Nigerian Research Journal of Chemical Sciences (ISSN: 2682-6054)
Volume 12, Issue 1, 2024

Biochemical Effects of Methanol Extract of *Picralima nitida* Seed on Alloxan-Induced Diabetic Male Albino Rats

*[1]Prince Ogochukwu Alaebo, [1]Ngozi Kalu Achi, [2]Udumma Nsofor Onuoha, [1]Chiemeziem Adanma Obike, [1]Uchechukwu Daniel Chukwu, [1]Wisdom Enyinna Chukwuka, [1]Chidera Clinton Udonu and [1]Praise Ebubechukwu Okoro

[1]Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria

[2]Department of Microbiology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria

*Corresponding Author alaebo.prince@mouau.edu.ng

Accepted: May 1, 2024. Published Online: May 7, 2024

ABSTRACT

This study evaluated the hypoglycemic and serum protein profile effect of methanol extract of *Picralima nitida* in normal and alloxan-induced diabetic rats. Thirty-six (36) male albino rats weighing 120-150 g were divided into six (6) groups (6 rats per group). Group 1 (normal control) received normal feed and water *ad libitum*; group 2 (negative control) were induced but were not treated; group 3 (positive control) were induced and received oral treatment of glibenclamide; groups (4-6) received induced and treatment with different doses of methanol seed extract of *Picralima nitida:* Group 4 (100 mg/kg/day); Group 5 (200g/kg/day) and Group 6 (400 mg/kg/day). Diabetes was induced in rats by an intraperitoneal injection of 120mg/kg/b.w alloxan. *P. nitida* was orally administered to diabetic rats for a period of 21 days. Blood glucose level was observed on day 1st, 7th, 14th and 21st. The extract caused a significant ($p< 0.05$) decrease of fasting blood glucose of both positive and alloxan-induced diabetic rats in all the treated groups. Serum kidney function marker was assessed. Creatinine, Urea and Creatinine-Urea ratio level in groups treated with 100 mg/kg and 200 mg/kg extracts were significantly decreased ($p< 0.05$), while the group treated with 400 mg/kg extract was significantly increased ($p< 0.05$) when compared to the controls. This may qualify *Picralima nitida* methanol seed extract for use in ethnomedical diabetic management. *Picralima nitida* methanol seed extract might also have nephroprotective potential.

Keywords: Diabetes, *Picralima nitida*, serum kidney, serum protein, albino rats

INTRODUCTION

Diabetes mellitus (DM) is a chronic hyperglycemic condition related to endogenous insulin deficit or absence. Type 2 DM is due to changes in carbohydrate, fat and protein metabolism that result to health complications [1]. Hyperglycemia, a physiologically abnormal state defined by persistently increased blood glucose levels, is a feature of diabetes mellitus, or simply diabetes [1]. The persistent and varied symptoms of hyperglycemia include abnormalities in the metabolism of carbohydrates, fats, and proteins [2]. Hyperglycemia is caused by abnormalities in either insulin secretion or insulin action, or both [3]. Diabetes has a complex etiology, a wide range of presentation patterns, and a complex course of progression [4]. Numerous physiological organs are impacted by hyperglycemia and the resulting metabolic dysfunctions of protein, fat, and carbohydrates, which interfere with their regular operation [5]. The adverse effects of hyperglycemia and its accompanying metabolic abnormalities on the normal structure and function of the micro and macro vasculature, which are at the foundation of organ structure and function throughout the body, are the primary cause of these disruptions, which develop gradually over time [6]. Complications in the micro and macro vasculature are caused by structural and functional changes in the organ system vasculature. These issues impact body organs, including the eyes, kidneys, heart, and nerves, causing organ damage, dysfunction, and eventually organ failure. Retinopathy is the result of eye-related issues, and eventually progresses to blindness. Nephropathy and possible renal failure are caused by issues related to the kidneys [7].

