preservatives. http://www.regional.org.au/au/allelopathy/2005/index.htm
11. Ezeonu, F.C., Chidume, G.I. and Udedi, S.C. (2001). Insecticidal Properties of Volatile Extract of Orange Peels. http://cat.insit.fr/?amodele=presentation
12. Steward, D. (1994). Toxic Effect of Limonene, Occupational Safety and Health Administration (OSHA). US Department of Labour.
13. Reitman, S. and Frankel, S. (1957). A Colourimetric Method for the Determination of Serum Glutamate-Oxaloacetate and Pyruvate Transaminase. Am. J. Clin. Path. 28:56.
14. Jaffe, B. (1972). What made the radical break? N. Engl. J. Med. 286-156.
15. Flack, C.P. and Woollen, J.W. (1984). Clin.Chem., 30-559
16. Maruna, R.F.L. (1958). Clin. chem. Acta. 2:581.
17. Terri, A.E., Sesin, P.G., Am. J. Clin. Path., 29:86(1958).
18. Skeggs, L.T., Jr and Hochstrasser, H. (1964). Multiple automatic sequential analysis. Clinical chemistry.10:918-936.
19. Auwioro, O. G. (2010). Histochemistry and tissue pathology principles and techniques, 2nd Edition. *accessed at ijs. oauife. edu. ng*, 561-568.
20. Cheesbrough, M. (2000). District Laboratory Practice in Tropical Countries. Part 2, Cambridge University Press.
21. Eroschenko, V.P. (2000). Atlas of Histology with Functional correlation ninth edition. Williams and Wilkins, Lippincott, P.R.
22. Biu, A.A., Yusufu, S.D., Rabo, J.S. (2010). African Scientist 11, no.4.
23. Sara, J., Boeke Marelle, G., Boersma Gerrit, M., Alink Joop, J.A., Van Loon Arnorld, Van Huis, Marcel Dicke, Ivonne, Rietjens, M.C.M. (2004). Safety evaluation of neem-derived pesticides. *Journal of Ethnopharmacology* **94**:25-41.
24. Gandhi, M., Lal, R., Sankaranarayana, A., Banerjee, C.K., Sharma, P.L. (1988). Acute toxicity study of the oil from Azadirachta indica seed (neem oil). Journal of Ethnopharmacology. 23:39-51.