



Research Article

JMR 2018; 4(2): 89-92

March- April

ISSN: 2395-7565

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www.medicinarticle.com

Received: 08-12-2017

Accepted: 26-02-2018

Effect of ethanol extract of ripe fruits of *Dennettia tripetala* on prostatic and testicular functions of male albino rats

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Abstract

The effects of ethanol extract of ripe fruits of *Dennettia tripetala* on prostatic and testicular functions of male albino Wistar rats were investigated. Twenty male albino rats weighing between 160 – 180g were divided into 4 groups with 5 animals in each group. Group 1 served as the control while Groups 2, 3 and 4 were orally administered 300 mg/kg, 600 mg/kg and 900 mg/kg of ethanol extract of ripe *Dennettia tripetala* fruits respectively daily for 28 days. Blood samples were collected through cardiac puncture, serum obtained for assay of prostate specific antigen (PSA) and testosterone levels while prostates and testes were excised and used for histological evaluation. The results showed a non-significant ($P > 0.05$) dose dependent decrease in the concentration of PSA. A non-significant decrease in testosterone levels in Groups 2 and 3 when compared to control was also recorded in this study while a significant decrease was observed in Group 4. Normal histological features were observed in the histology of the prostate while the presence of mature sperm cells in the photomicrograph of the testes of the treated groups suggest increased spermatogenesis following the administration of the extract. The results therefore suggest a beneficial effect of *Dennettia tripetala* in reducing PSA levels with possible improvement in spermatogenesis. It also showed no toxic effect on prostate and testes of the experimental animal.

Keywords: *Dennettia tripetala*, Prostate Specific Antigen (PSA), Testosterone, Prostate, Testes.

INTRODUCTION

Dennettia tripetala (commonly known as Pepper fruit) is a well-known plant in many communities in Southern States of Nigeria. It is widely grown in the rainforest zones of Nigeria and some part of the West. It is commonly known in different ethnic groups of Nigeria as; Ako (Edo), Mmimmi (Igbo), Nkarika (Ibibio) and Ata Igbere (Yoruba). It is widely consumed by the inhabitants of West Africa due to its distinctive spicy taste. The plant is usually found in Cocoa plantation where it is used as a means of demarcation of farm boundaries. *Dennettia tripetala* grows as a small shrub to a height of 12-15m and have a girth of 0.6m [1].

The fruits are green when developing but starts to turn red with ripening. The leaves are 3-6 inches long and 1.5 – 2.5 inches broad. They are elliptic in shape. The fruits are mainly made up of seeds and a bit of hard spicy flesh. The fruits, leaves, bark and root of the plant possess strong peppery and pungent taste. The various parts of the plants are usually used as spices and condiments because not only does it possess characteristics taste but also strong aroma. The fruits are commonly chewed raw in different forms (fresh green, fresh ripened brown, black dry fruits and dry seeds). The leaves are commonly used in pepper soup delicacies and as condiments in some special local dishes [1].

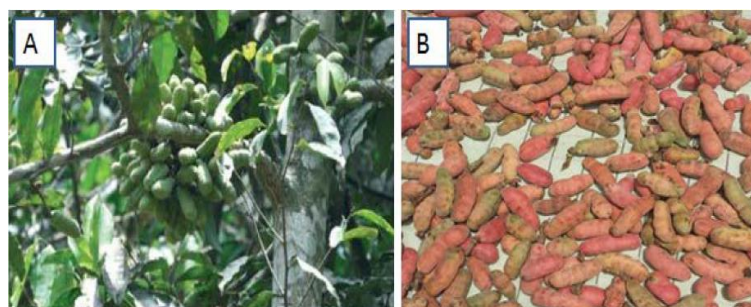


Figure 1: (A) *Dennettia tripetala* tree with leaves and unripe fruits. (B) Ripe (red) and unripe (green) *Dennettia tripetala* fruit [2].