Picralima nitida is a plant that belongs to the Apocynaceae family and the gentianales order. It may be found growing as bushes or homesteads in the tropical rain forest of Africa [8]. Numerous seeds are present in the fruit (pod), most of which are immersed in pulp *P. nitida* seed is known as Akuamma seed in Ghana, Osi-igwe seed in Igboland (Eastern Nigeria), Eso Abere in Yoruba land (Western Nigeria) [9]. Extracts from this plant possess various biological activities including antimalarial, antileishmanial, trypanocidal, larvicidal, antipyretic, analgesic, anticoagulant, anti-inflammatory, anti-diarrhoeal, hypoglycaemic and antimicrobial properties [8]. There is no much work done on the toxic effect of the extract on the long-term treatment, Hence, the present study examined the antidiabetic and hepatoprotective effect on methanol extract of *Picralima nitida* seed on alloxan-induced diabetic male albino rats.

Plate1: *Picralima nitida* fruits [10]

Plate 2: *Picralima nitida* seeds [11]

MATERIALS AND METHODS

Plant material

The fruits of *Picralima nitida* were harvested in Ikeduru village in Imo State Nigeria in the rainy season. The seeds were removed from the fruits, dried in the lab under room temperature for 2 weeks, then were identified at the Plant Science and Biotechnology Department of College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, where a voucher specimen (IHF 26123) was deposited in the Departmental herbarium.

Extraction

The powdered seeds of *Picralima nitida* weighing about 900 g were soaked in methanol and distilled water of 160:40 respectively and left to stand for three days with occasional shaking.

This was filtered using a Whatman No. 1 filter paper. The filtrate was subsequently evaporated to obtain the dry matter using a rotary evaporator under reduced pressure at 40 ^{o}C.

Experimental animals

Albino rats (36 in number) weighing 110-150 g were used for the study. The animals were obtained from Dr. Daniel's animal house in the Department of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Abia State. The animals were acclimatized to laboratory conditions for two weeks, under standard conditions with free access to food and water till the end of the experiment.

Induction of diabetes

Following a period of fasting and acclimatization, the animals were given an intraperitoneal (IP) injection of 120 mg/kg body weight of an alloxan monohydrate solution to induce diabetes. Animals with blood glucose level higher than 150 mg/dl were considered diabetic after 3 days of induction using fasting blood sugar method and were selected for the study.

Experimental design

After acclimatization, 36 animals were chosen for the experimentation. These were divided into 6 groups with 6 animals each. The groups are:

1. Normal Control Group (received Feed + H_2O *ad libitum*)
2. Negative Control Group (received Alloxan + Feed + H_2O *ad libitum*)
3. Positive Control Group (received Alloxan + Standard drug (Glibenclamide) + Feed + H_2O *ad libitum*)
4. Experimental Group (Alloxan + 100 mg/kg extract *P. nitida* + Feed + H_2O *ad libitum*)
5. Experimental Group (Alloxan + 200 mg/kg extract *P. nitida* + Feed + H_2O *ad libitum*)
6. Experimental Group (Alloxan + 400 mg/kg extract *P. nitida* + Feed + H_2O *ad libitum*)

The treatments lasted for 21 consecutive days.

Ethical consideration

Throughout the experiment, all the rats were housed at 25 ^{o}C in clean cages under normal daylight/day humid conditions. The rats freely fed (vital feed pellets) and clean water was made available throughout the process according to the guidelines approved by the Departmental

committee on animal use, Michael Okpara University of Agriculture, Umudike, on handling of experimental rats.

Determination of blood glucose

The level of blood glucose was determined using glucose oxidase methods, by collecting 0.5 ml of blood from the tail after a mild anesthesia using ether. Fasting blood sugar level was calculated and evaluated in mmol/L using a digital glucometer (Accu-check® Advantage, Roche Diagnostic, Germany). Animals were made to fast for a period of 16 hours prior to blood collection.

Preparation of drugs

A method described and modified by Alaebo *et al* (2022b) was used in the preparation of the extract. To make 500 mg/ml of stock solution, 500 mg of glibenclamide (glyburide) was made by crushing the tablet in a glass mortar and dissolving it in 1 milliliter of distilled water. Glibenclamide (glyburide) was orally administered to the animals at a dose of 500 mg/kg body weight.

