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The intraocular pressure reducing- potential of topically administered aqueous extract of *Dennetia Tripetala* on Wistar strain albino rats

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Abstract

The second section of manuscript should contain Abstract and Keywords. Abstract should be informative, self-explanatory and easy to understand.

Plant parts have continued to attract attention in the global search for the treatment of many diseases affecting humans. *Dennetia tripetala* (mmimi) is a well-known spicy indigenous forest fruit from the family annonacea and has been found to contain lots of minerals, vitamins, alkaloids and trace elements which are of medicinal importance. This study investigated the effect of topical ripe and unripe *Dennetia tripetala* seed extracts on the intraocular pressure of albino Wistar rats. Fifteen healthy male albino Wistar rats were used for the study. The rats were randomly divided into 3 groups (A, B, C), each group consisting of 5 rats. Group A rats were treated with one drop of ripe seed extract in their right eye and one drop of Timolol eye drop in their left eye. Group B rats received one drop of unripe seed extract in their right eye and one drop of Timolol eye drop in their left eye. Group C rats served as control (received one drop of water in OD). The I0P of each rat was measured pre and post instillation of one drop volume of the solutions at 30 mins interval for 120 minutes. The findings showed peak reduction of IOP after 60 mins (6.02 mmHg, representing 31.2 % reduction) of instillation one drop volume of ripe *D. tripetala* seed extract and reverted towards baseline (19.16 mmhg). The peak effect of ripe *D. tripetala* was found to be statistically significant (P =4.558). Unlike ripe *D. tripetala* seed extract, the unripe extract induced consistent IOP reduction till 120 minutes (5 % after 30 minutes, 16.5 % after 60minutes and 17.9 % after 120 minutes) from the baseline (18.84 mmHg). The reduction in IOP after 60 minutes (P = .4145) and 120 minutes was statistically significant (P= 2.5448). When compared with Timolol, Timolol produced steady and highest IOP reduction (15 %, 23.5 %, and 33.3 % from the baseline) 30 minutes, 60 minutes and 120 minutes post instillation respectively. Topical administration of aqueous extract of ripe *D. tripetala* seed significantly reduced intraocular pressure in albino Wistar rats, suggesting anti-glaucoma effect of the extract.

Keywords: Medicinal plant; Intraocular pressure; Glaucoma; *D. tripetala*; Timolol

1 Introduction

In Nigeria and most other developing countries, patients tend to prioritize alternative medicines over conventional medicine mostly due to inaccessibility of healthcare by the low and medium class of the society. Eye diseases, including conjunctivitis, cataract, glaucoma, eye allergies, eye inflammation etc. also inclusive. The problem of high cost and adverse drug effects of modern drugs nowadays has led to increased demand for herbal remedies in the treatment of

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eye diseases. *Dennettia tripetala* is one the numerous indigenous plants gaining popularity in Igbo traditional medicine therapy. It is commonly called 'pepper fruit' in English, and mmimi in Igbo language. The plant usually produces fruit between the months of March and May. Ejechi & Akpomedaye, (2005) [1] and Enwere, (1998) [2] reported that, its seeds are dried under the sun by local trader, so as to ensure its availability pending the next harvest.

