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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

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Authors' contributions

This work was carried out in collaboration among all authors. Author UDI designed the study, wrote the protocol/first draft of the manuscript, and monitored the bench work. Authors CJE, UDI and LCO managed the literature searches. Authors LCO, TOO and NPO modified the protocol, managed the manuscript preparation and study analysis. Author MSCR performed the statistical analysis, while author APB supervised the entire process of the research work to final stage. All authors read and approved the final manuscript.

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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 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 peculiar ocular, cardiovascular and public health care management.

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 [17-19]. *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. Image of *Dennettia tripetala* fruit [23]

2. MATERIALS AND METHODS

2.1 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 in the month of May 2023, 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.

2.2 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.

2.3 Acute Toxicity Test

Acute toxicity test - Phase 1 (10 mg/kg, 100mg/kg and 1000mg/kg) *p.o* and Phase 2 (1600mg/kg, 2900mg/kg and 5000mg/kg) *p.o* was carried out with 12 rats with average weight of 170g rats using Lorcke's method as described by Idyu et al. [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.

2.4 Administration of Cholesterol, IOP and Administration of Timolol Extract

Powdered keshi cholesterol with batch No: 015052201was purchased from Orchard pharmacy, Owerri. Hypercholesterolemia was induced by addition of cholesterol powder, bile salt and animal lard to the standard diet 1% for 10 days according to the method of Pengzhanet al. [25] with slight modification. Each rat group was treated with the mixture daily except rats in the control group.

The rats were grouped as follows:

Group A: Received no treatment (control).
Group B: Treated with Cholesterol *p.o* only.
Group C: Treated with Cholesterol *p.o* + 200mg ASEDt *p.o*.
Group D: Treated with Cholsterol *p.o* + 500mg ASEDt seed *p.o*.
Group E: Treated with Cholesterol *p.o* + 0.5% timolol topically into the eye.

2.5 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].

2.5.1 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 (Cobas c111-Roche).

2.5.2 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 (Cobas c111-Roche).

2.5.3 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 (Cobas c111-Roche).

2.5.4 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 (Cobas c111-Roche).

2.5.5 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.

2.6 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

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. [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.

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

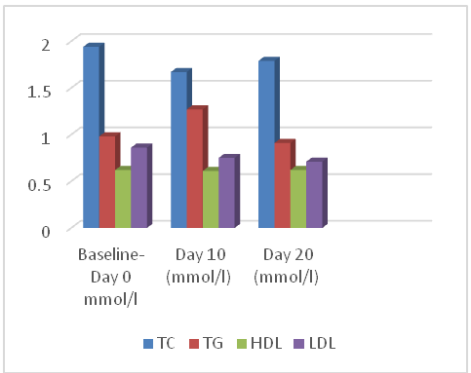

Fig. 1a. MSC of Lipid Parameters for the rats in Group

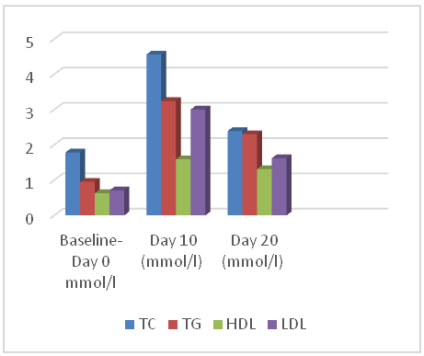

Fig. 1b. MSC of Lipid Parameters for the rats in Group B

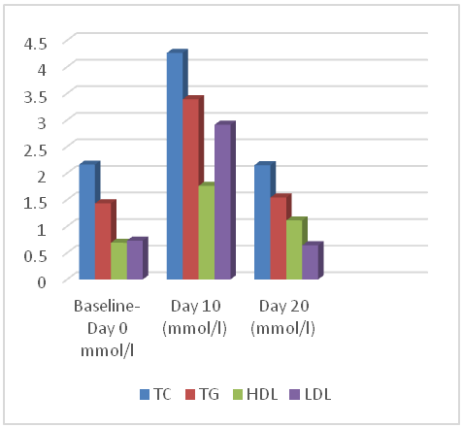

Fig. 1c. MSC of Lipid Parameters for the rats in Group C

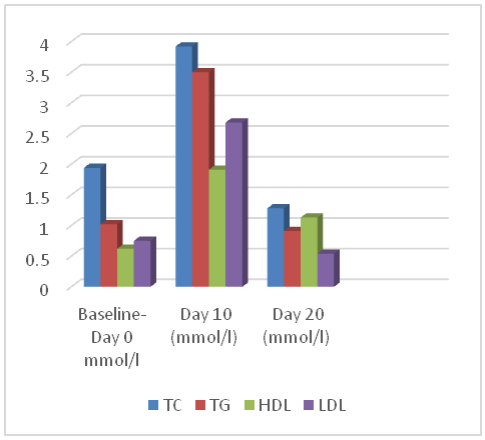

Fig. 1d. MSC of Lipid Parameters for the rats in Group D

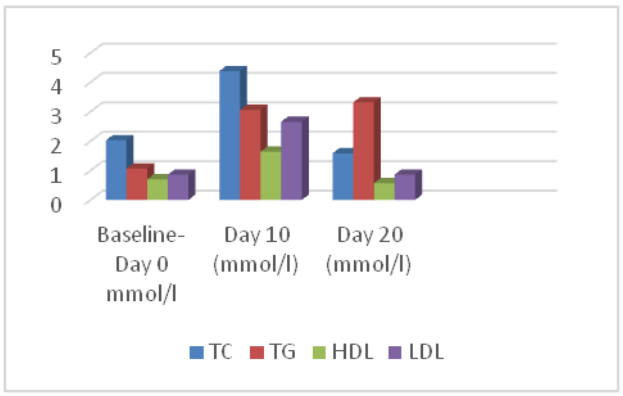

Fig. 1e. MSC of Lipid Parameters for the rats in Group E

Fig. 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 – Figs. 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.

CONSENT

It is not applicable.

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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