Original Research Article

Effect of Orally administered *Dennettia tripetala* (Pepper fruit) (Aq) extract on the intraocular pressure and serum concentration of lipid parameters of Wistar strain albino rat

ABSTRACT

Background: Plant parts have continued to attract attention in the global search for natural means of treatment of many diseases affecting humans and animals including glaucoma. **Aims**: The present study was designed to evaluate the effect of Aqueous seed extract of *Dennettia tripetala* (ASEDt) on the intraocular pressure (IOP) and the serum concentration of lipid parameters of Wistar strain albino rats (male and female).
Methodology: This study was conducted in the Optometry and Anatomy department of Abia state University Uturu, Nigeria where a total of 37 males + females albino Wistar rats (12 weeks old), weighing 200 – 230g were used (12 for acute toxicity studies and 25 for the experiment proper) for a period of 35 days. The 25 rats were further divided into 5 groups (A-E) of 5 animals each according to various treatments A- No treatment, B-10mg/ml Cholesterol p.o, while C, D and E were treated with 10mg/ml Cholesterol p.o, plus administration of 200mg DT seed (aq) extract, 500mg DT seed (aq) extract and 0.5% timolol topically into the eye respectively. Intraocular pressure as well as the serum concentration of lipid parameters of the control group viz; Total cholesterol (TC), Triglycerides (TG), High density lipoprotein (HDL) and Low-Density Lipoprotein (LDL) was measured at day 1 (Baseline) 10 and 20 of experiment. **Results**: Topical administration of 200mg/kg and 500mg/kg DT seed (aq) extract caused a significant reduction of IOP in the rats. This reduction was higher with 500mg. Furthermore, both 500mg and 200mg (ASEDt)A caused a reduction in the levels of TC, TG, LDL and increased level of HDL.
Conclusion: Aqueous seed extract of *Dennettia tripetala* caused a dose dependent reduction of intraocular pressure and ameliorated the serum concentration of lipid parameters in both male and female Wistar strain albino rats, thus suggesting that it could be beneficial in ocular, cardiovascular and public health.

Keywords: Cholesterol, intraocular pressure, glaucoma, *Dennettia tripetala*, Lipid parameters

1. INTRODUCTION

Glaucoma is a form of progressive optic neuropathy and the second leading cause of blindness globally [1]. It is estimated that 57.5 million people worldwide are affected by primary open-angle glaucoma [2]. Reports by Tham *et al.,* [3] and Allison *et al.,* [4] stated that it is expected that approximately 76 million people will suffer from glaucoma by 2020 and that this number is estimated to reach 111.8 million by 2040. Those with high risk of glaucoma includes people ≥ 60years of age, family members of those already diagnosed with glaucoma, steroid users, diabetics, as well as those with high myopia, hypertension, central cornea thickness of <5 mm and eye injury [5]. The two major types of glaucoma are

primary and secondary glaucoma. These are also subdivided into open-angle and angle-closure according to the underlying anatomy and pathophysiology. One of the most important risk factors for glaucoma is elevated intraocular pressure (IOP), and its reduction is the only proven treatment to reduce the development and progression of the disease [6]. Identifying potential systemic associations with IOP may provide further insight into the pathophysiologic features of glaucoma [7], in other words, the only known treatment of the disease is reduction of intraocular pressure (IOP), which has been shown to reduce glaucoma progression in a variety of large-scale clinical trials [8]. The availability of topical antiglaucoma drugs including prostaglandin analogues [9], carbonic anhydrase inhibitors [10], beta-receptor antagonists e.g., Timolol [11], adrenergic agonists [12], and parasympathomimetics [13] and for systemic therapies, Carbonic anhydrase inhibitors - Systemic carbonic anhydrase inhibitors, such as acetazolamide [14] and Osmotics - Hyperosmotics such as mannitol or glycerol [10] commonly used in clinical routine allows for individualized treatment taking risk factors, efficacy, and safety into account [8]. Drugs used to treat systemic disease may raise the IOP. For instance, response usually occurs within a few weeks of initiating steroid therapy, but it can present at any time thereafter. Patients treated with corticosteroids may develop elevated IOP and glaucomatous optic nerve damage, as a result, steroids should be used thoughtfully, and patients should be observed for steroid-induced IOP spikes [15] and [16], reported the demonstration of a potential association between elevated IOP and hyperlipidemia as one of the casual cardiovascular risk factors.