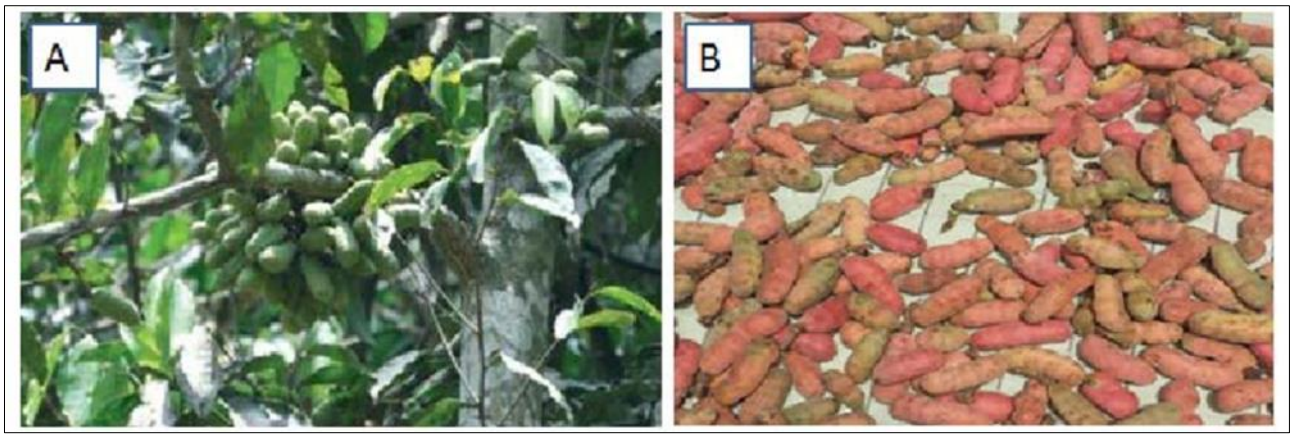

Figure 1 (A) *D. tripetala* tree with leaves and unripe fruits. Image courtesy: World Agroforestry Center and Rubber Research Institute of Nigeria (B) Ripe (red) and unripe (green) *D. tripetala* fruits [3]

Various parts of *Dennettia tripetala* including the leaves, seeds, bark, stem and stem bark as food additives, fuel and traditional medicine for treatment of fever, cough, asthma, catarrh, toothache, diarrhea and rheumatism [4] (Oyemitan *et al.,* 2006) [5]. The nutritional and medicinal benefits of this plant is not farfetched as Isegholi, (2015) [3] and Ihemeje *et al.,* (2013) [6] reported Its fruit to be rich in fatty acids, carbohydrates, proteins, calcium, potassium, magnesium, phosphorus, niacin, riboflavin, thiamine and vitamins A, C and E. Glaucoma refers to a group of conditions that have a characteristic optic neuropathy associated with visual field defects and elevated intraocular pressure (IOP) [7, 8]. Common amongst the types of glaucoma is open angle glaucoma, in which the drainage angle for fluid within the eye remains open while closed/narrow angle and normal tension glaucoma is the least common. The sudden presentation may involve severe eye pain, blurred vision, mid-dilated pupil, redness of the eye, and nausea [9]. An estimated 57.5 million people worldwide are affected by Primary open-angle glaucoma (POAG) with a global prevalence of 2.2 % [10]. In Europe, 7.8 million people were affected by POAG and the total prevalence is 2.51 % [11]. The most common type of glaucoma in the UK is POAG, affecting 2 % of individuals older than 40 years and 10 % of individuals older than 75 years, particularly African-Caribbean people; PACG is not as prevalent and only affects 0.17 % of individuals younger than 40 years, particularly East Asians [12]. Reports by Allingham *et al.,* 2005 [13] and Kyari *et al.,* 2015) [14] stated that the high age-specific prevalence of glaucoma in Nigerian adults aged ≥ 40 years suggests that the severity of glaucoma begins at an earlier age and at a more aggressive course in blacks than in Caucasians and some Asians. Intraocular pressure (IOP) is the fluid pressure of the eye. Its level is determined by the quantity of aqueous humour produced by the ciliary body and its drainage via the trabecular meshwork and uveoscleral outflow. The reason for this is because the vitreous humour in the posterior segment has a relatively fixed volume and thus does not affect intraocular pressure regulation. Some reported factors that affect glaucoma includes socioeconomic differences or inequalities have affected glaucoma services [15] and genes involved in the IOP regulation which was further reported to be more among the Igbo tribe (a rather homogenous ethnic group) of Nigeria, owing to highest prevalence of glaucoma among some sampled African populations [14].