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The leaves are also used by local herbalists in combination with other medical plants to treat various ailments including fever, infantile convulsion, typhoid, cough, worm infestation and stomach upset [3]. There are also reports that the fruits are sometimes used for masking mouth odour [3]. The fruits of the plant have been reported to be popularly used as stimulants [4, 5, 3]. *Dennettia tripetala* fruits have been reported to contain important nutritive substances such as vitamins, minerals and fiber of essential oil [6]. It was also indicated that the rich presence of essential oil (oleoresins) determines the aromatic flavouring, coloring and pungent properties of pepper fruits. Nwaogu *et al.* investigated the phytochemical contents of *Dennettia tripetala* and reported the presence of saponins, flavonoids, tannins, and cyanogenic glycosides [7]. Also Adedayo *et al.* reported the presence of flavonoids in *Dennettia tripetala* [8]. The intake of flavonoids in any fruits or vegetables tends to reduce cancer risk [9, 10]. Furthermore, biochemical screening of *Dennettia tripetala* revealed the presence of the following components; Crude fiber, fats and oil, Carbohydrate, crude protein, moisture, caloric values, hydrogen cyanide. It is also rich in minerals such as: thiamine, riboflavin, niacin, and ascorbic acid. *Dennettia tripetala* contains a hexanolic constituent (n-hexane) which is toxic to larvae.

The essential oil and phenolic acid extract of *D. tripetala* has been reported to inhibit the growth of food-borne microorganisms such as *Staphylococcus aureus*, *Salmonella* sp., *Escherichia coli* and a host of other microorganisms [1]. The leaves of *D. tripetala* were found to be effective in inhibiting the growth of the rot-causing fungus *Sclerotium rolfsii* in cocoyam both in vitro and in vivo [11]. The oil is also reported to relieve inflammation in rodents with edema to levels comparable with that of dexamethasone [12]. Anaga and Asuzu (2010) showed that *D. tripetala* can reduce the plasma glucose level in drug-induced hyperglycemic rats to levels comparable with that of normal rats [13]. The aqueous extract of unripe *D. tripetala* has been reported possess greater antioxidant ability compared to ripe DT as typified by higher reducing power, greater ability to scavenge ABTS, DPPH, and OH, as well as higher Fe reducing and chelating potential [8]. Furthermore, other studies have documented the *in vivo* free radical scavenging and *in vitro* antioxidant potentials of *Dennettia tripetala* [14, 15]. The presence of antioxidants such as flavonoids and ascorbic acid in *D. tripetala* are responsible for the antioxidant potentials of the plant. Recent studies by Akpakpan *et al.* has reported that *D. tripetala* at doses ranging between 262.20mg/kg and 786.61mg/kg is not toxic to the liver and kidney of normal albino Wistar rats [2]. The present study evaluated the effect of ethanol extract of crude extract of *Dennettia tripetala* on the function of prostate and testes of healthy albino wistar rats.

MATERIALS AND METHODS

Denettia tripetala

Ripe fruits of *Denettia tripetala* was obtained from Oboh Market in Etim Ekpo Local Government Area, Akwa Ibom State. The fruit samples were authenticated by a Botanist in Department of Botany and Ecological Studies, University of Uyo, Akwa Ibom State. The fruits were processed as described by Akpakpan *et al.* [2]. They were washed, air dried at room temperature and grounded into powder with mortar and pestle. The powdered fruit was macerated in 80% of ethanol for 3 days and filtered, the filtrate was placed on the water bath for complete evaporation. The concentrated extract was stored in a freezer at -4°C.

Experimental Animals and Design

Twenty male albino rats weighing between 160 – 180g were obtained from Animal House of College of Health Sciences, University of Uyo,

Nigeria. The animals were allowed to acclimatize under standard laboratory conditions and given access to commercial food and clean drinking water *ad libitum*. The animals were divided into 4 groups with 5 animals in each group. Group 1 served as control while Groups 2, 4 and 4 (extract treated groups) received orally the ethanol extracts of *Dennettia tripetala* at doses of 300 mg/kg, 600 mg/kg and 900 mg/kg body weight respectively. The extract was administered daily for 28 consecutive days.

Collection of Blood and Tissue Sample

After the last administration, the animals were allowed to fast overnight and then sacrificed under chloroform anesthesia. Whole blood was obtained by cardiac puncture into a sterilized sample bottles. The blood collected was allowed to clot by standing at room temperature for one hour and centrifuge at 2500g for ten minutes. The serum obtained was used for assay of testosterone and prostate specific antigen concentrations. The organs; testes and prostates were excised from the animals and preserved in 10% buffered formalin for histological studies.