The use of medicinal plant in the contemporary world cannot be over emphasized. Plant parts such as seeds, stems, leaves, roots and bark etcetera have continued to attract attention in the global search for the treatment of many diseases affecting humans [17, 18, 19]. *Dennettia tripetala* (DT), is widely grown in the rain forest zones of Nigeria and some parts of West Africa. It is classified as Kingdom: Plantae; Phylum: Magnoliophyta; Class: Magnolidae; Order: Magnoliales; Family: Annonaceae; Genus: Denettia; Species: *Dennettia tripetala*. Its seeds are usually dried for preservation by local traders thus ensuring its availability [20, 21]. The fruit and seeds are edible and are consumed because of the spicy nature. Phytochemical screening of the ethanolic extract revealed the presence of tannins, alkaloids, steroids, flavonoids, cardiac glycosides, saponins, and terpenoids [22]. These constituents provide a scientific basis for the use of DT in traditional medicine, as it is claimed to be used in the treatment of diabetes, antimicrobial, anti-inflammatory etc.

This study aimed to determine the effect of orally administered aqueous extract of *Dennettia tripetala* seed on cholesterol induced intraocular pressure of Wistar strain albino rats.

Image 1 of *Dennettia tripetala* fruit [23]

2. MATERIAL AND METHODS

Study Area, Collection of Plant Material and Processing

The study was carried out in the Department of Optometry and Anatomy, Abia state University Uturu, Nigeria. Plant sample of *Dennettia tripetala* was collected obtained from Okporo-Orlu, Imo State Nigeria, was identified and authenticated at the Department of Animal and Environmental biology, Imo state University Owerri, Nigeria. Matured fruits were collected from same location, seeds were removed from the succulent pericarp, chopped into small pieces and dried under room temperature for 4 weeks. The dried plant material pulverized into powder using mortar and pestle. 500g of the powder was then soaked in 1.5 liters of distilled water for 48 hours, filtered with a cheesecloth sieve, after which the filtrate was concentrated using an electric oven at 3^{o}C for 72 hours The residue was weighed and kept in an air tight plastic container in the refrigerator until use.

Animal Procurement and Preparations

A total of 37 males albino Wistar rats (12 weeks old), weighing 200 – 230g were used for the study (12 for acute toxicity studies and 25 for the experiment proper). The rats were obtained from the Animal House of the Department of Biological sciences, Anambra state University, Nigeria, housed in standard cages and kept in the Animal House of Anatomy Department, Imo State University, Owerri, Nigeria where they were maintained at 26-29^{o}C, 12/12hours light/dark cycle, fed with pelleted animal feed chow (Pfizer livestock co. Ltd, Aba, Nigeria) and tap water *ad libitum* for a period of 14 days acclimatization.

Acute Toxicity Test

Acute toxicity test (Phase 1 and 2) was carried out with 12 rats with average weight of 170g rats using Lorcke's method as described by Idyu *et al*., (2015) [24]. LD50 of ASEDt was ≥10, 000 mg/kg body weight, thus 200mg/kg and 500 mg/kg of ASDt were used for the experiment.

Induction of Cholesterol, IOP and Administration of Timolol Extract

Cholesterol mixed with 1ml of distilled water was administered with Orogastric cannula attached to a 5ml syringe for 10 days. Each rat received 1ml of the mixture daily for 10 days except rats in the control group. Extract administration commenced immediately after cholesterol was administered for 10 days. The extract was administered through Orogastric feeding according to the methods of Eguajoe *et al.,* [25]. Cholesterol and intraocular pressure were induced to rats in this group by feeding each rat with 10mg of cholesterol dissolved in 1ml of distilled water daily for ten days. This was done to increase the cholesterol level of the rats.

The rats were grouped as follows:

Group A: Received no treatment (control).

Group B: Treated with Cholesterol (10mg) *p.o* only.

Group C: Treated with Cholesterol (10mg) *p.o* + 200mg ASEDt topically.

Group D: Treated with Cholsterol (10mg) *p.o* + 500mg ASEDt seed topically.

Group E: Treated with Cholesterol (10mg) *p.o* + 0.5% timolol topically into the eye.