Current consensus among eye care professionals defines normal intraocular pressure as that between 10 mmHg and 20mmHg [16] [17]. The average value of intraocular pressure is 15.5 mmHg with fluctuations of about 2.75 mmHg [18]. Sudden increases in IOP can cause mechanical stress and ischemic effects on the retinal nerve fiber layer, while sudden decreases in IOP can cause micro-bubbles to form from dissolved gases in microvasculature with resultant gas emboli and ischemic tissue damage [19]. The suspicion is that elevated IOP causes direct mechanical damage to the retinal ganglion cell axons. Alternatively, there have been suggestions that the elevated IOP produces a shearing of the dorsal attachments of the astrocytes from the optic nerve head, resulting in a loss of metabolic support to the optic nerve head [20]. Other possible mechanisms include ischemic damage due to compression of blood vessels supplying the optic nerve head.

Eye doctors lower intraocular pressure using drugs, surgery or by a combination of both techniques. Some of the drug groups include: *Beta-blockers*, e.g., Timolol maleate, betaxolol, carteolol [21], *Miotics*, e.g., pilocarpine and carbachol

[22], *Prostaglandin analogues* e.g., latanoprost, bimatoprost and travoprost [23,24], *Carbonic Anhydrase inhibitors*, e.g., acetazolamide (systemic diamox) and dorzolamide [25, 24]. Others are: *Adrenergics/Sympathomimetics*, e.g., epinephrine and dipivefrine, brimonidine [26], *Hyperosmotic agents,* e.g., glycerine and mannitol [27], *Cholinergic agonists,* Parasympathomimetic agents, most commonly pilocarpine, are rarely used as first-line therapy today, but are considered third-line treatment options. When added to bimatoprost at concentrations of 2 %, 4 %, and 6 %, pilocarpine was reported to be neither additive nor antagonistic to the ocular hypotensive efficacy of bimatoprost [28].

The phytochemicals present in the fruits of *D. tripetala* are tannins, alkaloids, steroids, terpenes, flavonoids, balsams (resin) phenol and cardiac glycosides [29, 30].

Considering the fact that *Dennettia tripetala* is used among others for therapeutic purposes, its topical effect may be oblivious. Therefore, this work was geared towards determining the effect of *Dennettia tripetala* extract (aq) on the IOP of Wister strain albino rats.

2 Material and methods

2.1 Study Location

This study was carried out in the Department of Optometry and the animal research faculty of the Department of Anatomy, Imo State University Owerri, Nigeria.

2.2 Experimental Animals Procurement and Preparation

Fifteen healthy (also with no obvious external eye disorder) male albino Wistar strain albino rats (12 weeks old), weighing 200 – 230 g were purchased from the school Animal farm of Imo State University Owerri, Nigeria, housed in standard cages at the Animal Research unit of Anatomy Department of same institution in groups of five rats per cage and maintained at 26-29 ^{o}C, also with 12/12hours light/dark cycle. They were fed with normal rat chow (Pfizer livestock co. Ltd, Aba, Nigeria) and clean water *ad libitum*. The rats were allowed to acclimatize for 14 days.

2.3 Procurement, Identification, Authentication and Preparation of Plant Material

The fruits of *D. tripetala* were purchased from Relief Market in Owerri metropolis, Imo State, Nigeria. A sample of the fruit was identified and authenticated at the herbarium section of the Department of Plant Science and Biotechnology, Imo state University, Owerri, Nigeria. Samples of the fruit was sorted, washed with clean water and dried, then seeds manually separated from the fruits into ripe and unripe, separately blended without water, filtered using a clean sieve and finally filtered using No. 1 Whatman filter paper. and measuring cylinders. The ripe and unripe filtrates were poured into separate clean beakers, labelled appropriately and kept as extracts in a refrigerator at 4 °C till ready for use.

2.4 Animal Grouping and Extract/Drug Administration

The 15 rats were randomly divided into 3 groups of 5 animals each as follows:

- Group A - Administered with one drop of ripe *D. tripetala* seed extract in their right eye (*OD*) and one drop of 5% Timolol eye drop in their left eye.
- Group B - Administered with one drop of unripe *D. tripetala* seed extract in their right eye and one drop of 5 % Timolol eye drop in their left eye.
- Group C (Control Group) - Administered with one drop of distilled water on both eyes.

Administration of extracts and drug was done in the mornings prior to food administration.