Assay Methods for Testosterone and PSA

The serum testosterone was determined using testosterone Elisa kit for the quantitative determination of testosterone concentrations in serum. The testosterone Elisa kit is a solid phase enzyme-linked immunosorbent assay based on the principle of competitive binding. The concentration of prostate specific antigen in the serum was assayed by PSA ELISA method. The PSA ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes an anti-PSA antibody directed against PSA for solid phase immobilization (on microliter wells).

Histological Evaluation of the Testes and Prostate

The organs were subject to routine histological evaluation using haematoxylin and eosin staining methods. Sections from the testes and prostates were fixed with 10% buffered formalin, embedded with paraffin. After routine processing, paraffin sections of each tissue were cut into 5µm thickness and stained with haematoxylin and eosin [16]. The photomicrographs were taken with a digital camera attached to a light microscope.

Statistical Analysis

Data are presented as mean ± standard error of the mean and were analyzed using one-way analysis of variance (ANOVA) followed with a *post-hoc* test (least significance difference) using SPSS version 20.0. *P* =.05 was considered as statistically significant.

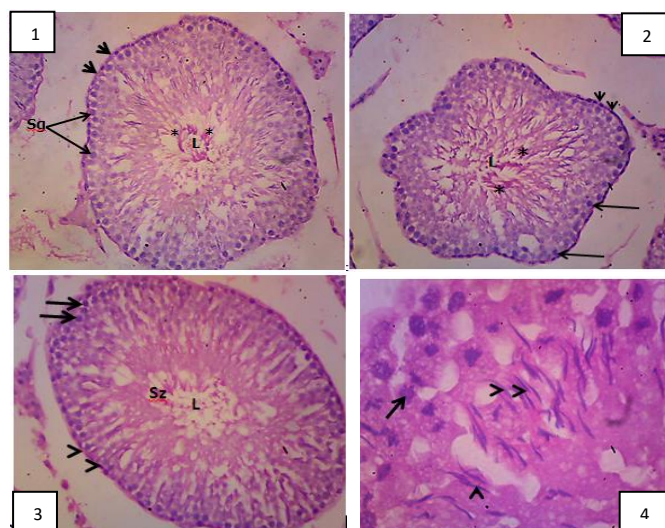
RESULTS

Table 1: The concentration of testosterone and PSA of albino Wistar rats administered with ethanol extract of ripe fruits of *Dennettia tripetala*

Groups	Testosterone (mg/ml)	PSA (ng/ml)
Group 1 (Control)	3.64 ± 0.06	0.41 ± 0.03
Group 2 (300 mg/kg bw)	3.29 ± 0.23	0.41 ± 0.03
Group 3 (600 mg/kg bw)	3.11 ± 0.27	0.38 ± 0.03
Group 4 (900 mg/kg bw)	2.93 ± 0.20 ^a	0.37 ± 0.03

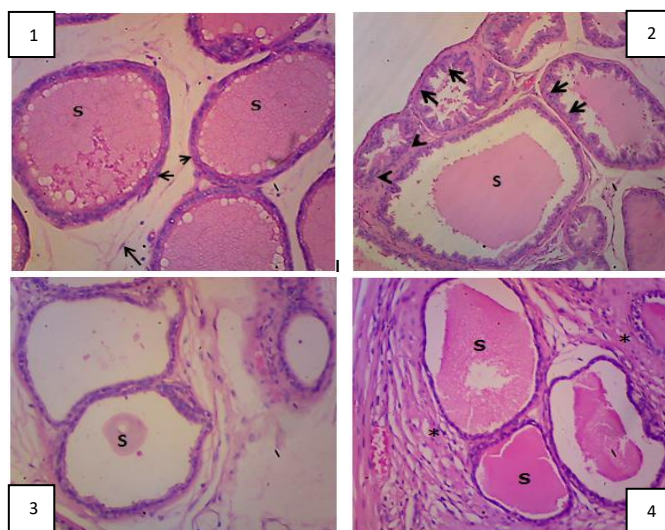
Data presented as Mean ± Standard Error of Mean. Bw = body weight
^a = significant difference at *P* < 0.05 when compared to the control