Collection of Blood for Cholesterol Analysis

Collection of Blood for Cholesterol Analysis

Blood samples were collected three times from the experimental animals, viz; before and after cholesterol administration as well as after administration of aqueous extract of DT and timolol. This was done via dorsal pedal vein according to the methods described by Parasuraman *et al*., [26]. The rats were anaesthetized under chloroform vapor in a plastic container that was slightly covered with black cellophane to ensure the rats inhale the chloroform for 1 min. After anaesthetizing the rat, the rat was placed on his back with finger placed at the level of the lowest ribs without applying pressure on the rat. The heart is roughly 1 cm above this point, slightly right [27]. A 23G needle with 5ml syringe was positioned at 45 degrees angle. The needle was inserted between the two ribs of the rat and blood was seen coming out from the needle and that signified that the needle was inside the heart. 1 ml blood was collected from each rat for cholesterol assessment [27].

Determination of Total Cholesterol

Cholesterol Oxidase Method according to Allain *et al.,* [28]. The absorbance of the sample, standard and control were measured at 505nm against the reagent blank using semi autoanalyzer spectrophotometer.

Determination of Triglyceride

Glycerophosphate Oxidase Method according to Fossati *et al.,* [29]. The absorbance of the sample, standard and control were measured at 546nm against the reagent blank using semi autoanalyzer spectrophotometer.

Determination of High-Density Lipoprotein

Precipitation method using phosphotungstic acid and magnesium ions according to Assmann *et al.,* [30]. The absorbance of the sample, standard and control were measured at 505nm against the reagent blank using semi autoanalyzer spectrophotometer.

Determination of Low-Density Lipoprotein

Precipitation with heparin according to Nauck *et al.,* [31]**.** Absorbance of the sample, standard and control were measured at 505nm against the reagent blank using semi autoanalyzer spectrophotometer.

Measurement of Intraocular Pressure

A mitten fabric was used to restrain each rat in order to avoid inducing pressure on the animal while holding slightly on the neck as described by the method of Camilo *et al.,* [32]. Having done this, both eyes of each rat were anaesthetized with Tetracaine hydrochloride eye drop and their tear film stained with fluoresce in strips. Following, the IOP was measured by gently tapping the cornea with the transducer of the Tono-Pen in a perpendicular orientation. A click sound is heard for each applanation, signifying a reading was taking. Intraocular pressure was measured at day 1 (Baseline), 10 days after acclimatization and 20 days after acclimatization.

Statistical Analysis

The descriptive method was used to compare the mean and graphical display of the contributions of topically ASEDt sample on cholesterol induced intraocular pressure of the experimental animals. Collected data was subjected to a regression analysis to ascertain the contributions of cholesterol treatment and timolol to the baseline using the Statistical packages for social sciences (SPSS) version 23.

3. RESULTS AND DISCUSSION

Table 1: Mean IOP Measurements of Rats in various Groups (Treatments)

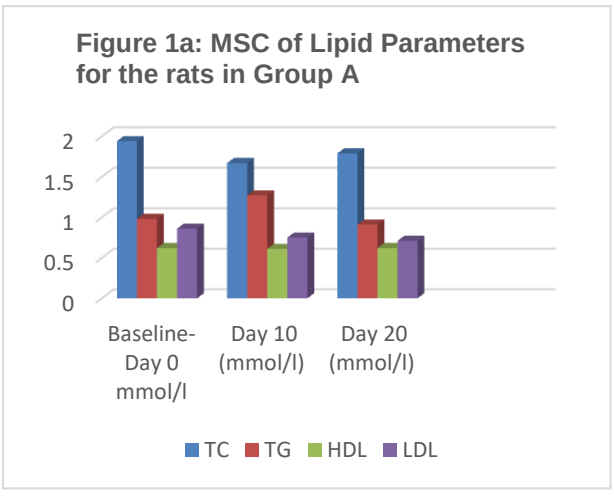
Groups Rats (n =5)	Mean IOP (mmHg) Baseline	Mean (IOP) MmHg 10 days after acclimatization	Mean (IOP) MmHg 20 days after acclimatization
A	10.33±*0*.32	10.35±*0*.266	10.24±*0*.113
B	9.7±*0*.36	18.07±*2*.53	14.1±*0*.46
C	10.77±*0*.38	19.45±*1*.60	10.90±*0*.136
D	11.05±*0*.55	19.39±*0*.75	8.97±*0*.039
E	10.13±*0.26*	17.08±*0.60*	9.83±*0.25*