Statistical analysis

Statistical analysis of data was carried out using one way analysis of variance (ANOVA) in Statistical package for social sciences (SPSS) version 20.0. The analysis data was reported as mean ± standard error of mean (SEM). Significant difference using Tukey's Post Hoc test was accepted at 95% confidence level of probability i.e., at $p< 0.05$.

RESULTS AND DISCUSSION

Table 1 shows that the treatment with *P.nitida* seed extract at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg respectively significantly ($P>0.05$) decreased the level of blood glucose as compared to negative control.

Table 1: Antidiabetic effects of the *Picralima nitida* seed extract on fasting blood glucose level of treated rats

Group/Treatment	Mean fasting blood glucose level (mg/dL) 0	Week One	Week Two	Week Three
1. Normal control	112.33 ± 9.94*	107. 00 ±2.52*	99.33 ± 3.48*	100.21 ± 3.48*
2. Negative control (diabetic but not treated group)	416.00 ± 77.67	403.33 ± 99.85	419.67±74.31	419.67±74.31
3. Positive control (Diabetic, treated with Glibenclamide)	201.67 ± 39.23*	172.00±52.05*	108.33±11.89*	75.33±11.89*
4. *Picralima nitida* Extract, 100 mg/kg	266.67 ± 23.75*	165.67±77.40*	109.00 ± 0.00*	85.33±11.89*
5. *Picralima nitida* Extract, 200 mg/kg	331.33 ± 100.40*	212.33±89.35*	99.00 ± 17.90*	88.33±11.89*
6. *Picralima nitida* Extract, 400 mg/kg	415.33 ± 136.87*	227.67±32.71*	106.50 ± 6.50*	98.33±11.89*

*$p < 0.05$ when compared with the negative control group, for 0, 7th, 14th and 21st day

Table 2 presents the activities of kidney function markers in rats administered *Picralima nitida* methanol seed extract and is indicative that there was a significant decrease in creatinine in all the experimental rats administered 100mg/kg, 200 mg/kg and 400 mg/kg respectively when compared to the negative control. There was significant decrease in serum urea for 100 mg/kg, 200 mg/kg and 400 mg/kg respectively when compared to the negative control group. Urea-Creatinine activities also showed significant decrease in serum kidney for 100 mg/kg, 200 mg/kg and 400 mg/kg when compared to the negative control.

Table 2: Effect of *Picralima nitida* methanol seed extract on serum kidney function marker (creatinine, urea and urea-creatinine ratio)

GROUP	Creatinine (mg/dL)	Urea (mg/dL)	Urea-Creatinine ratio µmol/L
1. Normal control	1.12 ± 0.08*	27.27 ± 2.39*	63.26 ± 0.49*
2. Negative control	1.42 ± 0.03	98.59 ± 1.07	111.78 ± 3.15
3. Positive control	0.79 ± 0.08*	56.89 ± 2.28*	88.17 ± 2.19
4. *P. nitida,* 100 mg/kg	0.96 ± 0.03*	32.98 ± 0.40*	64.57 ± 0.62*
5. *P. nitida*, 200 mg/kg	0.76 ± 0.05*	23.34 ± 1.49*	69.65 ± 6.56*
6. *P. nitida*, 400 mg/kg	1.01 ± 0.08*	27.05 ± 1.34*	67.87 ± 0.81*

*$p < 0.05$ when compared with the control group.

Table 3 presents the data on changes in total protein, albumin and globulin concentrations of rats following treatment with *Picralima nitida* methanol seed extract.

Table 3: Effect of *Picralima nitida* methanol seed extract on total protein, albumin and globulin concentrations of rats

GROUP	TP (g/dL)	ALB (g/dL)	GLB (g/dL)
1. Normal control	6.16 ± 0.15*	4.23 ± 0.00*	2.92 ± 0.15*
2. Negative control	8.26 ± 0.02	5.55 ± 0.03	4.01 ± 0.05
3. Positive control	6.46 ± 0.06*	3.37 ± 0.12*	3.09 ± 0.08*
4. *P. nitida,* 100 mg/kg	6.75 ± 0.07*	3.76 ± 0.03*	2.99 ± 0.08*
5. *P. nitida,* 200 mg/kg	6.92 ± 0.03*	4.04 ± 0.04*	2.88 ± 0.03*
6. *P. nitida,* 400 mg/kg	6.91 ± 0.09*	3.89 ± 0.04*	3.02 ± 0.12*