2.5 Measurement of IOP

A mitten fabric was used to restrain the animals in order to avoid inducing pressure on the rats while holding them slightly on the neck. Both eyes of each rat were anaesthetized with Proparacaine hydrochloride eye drop and their tear film stained with fluorescein strips. The IOP of the rats, pre-instillation was measured using Schiotz tonometer and recorded as the baseline value. This is done by gently tapping the cornea with the footplate of the tonometer in a perpendicular orientation. Three (3) readings were obtained for each eye and an average taken and the equivalent IOP obtained from the tonometer chart. The IOP was measured post-instillation of the extract, after 30 minutes of instillation to allow for absorption and assimilation at 60 minutes and 120 minutes.

2.6 Statistical Analysis

Both descriptive and inferential methods of data analysis were used. This study was then subjected to a regression analysis to ascertain the contributions of *D. tripetala* extract treatment and Timolol to the baseline. The Statistical packages for social sciences (SPSS) version 23 was used for analysis.

3 Result and Discussion

Table 1 SEM and Percentage changes in IOP due to administration of ripe *D. tripetala* seed extract with time in the experimental rats

Time interval (minutes)	Baseline SEM of IOP (mmHg)	SEM of IOP (mmHg)	SEM of change in IOP (mmHg)	Percentage change in IOP (%)
30	19.51±1.84	16.42±2.29	12.88	15.8
60	19.50±1.84	14.76±2.97	4.54	24.3
120	19.50±1.84	12.88±2.20	6.42	33.9

Key: Sem = Standard Error of Mean, IOP = Intraocular Pressure

Administration of one drop of ripe *D. tripetala* seed extract reduced the IOP of albino Wistar rat though not sustained. At 30minutes post administration of the extract, the IOP was reduced by 9.64 % (1.86 mmHg) from the mean baseline of 19.16 mmHg. This decrease in IOP was consistent at 60 minutes (a 31.2 % reduction, 6.02 mmHg) and then started reverting towards baseline. This decrease was therefore not found to be sustained after 60 minutes post instillation of the seed extract. The IOP returned almost to baseline after 120 minutes (a mean of 17.66 mmHg) post instillation of one drop of ripe *D. tripetala* seed extract. One drop of ripe *D. tripetala* seed extract reduced the IOP of albino Wistar rat though not sustained beyond 60 minutes post instillation -*Table 1.*

Table 2 SEM and Percentage changes in IOP due to administration of unripe *D. tripetala* seed extract with time in the experimental rats

Time interval (minutes)	Baseline SEM of IOP (mmHg)	SEM of IOP (mmHg)	SEM of change in IOP (mmHg)	Percentage change in IOP (%)
30	19.16±2.02	17.44±1.81	1.86±	9.64
60	19.16±2.02	13.28±2.05	6.02±	31.2
120	19.16±2.02	17.66±1.84	1.64 ±	8.5

Key: SEM = Standard Error of Mean, IOP = Intraocular Pressure

The administration of one drop of unripe *D. tripetala* seed extract reduced the IOP of albino Wistar rat. At 30 minutes post administration of the extract, the IOP was reduced by 5 % (0.98 mmHg) from the mean baseline of 18.84 mmHg. This decrease in IOP was consistent at 60 minutes (a 16.5 % reduction, 3.1 mmHg) and at 120 minutes (a 17.9 % reduction, 3.38 mmHg) from the baseline. Equal volume of unripe *D. tripetala* seed extract caused sustained reduction of the IOP of albino Wistar rat. At 30 minutes, the IOP was reduced by 5 %, with the rate of IOP reduction to increasing to 16.5 % (3.1 mmHg) at 60 minutes and 17.9 % reduction at 120 minutes (3.38 mmHg) from the baseline. There was a statistically significant ($P< 0.05$) reduction of IOP after 30 minutes, 60 minutes and 120 minutes. This reduction in IOP according to Timothy and Okere (2008) [31] was probably due to the ascorbic acid, magnesium, flavonoid, melatonin, thiamin, vitamin B, lipoid acid content of *D. tripetala* seed. Ascorbic acid had been established to support the osmotic influx of water following osmolarity elevation of blood artificially leading to the fall in IOP [32]. According to Trygre (2003) [33], 1.50 % of the anterior chamber contents are renewed each minute and the half-life of anterior chamber aqueous is some 45 minutes corresponding to a daily production of about 2.80 ml. Therefore when *D. tripetala* seed extract was instilled and absorbed into the eye its constituent ascorbic acid lowered the eye pressure through increased blood osmolarity. The presence of Ascorbic acid in the anterior chamber protected the cells of trabecular meshwork collagen fibres preventing its shrinkage, narrowing and blockage by pigment cells which normally circulate within the aqueous and drain out via the trabecular meshwork. Timothy and Okere (2008) [31] suggested that the increase in IOP