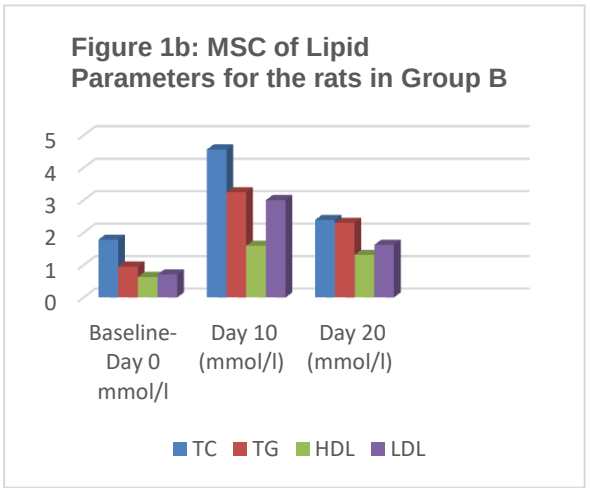
Key: A= Received no (Control) treatment, B= Treated with Cholesterol, C= Treated with Cholesterol + 200mg aqueous extract of DT seed orally, D= Treated with Cholesterol + 500mg aqueous extract of DT seed orally and E: Treated with Cholesterol + 0.5% timolol topically into the eye.

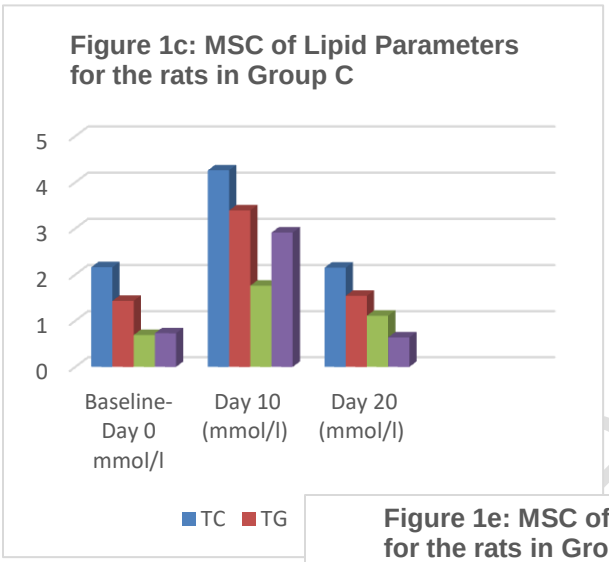
In this present study, the intraocular pressure of experimental rats was assessed before and after 10 days administration of cholesterol (10mg) agent and after 10 subsequent days of topical administration of 200mg/kg and 500mg/kg ASEDt and 0.5% timolol maleate. The mean IOP (mmHg) increased in the experimental rats after 10mg of cholesterol administration compared to control. The mean differences were statistically significant (*P* <.05) with P= 0.00. The increase in IOP of the cholesterol administered animals may likely be due to elevated in cholesterol level recorded in those groups. This is in tandem with the report by Shiming *et al*., (2019) [33] stating that an increase of 10mg/dl in blood TG Levels, TC or LDL would increase IOP. thus, hypercholesterolemia can cause ocular hypertension, which is one of the major risk factors for development of primary open-angle glaucoma [34] - *Table 1*

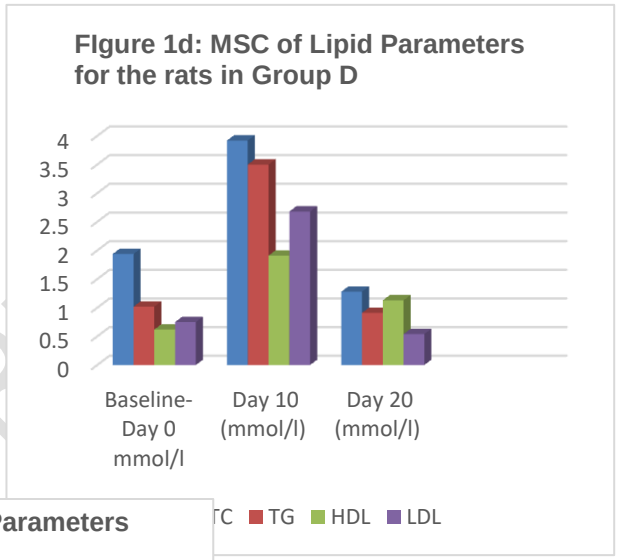
The administration of ASEDt (200mg/kg and 500mg/kg) to the cholesterol-administered groups C and D significantly reduced the IOP (*P* <.05) with *P* = at 0.03 and 0.02 respectively. This reduction was higher with 500mg/kg than that of 200mg/kg. The reduction in IOP suggests a cholesterol lowering effect of ASEDt possibly due to the presence of beta sitosterol and tocopherols in the seeds. Beta sitosterol is a natural plant sterol which maintains healthy cholesterol levels which carried out by interfering with cholesterol absorption. Furthermore, *Dennettia tripetala* leaf possesses flavonoids, phenolics, terpenoids and steroids as part of the phytochemical constituents; these bioactive compounds are well known for their hepatoprotective potency [35]. Intraocular pressure reducing property of *Dennettia tripetala* can be attributed to flavonoid terpenoids and steroids as these possess biochemical and antioxidant properties that could have helped to enhance ocular blood flow and in-turn reduces intraocular pressure. The mean IOP of the experimental rats was significantly (*P* <.05) reduced in the group treated with 0.5% timolol maleate with *P* =0.03. This suggests that Its Mechanism of action could via reducing the rate of aqueous humour formation through reduction of blood flow to the ciliary process.