*$p < 0.05$ when compared with the negative control group, TP = total protein, ALB = albumin, GLB = globulin

In total protein, there were no significant differences in all the treated groups (100 mg/kg, 200 mg/kg and 400 mg/kg body weight) when compared to the normal control but there was significant decrease in all the treated groups when compared to the negative control. In albumin there were no significant differences in all the treated groups (100 mg/kg, 200 mg/kg and 400 mg/kg body weight) when compared to the control but there was significant increase in all the treated groups when compared to the negative control. Also in globulin, there were no significant differences in all the treated groups (100 mg/kg, 200 mg/kg and 400 mg/kg body weight) when compared to the normal control but there was significant decrease in all the treated groups when compared to the negative control.

Table 4 presents the data on changes in total bilirubin, direct bilirubin and conjugated bilirubin concentrations of rats following treatment with *Picralima nitida* methanol seed extract.

Table 4: Effect of *Picralima nitida* methanol seed extract on total bilirubin, direct bilirubin and conjugated bilirubin concentrations of rats

GROUP	TBIL (mg/dL)	DBIL (mg/dL)	CBIL (mg/dL)
1. Normal control	0.30 ± 0.02	0.27 ± 0.02*	0.03 ± 0.01*
2. Negative control	0.26 ± 0.02	0.08 ± 0.02	0.18 ± 0.01
3. Positive control	0.27 ± 0.01	0.10 ± 0.02	0.18 ± 0.02
4. *P. nitida*, 100 mg/kg	0.29 ± 0.01	0.08 ± 0.02	0.21 ± 0.02
5. *P. nitida,* 200 mg/kg	0.27 ± 0.01	0.12 ± 0.03	0.16 ± 0.05
6. *P. nitida*, 400 mg/kg	0.27 ± 0.01	0.10 ± 0.02	0.18 ± 0.02

*p < 0.05 when compared with the negative control group, TBIL = Total bilirubin, DBIL = Direct bilirubin, CBIL = conjugated bilirubin

In total bilirubin, there were no significant differences in all the treated groups (100 mg/kg, 200 mg/kg and 400 mg/kg body weight) when compared to the normal control. In direct bilirubin there were no significant differences in all the treated groups (100 mg/kg, 200 mg/kg and 400 mg/kg body weight) when compared to the normal control. Also in conjugated bilirubin, there were no significant differences in all the treated groups (100 mg/kg, 200 mg/kg and 400 mg/kg body weight) when compared to the normal control.

Bilirubin is a yellowish -brown substance that is formed when red blood cells are broken down. Normal levels of total bilirubin, direct bilirubin and conjugated bilirubin indicate that the liver is functioning properly and breaking down red blood cells normally. Elevated levels of any of these types of bilirubin can indicate liver disease, bile duct obstruction or other health problems. *Picralima nitida* methanol seed extract could be helping to regulate the levels of bilirubin, keeping them within a normal range. It is also possible that this extract could have other effect on the liver, like protecting it from damage or helping it to regenerate.

The study shows that treatment with the extract significantly improves the glycemic profile of animals treated at all doses of 100 mg/kg, 200 mg/kg and 400 mg/ kg compared to negative control. This effect is more noticeable in the glibenclamide-treated group. However, there is an increase of the Area Under the Curve (AUC) of the groups treated respectively with the extract at the dose 400 mg/kg. The AUC of the group of animals treated with glibenclamide,

on the other hand, shows a significant decrease, both in comparison with the control group. Glibenclamide is an insulin secretor, it works by stimulating beta cells to produce insulin, and its better effectiveness could be explained by this mode of action. In this study, a reduction in the fasting blood glucose levels with seeds extract of *Picralima nitida* in all doses showed reduction in glucose levels of hyperglycemic animals and was similar to that of the rats treated with glibenclamide (10 mg/kg) when compared with normal and negative control rats.