after 60 minutes and 120 minutes was probably due to the amount of ascorbic acid and magnesium in 0.75 g was not enough to continue with the reduction of I0P. Furthermore, Gasper (1995) [34] noted that magnesium supplementation improves blood supply to the eye and assisted with vision in glaucomatous patients. Flavonoid, another constituent of is attributed to reduce IOP. Head and Kathleen (2001) [35], explained that flavonoids assist with collagen stabilization and synergizes the effect of vitamin C. The effect of flavonoid in IOP reduction is thought to be as a result of the reduction in excessive permeability of blood aqueous membrane within the eye. The presence of lipoic acid (fatty acid) probably helped in the reduction of IOP by increasing glutathione in red blood cells and lacrimal fluid of glaucomatous patients thereby reducing the intraocular pressure. Finally, the presence of melatonin, thiamine (vitamin B) and vitamin B perhaps also contributed to the reduction in IOP. This is due to the fact that melatonin levels have been found to decrease in glaucomatous patient and normal diurnal rhythms of IOP fluctuation reflect melatonin rhythms. The difference in sustainability of IOP reduction for ripe pepper fruit versus unripe pepper fruit could be accounted for by the larger flavonoid content of unripe pepper fruit.

Table 3 SEM and Percentage changes in IOP due to administration of 5% timolol with time in the experimental rats

Time interval (minutes)	Baseline SEM of IOP (mmHg)	SEM of IOP (mmHg)	SEM of change in IOP (mmHg)	Percentage change in IOP (%)
30	18.84±1.99	17.86±1.95	0.98	9.64
60	18.84±1.99	15.74±2.06	3.1	31.2
120	18.84±1.99	15.46±2.2	3.38	17.9

Key: SEM = Standard Error of Mean, IOP = Intraocular Pressure

The administration of one drop of Timolol malate 0.5 % steadily reduced the IOP of albino Wistar rat. At 30 minutes post instillation, the IOP was reduced by 15 % (2.88 mmHg) from the mean baseline of 19.50 mmHg. This decrease in IOP was consistent at 60 minutes (a 23.5 % reduction, 4.54 mmHg) and at 120 minutes (a 33.3 % reduction, 6.42 mmHg) from the baseline. The group treated with Timolol maleate, showed maximum reduction in IOP 120 minutes after administration. The relative difference in reduction in intraocular pressure was statistically significant (P= 0.05). This is consistent with the work done by (Liu *et al*., 2004 [36], who reported Timolol to lower IOP by reducing aqueous humor flow. Following instillation, the effect of Timolol is known to peak at 2hours. Different results in IOP readings obtained by Liu *et al*. (2004) [36], may be mainly referable to their different methodology in the study design and their sample size (18 patients).

4 Conclusion

Topical administration of aqueous extract of ripe *D. tripetala* seed significantly reduced intraocular pressure in albino Wistar rats, suggesting anti-glaucoma effect of the extract.

Compliance with ethical standards

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Disclosure of conflict of interest

All Authors declare no competing interest.

Statement of ethical approval

Ethical clearance was obtained from the Research and Ethics Committee of the College of Health Sciences, Abia State University, Uturu, Nigeria. Study was also carried out in accordance with the Association for Research in Vision and Ophthalmology (ARVO) statement for the use of animals in Ophthalmic and Vision Research.

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