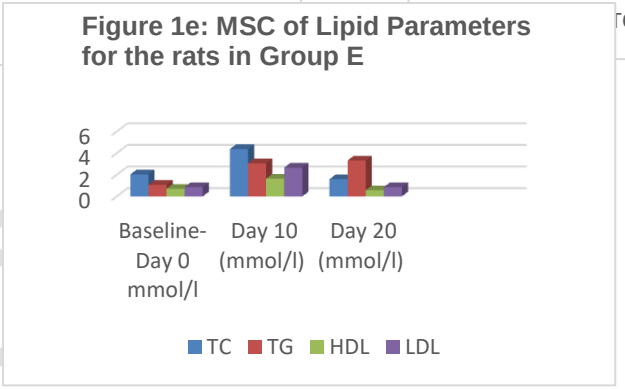
Figure 1: Mean Serum Concentration (MSC) of Lipid Parameters for the rat Groups











***Key**: TC= Total cholesterol, TG= Triglycerides, HDL= High density lipoprotein and LDL= Low density lipoprotein in mmol/l.*A= Received no treatment (Control), B= Treated with Cholesterol, C= Treated with Cholesterol + 200mg aqueous extract of DT seed orally, D= Treated with Cholesterol + 500mg aqueous extract of DT seed orally and E: Treated with Cholesterol + 0.5% timolol topically into the eye

Moreover, the mean serum concentration of TC, TG, HDL and LDL significantly increased in the experimental groups after 10 days of cholesterol administration compared to control group – *Figure 1a-e*.

Furthermore, the increase in the lipid profile parameters may likely be attributed to increase in circulating cholesterol as regards to its administration to the experimental rats. This finding corresponds to the report of Adeyemi and Orekoya (2014) [36], that oral herbal (Fijk) remedy caused a dose-dependent elevation in the plasma atherogenic index.The mean serum concentration of TC, TG, HDL and LDL showed significant alterations in the experimental rats after extract administration. However, the mean serum TC concentration was significantly reduced in group treated with both 200mg/kg and 500mg/kg of ASEDt and timolol maleate. This reduction may likely be attributed to the possible ameliorative effect of ASEDt while the significant increase in HDL lev. el recorded in the experimental group may likely be attributed to polyunsaturated fatty acid content of ASEDt. Our results further establish significant decrease in the TC and HDL with administration of 0.5% timolol as reported by Rahman *et al*., [37].

4. CONCLUSION

Aqueous seed extract of *Dennettia tripetala* caused a dose dependent reduction of intraocular pressure and ameliorated the serum concentration of lipid parameters in both male and female Wistar strain albino rats, thus suggesting that it could be beneficial in ocular and cardiovascular health.

ETHICAL APPROVAL

Ethical clearance was obtained from the Research and Ethics Committee of the College of Health Sciences, Abia State University, Uturu, Nigeria. All animals were treated in line with guidelines, stipulated by the National Institute for Health Guide on the Care and Use of Laboratory Animals (1985). They were also in accordance with the Association for Research in Vision and Ophthalmology (ARVO) statement for the use of animals in Ophthalmic and Vision Research.