Glucocorticoids raise blood sugar levels through a variety of processes, including enhanced hepatic glucose synthesis (gluconeogenesis), reduced peripheral glucose absorption into muscle and adipose tissue, and breakdown of muscle and fat to supply extra substrates for glucose synthesis [12]. Also, prolonged glucocorticoid exposure is associated with the development of severe insulin resistance and metabolic dysfunction [12]. The present study also confirms the same findings. *Picralima nitida* aqueous seeds extract lowered glycemia of rats in a dose-dependent manner when compared to rats treated with dexamethasone only [13] which is in tandem with our results.

Kidney is the largest organ, accounting for approximately 2% to 3% of average body weight [14]. It functions as a center for metabolism of nutrients, excretion of waste metabolites and controls the flow and safety of substances absorbed from the digestive system before distribution to the systemic circulatory system [15]. According to the World Health Organization, an estimated 354 million people were reported to be living with hepatitis infection and for most, testing and treatment remain beyond reach [16]. The symptoms of liver disease may include jaundice, abdominal pain and swelling, swelling in the legs and ankles, itchy skin, dark urine colour among other signs [17]. Kidney damage induced by alloxan is a commonly used model for the screening of nephroprotective drugs [6]. When rats are diabetic it induces hepatotoxicity by metabolic activation [18]. Alloxan induction selectively causes toxicity in kidney cells maintaining semi-normal metabolic function [19; 20].

In the present study, a significant reduction in the serum levels of creatinine, urea, and urea-creatinine was observed. These enzymes are markers of kidney toxicity induced by various chemicals and toxicants. A report by Alaebo *et al.* [21] has indicated that the kidney toxicity markers, creatinine and urea, vary significantly in acute kidney injury. According to their reports, creatinine rose rapidly with acute kidney injury and the creatinine level steadily increased over

time, reaching similar levels to AST by 24–48 h. However, in chronic kidney injury, the creatinine level tends to rise and reach high urea/creatinine ratios during kidney damage [22, 23]. In our study, the reduced levels of creatinine, urea, and urea-creatinine are indicative of the prevention of *Picralima nitida* toxicity in rats. Therefore, serum levels of the enzymes are useful indicators of extent of kidney damage [9]. The non-significant effect of urea in rats fed standard basal diet or standard basal diet plus *Picralima nitida* seed extract is an indication that the kidney of the animals was not damaged. The normal values of creatinine recorded in albino rats fed standard basal diet or standard basal diet plus *Picralima nitida* may strongly indicate that the administration of *Picralima nitida* seed extract did not cause injury to kidney of all the albino rats treated with the extract.

High level of total protein which is hyperproteinemia can have a variety of causes including dehydration, liver disease and some types of cancer. The symptoms can include increased thirst, increased urination and fatigue.

From this research, *Picralima nitida* methanol seed extract possessed some phytochemicals that have a regulatory effect, keeping the level of the total protein within the normal range. This could be beneficial for people with diabetes as the plant extract has potential therapeutic use. Albumin and globulin are both proteins found in the blood and they play an important role in the body. Albumin is involved in maintaining blood volume and transporting substances like hormones and nutrients while globulin is involved in the immune response. Albumin helps to keep the right amount of fluid in the blood vessels, which can prevent swelling and other symptoms of fluid retention. It also helps to maintain the right balance of electrolytes like sodium and potassium in the body [24]. Globulin helps to fight off infections and other diseases by activating the immune system. It is impressive that *Picralima nitida* methanol seed extract could have such a wide range of effects on the body.

From the findings of the present study, the compounds of the *Picralima nitida* methanol seed extract could act as enzyme inhibitors which means they could bind to enzymes and prevent them from carrying out their usual function. They could act as enzyme activators which would increase the activity of enzymes.

This study was limited to the seed of the extract only. It was also limited to the antihyperglycemic and serum kidney markers.