REFERENCES

[1] Dey A, Manthey AL, Chiu K, Do CW. Methods to Induce Chronic Ocular Hypertension: Reliable Rodent Models as a Platform for Cell Transplantation and Other Therapies. Cell Transplant. 2018 Feb; 27(2):213-229. doi: 10.1177/0963689717724793.
[2] Wiggs JL, Pasquale LR. Genetics of glaucoma. Hum Mol Genet. 2017 Aug 1;26(R1): R21-R27. doi: 10.1093/hmg/ddx184.
[3] Tham YC, Li X, Wong TY, Quigley HA, Aung T, Cheng CY. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. *Ophthalmology.* 2014; 121:2081–2090.
[4] Allison K, Patel D, Alabi O Epidemiology of Glaucoma: The Past, Present, and Predictions for the Future. *Cureus*. 2020; *12*(11), e11686.
[5] McMonnies, CW Glaucoma history and risk factors. *J Optom*. 2017; 10:71–78
[6] Stein JD, Khawaja AP, Weizer JS. Glaucoma in adults screening, diagnosis, and management: a review. JAMA. 2021;325(2):164e174.
[7] Kian M. Madjedi, Kelsey V. Stuart, Sharon Y.L. Chua, Robert N. Luben, Alasdair Warwick, Louis R. Pasquale, *et al*. The Association between Serum Lipids and Intraocular Pressure in 2 Large United Kingdom Cohorts, Ophthalmology, Volume 129, Issue 9, 2022, Pages 986-996, ISSN 0161-6420, https://doi.org/10.1016/j.ophtha.2022.04.023.

[8] Schmidl D, Schmetterer L, Garhöfer G, Popa-Cherecheanu A. Pharmacotherapy of glaucoma. J Ocul Pharmacol Ther. 2015 Mar;31(2):63-77. doi: 10.1089/jop.2014.0067. Epub 2015 Jan 14. PMID: 25587905; PMCID: PMC4346603.
[9] Cunniffe MG, Medel-Jiménez R, González-Candial M. Topical antiglaucoma treatment with prostaglandin analogues may precipitate meibomian gland disease. Ophthalmic Plast Reconstr Surg. 2011 Sep-Oct;27(5):e128-9. doi: 10.1097/IOP.0b013e318201d32f.
[10] Sambhara D, Aref AA. Glaucoma management: relative value and place in therapy of available drug treatments. Ther. Adv. Chronic. Dis.2014; 5:30–43.
[11] Brooks AM, Gillies WE. Ocular beta-blockers in glaucoma management. Clinical pharmacological aspects. Drugs Aging. 1992 May-Jun;2(3):208-21. doi: 10.2165/00002512-199202030-00005.
[12] Philipp M, Brede M, and Hein L.Physiological significance of alpha(2)-adrenergic receptor subtype diversity: one receptor is not enough. Am. J. Physiol. Regul. Integr. Comp. Physiol. 2002; 283:287–295.
[13] Costagliola C, dell'Omo R, Romano MR, Rinaldi M, Zeppa L, Parmeggiani F. Pharmacotherapy of intraocular pressure: part I. Parasympathomimetic, sympathomimetic and sympatholytics. Expert Opin Pharmacother. 2009 Nov;10(16):2663-77. doi: 10.1517/14656560903300103.
[14] Aslam S, Gupta V. Carbonic Anhydrase Inhibitors. [Updated 2023 Apr 17]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557736/
[15] Siddique SS, Suelves, AM, Baheti U, Foster CS. Major review: glaucoma and uveitis. *Surv Ophthalmol*. 2013; *58*(1):1-10.
[16] Wang S, Bao X. Hyperlipidemia, blood lipid level, and the risk of glaucoma: a meta-analysis. Invest Ophthalmol Vis Sci. 2019;60(4):1028e1043.
[17] Evans WC. Trease and Evans' Pharmacognosy. 16th Edition. London: WB Saunders Company Ltd; 2008.
[18] Sofowora A, Ogunbodede E, Onayade A. The role and place of medicinal plants in the strategies for disease prevention. Afr J Tradit Complement Altern Med. 2013 Aug 12;10(5):210-29. doi: 10.4314/ajtcam. v10i5.2. PMID: 24311829; PMCID: PMC3847409
[19] Sakar, S., Zaidi, S., Chaturvedi, A.K., Srivastava, R., Dwivedi, P.K. & Shukla, R. (2015). Search for a herbal medicine: Anti-asthmatic activity of mehtanolic extract of Curcuma longa. *Journal of pharmacognosy and phytochemistry. 3*(6), 59-72.
[20] Ejechi BO, Akpomedaye DE. Activity of essential oil and phenolic acid extracts of pepper fruit (*Dennetia tripetala* G. Barker; Anonaceae) against some food-borne microorganisms. Afr. J. Biotechnol. 2005; 4:258–261.
[21] Okwu DE, Morah FNI. Mineral and nutritive value of *Dennettia tripetala* fruits. Fruits. 2004; 59:437–442. doi: 10.1051/fruits:2005006.
[22] Elekwa I, Okereke SC, Chukwudomo CS. Phytochemical screening and GC-MS analysis of the essential oil of *Dennettia tripetala* (Pepper fruit) seeds. ABSU J. Environ. Sci. Tech. 2011; 1:93–98.
[23] Blessing Okpala https://globalfoodbook.com/18-wondrous-benefits-of-pepper-fruit-dennettia-tripetala Published on 2016 M04 30 | |accessed 12th June, 2023
[24] Idyu, I.I., Deshi, E.F., Idyu, V.C., Ogundeko, T.O. (2015). The Anti-diarrhoeal Effect of Ethanolic-Bark Extract of *Sterculia setigera* in Mice. *Asian J. Sci.Tech*. 6(5), pp. 1397 1400.
[25] Eguajoe M, Aigbiremolen AA, Aigbiremolen RN, Ativie M E, Aisuodionoe OM, Odigie JC. Effect of Aqueous Extract of *Persea Americana* Seed on Blood Glucose in Alloxan-induced Diabetic Wistar Rats. *Asian Journal of medicine and health*. 2017; *9*(3): 1-10.
[26] Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother. 2010 Jul;1(2):87-93. doi: 10.4103/0976-500X.72350. Erratum in: J Pharmacol Pharmacother. 2017 Jul-Sep;8(3):153.
[27] Manoj, K., Sukumar D., Manoranjan, P.S., Amar, K, Bharti SR. Different blood collection methods from rats: A review. Balneo Research Journal. 2017; 8(1).