CONCLUSIONS

The administration of methanol extract of *Picralima nitida* seed on alloxan-induced diabetic albino rats significantly reversed the damage associated with alloxan-induced diabetes revealing its hypoglycemic, renal function integrity. The extract has great potential therapeutic benefits. The presence of phytochemicals and bio-compounds found in *Picralima nitida* seed could explain the observed pharmacological property of the studied extract. This study revealed the biochemical effects of methanol extract of *Picralima nitida* seed on alloxan-induced diabetic albino rats. Further research could be carried out on the haematological and histological effect of this extract and the mechanism of action of this extract.

REFERENCES

[1] Alaebo, P.O., Iloanusi, D.U., Njoku, G.C., Dike, V.C., Ekeleme, N.M., Anoliefo, C.L., Nkume, P. I. & Nwankwere, D.G. (2023a). Evaluation of Antidiabetic, Haematology and Hypolipidemic Effects of Annona *Muricata* (Annonaceae) Seeds in Alloxan-induced Diabetic Rats. *Asian Journal of Research in Cardiology Diseases,* (5)1, 30–37.

[2] Alaebo, P.O., Njoku, G.C., Udensi, G.C. & Ugwu, C.O. (2022a). Hypoglycemic and Hypolipidemic Effect of Dialum guineense (ICHEKU) Fresh Leaves on Alloxan-induced Diabetes in Male Albino Rats. *Asian Journal of Research in Cardiovascular Diseases,* 4(4), 1-7.

[3] Alaebo, P.O., Oriaku, C.E., Obike, C.A., Onyeabo, C. & Njoku, G.C. (2022b). Hypoglycemic Effect of Methanol Extract of Carica Papaya (Pawpaw) Leaves in Alloxan-Induced Diabetic Rats. *International Journal of Innovative Science and Research Technology,* 7(1), 627-631

[4] Banday, M.Z., Sameer, A.S. & Nissar, S. (2020). Pathophysiology of diabetes: An overview. *Avicenna Journal of Medicine*, 10(04), 174–188.

[5] Uhuo, E.N., Godwin, K.O., Alaebo, P.O. & Ezeh, H.C. (2022). Haematological and Biochemical Parameters Assessment of Alloxan-induced Diabetic Rats Treated with Ethanol Leaf Extract of Adansonia Digitata (Baobab) Leaf. *Animal Research International*, 19(2), 4469 – 4477.

[6] Alaebo, P.O., Ukpabi-Ugo, J.C., Onyeabo, C., Iloanusi, D.U., Ekeleme, N.M., Nkume, P.I., Ugwu, P., Esther, A.A. & Njoku, G.C. (2023b). Evaluation of the Antihyperglycemic and Antioxidant Effects of Methanol Crude Extract of Unripe Pulp of Carica papaya on Diabetic Male Albino Rats Induced by Alloxan. *South Asian Research Journal of Natural Products*, 6 (1), 39-48.

[7] Alaebo, P.O., Chukwu, C.N., Nwuke, C.P., Ezeigwe, O.C. & Ekwuno, P.O (2020a). Hepatoprotective and Antioxidant Effects of Methanol Extract of Soursop *(Annona muricata)* Seeds on Alloxan-induced Diabetic Wistar Rats. *Nigerian Research Journal of Chemical Sciences.* 8 (2), 199-210

[8] Ubulom, P.M.E. (2012). Larvicidal and antifungal properties of *Picralima nitida* (Apocynaceae) leaf extracts. *European Journal of Medicinal Plants*, 2(2), 132-139.

[9] Aguwa, C.N. (2001). Antidiabetic effect of *Picralima nitida* aqueous seed extract in experimental rabbit model. *Journal of Natural Remedies*, 1(2), 135-139.

[10] Alcover, C.F., Bernadat, G. & Kabran, F.A. (2020). Molecular networking reveals serpentinine-related bisindole alkaloids from *Picralima nitida*, a previously well-investigated species. *Journal of Natural Products,* 83(4), 1207–1216.

[11] Olufunsho, A., Coulidiaty, A.G.V., Oluyemi, A.G., Sunday, A., Omoseyindemi, B. & Busia, K. (2019). Toxicological evaluation of *Picralima nitida* in rodents. *Journal of Ethnopharmacology,* 236, 205–219.

[12] Olajide, O.A., Velagapudi, R., Okorji, U.P., Sarker, S.D. & Fiebich, B.L. (2014). *Picralima nitida* seeds suppress PGE2 production by interfering with multiple signalling pathways in IL-1β-stimulated SK-N-SH neuronal cells. *Journal of Ethnopharmacology,* 152(2), 377–383.

[13] Adegoke, B.M. & Oloyede, B.O. (2013). Antihyperglycemic and anti-hyperproteinemia activity of extracts of *Picralima nitida* seed and *Tapinanthus bangwensis* leaf on alloxan-induced diabetic rabbits. *International Journal of Innovation and Applied Studies*, 3(4), 1125-1131.

[14] Abdel-Misih, S.R. & loomston, M. (2010). Liver anatomy. *Surgery and Clinical North America,* 90(4), 643–653.

[15] Ozougwu, J.C. & Eyo, J.E. (2014). Hepatoprotective effects of Allium cepa extracts on paracetamol-induced liver damage in rat. *African Journal Biotechnology*, 13(26), 2679–2688.

[16] W.H.O. World Hepatitis Summit 2022. Available at: https://www.who.int/news-room/events/detail/2022/06/07/default-calendar/world-hepatitis-summit. Accessed: 13 March 2023.

[17] Allen, S.E. (2002). The liver: Anatomy, Physiology, Disease and Treatment. North Eastern University Press.

[18] Alaebo, P.O., Oriaku, C.E., Obike, C.A., Onyeabo, C., Njoku, G.C. & Ekwunoh, P.O. (2022c). Hypoglycemic Effect of Methanol Extract of Carica Papaya (Pawpaw) Leaves in Alloxan-Induced Diabetic Rats. *International Journal of Innovative Science and Research Technology,* 7(1), 627-631

[19] Omodamiro, O.D., Alaebo, P.O., Olukotun, B.G. & Chikezie, P.C. (2021). Hepatotoxicity Effects of Methanol Extract of *Gmelina arborea* leaves on Liver enzymes marker in Wistar Rats. *GSC Biological and Pharmaceutical Sciences.* 15(2), 059-066

[20] Alaebo, P.O., Chukwu, C.N., Nwuke, C.P., Ukpabi-Ugo, J.C., Ezeigwe, O.C. & Ekwuno, P.O. (2020b). Hypoglycemic and Hepatoprotective Effects of Crude Extract of *Averrhoa Carambola* Leaves in Alloxan-induced Female Diabetic Rats. *Nigerian Research Journal of Chemical Sciences,* 8 (2), 40-56

[21] Alaebo, P.O., Umeh, V.A., Njoku, G.C., Ezennaya, I.E., Anyadike, N.N., James, U.A., Ezeh, C.J. & Ugwu, P. (2022d). Antioxidant and Nephroprotective Effects of Honey in Alloxan-Induced Diabetic Rats. *Journal of Applied Life Sciences International,* 25(2): 26-32.

[22] Åberg, F., Danford, C.J., Thiele, M., Talbäck, M., Rasmussen, D.N., Jiang, Z.G., Hammar, N., Nasr, P., Ekstedt, M. & But A. (2021). A Dynamic Aspartate-to-Alanine Aminotransferase Ratio Provides Valid Predictions of Incident Severe Liver Disease. *Hepatological Community,* 5, 1021–1035.

[23] Yasmin, A., Rukunuzzaman, M., Karim, A.S.M.B., Alam, R., Hossen, K. & Sonia, Z.F. (2022). Ratio of aspartate aminotransferase to alanine aminotransferase and alkaline phosphatase to total bilirubin in Wilsonian acute liver failure in children. *Indian Journal of Gastroenterol*, 41, 224–230.

[24] Ezeigwe, O.C., Alaebo, P.O., Enemchukwu, B.N., Chukwuemeka, U.V., Ifedilichukwu, N.H., Uhama, K.C., Naomi, N. N. & Iloanya, E.L. (2020). Haematological and Biochemical effects of a combination of leaves of *Telfairia occidentalis* and *Mucuna pruriens* in male rats. International *Journal Bioscience,* 16(1), 139-149.