[28] Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clinical Chemistry*. 1974; *20*(4): 470-475.
[29] Fossati P, Lorenzo P. Serum triglycerides determined colorimetrically with anenzyme that produces hydrogen peroxide*. Clinical Chemistry*. 1982; 28: 2077.
[30] Assmann G, Schriewer H, Schmitz G, Hägele EO. Quantification of high-density Lipoprotein cholesterol by precipitation with phosphotungestic acid/$MgCl_2$. *Clinical Chemistry*. 1983; *29*(12): 2026-2030.
[31] Nauck MG, Warnick R, Rifai N. Methods for Measurement of LDL-Cholesterol: A Critical Assessment of Direct Measurement by Homogeneous Assays versus Calculation. Clinical Chemistry. 2002; 48(2):236 –254.
[32] Camilo LC, Leslie AW, Anthony MS, David FB, Bruce EC. Intraocular pressure measurement in the conscious rat. *Acta Ophthalmologica Scandinavica*. 1999; *77*: 33-36.
[33] Shiming W, Xiang. Hyperlipidemia, Blood lipid level and the risk of glaucoma; A meta-analysis. J. Investigative Ophthalmol & Vis Sci. 2019; *60*(4), 34-56.
*[34] Stewart, W.C., Sine, C., Sutherland, S., Stewart, J.A. (1996). Total cholesterol and high-density lipoprotein levels as risk factors for increased pressure. Am. Journal of ophthalmol.*1996; *122: 575-577*.
[35] Alaebo O, Njoku GC, Anumudu OF, Ugwu PA, Norah N, Udensi CG *et al*. Evaluation of The Biochemical and Toxicological Profile of Methanol Extract of *Dennettia tripetala* (Pepper Fruit) Fresh Leaves on Some Selected Parameters in Male Albino Rats. Anim Res Int. 2022; 19(3): 4571 – 4580.
[36] Adeyemi OS, Orekoya BT. Lipid Profile and Oxidative Stress Markers in Wistar Rats following Oral and Repeated Exposure to Fijk Herbal Mixture. J Toxicol. 2014; 2014:876035. doi: 10.1155/2014/876035.
[37] Rahman M, Islam MK, Hossain MK, Al-Mamun, Khan N, Islam S. Serum Lipid Profiles of Primary Open Angle Glaucoma Patients Treated with Topical Timolol. 46J Rang J Med Col. September 2022; 7(2):40-46.