Histology of the Testes



Photomicrographs of Wistar Rat Testes: 1 (Group 1 – Control): Normal architecture of testes showing average sized seminiferous tubules (ST) with spermatogenic cell layers, highly basophilic spermatogonia (Sg) and spermatogenesis progressing from the basal germinal layer (arrow head) to mature spermatozoa (*) with their tail extending into the lumen (L). 2 (Group 2 – 300 mg/kg of DT fruit extract): Normal architecture of testes with normal features and irregular border. 3 (Group 3 – 600 mg/kg of DT fruit extract); Normal architecture of testes with normal features. 4 (Group 4 – 600 mg/kg of DT fruit extract); Normal architecture of testes with normal features, Primary spermatocyte (arrow) and evidence of increased mature spermatozoa (Notched arrow). H and E Staining Technique, Mag x100.

Histology of the Prostate



Photomicrograph of Prostatic tissues of Wistar Rats: 1 (Group 1 – Control); showing numerous prostatic acini filled with homogeneous acidophilic intraluminal secretion (S), lining epithelium (arrow head) consist of single layer of both flattened and cuboidal cells with few connective tissues, fibrocyte (arrow) and sprouting epithelium (notched arrow). 2 (Group 2 – 300 mg/kg of DT fruit extract): Prostatic tissue showing benign gland having undulating luminal contour. The tall columnar epithelial cell have a pale clear cytoplasm, basal cell can be identified. Some acini show intraluminal secretion (S). Also seen between the acini is fibromuscular stroma (notch arrow). 3 (Group 3 – 600 mg/kg of DT fruit extract): Prostatic tissue showing benign gland having undulating luminal contour (arrow). The tall columnar epithelial cell have a pale clear cytoplasm, basal cell can be identified. Some acini

were cystically dilated and show intraluminal prostatic concretion (corpora amyloacea) (S). Reduced fibromuscular stroma (*) are seen between the acini. The are areas of nodular hyperplasia (H). 4 (Group 4 – 900 mg/kg of DT fruit extract): Prostatic tissue. line by simple cuboidal epithelium supported by prominent fibromuscular stroma (*). Some acini show many papillary folds (arrow head) and occupied acidophilic secretions (S). H and E Staining Technique, Mag x100.

DISCUSSION

Preliminary phytochemical screening carried out by Ihemeje *et al.* indicated that *Dennettia tripetala* fruits contain alkaloids, flavonoids, tannins and saponins in its ethanol extracts [17]. These phytochemicals are known to perform several general and specific functions in plants, and may exhibit different biochemical and pharmacological actions in different species of animals when ingested. These actions range from cell toxicity to cell protective effects.

Testosterone is the primary male sex hormone and an anabolic steroid. In men, testosterone plays a key role in the development of male reproductive tissues such as the testes and prostate, as well as promoting secondary sexual characteristics such as increased muscle and bone mass, and the growth of body hair [18]. In addition, testosterone is involved in health and well-being [19] and the prevention of osteoporosis [20]. Insufficient levels of testosterone in men may lead to abnormalities including frailty, muscle weakness and age related bone loss [21].

Testosterone is needed for growth and acts in the seminiferous tubules to initiate and maintain spermatogenesis in association with follicle stimulating hormones [22]. The reduced levels of testosterone and normal histological features of the testes as well as enhanced spermatogenesis observed in this study suggest that the extract may promote the activity of the enzyme 5 α reductase which converts testosterone to its active form dihydrotestosterone resulting in increased level of spermatogenesis in the animals especially Group 4 with the lowest level of testosterone. The extract of ripe *Dennettia tripetala* fruit in this study has been shown not to negatively affect the histomorphology of the testes. This is similar to the effect of *Hippocratea africana* root bark extract on the histology of the testes as reported by Ndem and Johnson [23]. However, while the testosterone levels were observed to be decreased with *Dennettia tripetala* extract, *Hippocratea africana* administration was reported to result in increased testosterone levels in albino Wistar rats. High levels of testosterone has been reportedly utilized in the induction of prostate enlargement and hyperplasia in experimental animals [24].

The prostate gland provides the semen with vitamins and other nutrients, thus maintaining its vitality during the journey up the female reproductive system. Prostate specific antigen is an organ specific marker occurring abundantly in seminal fluid. Although its physiological actions are unknown, it is considered to play a role in liquefaction of coagulated seminal fluid. It is specific to prostatic epithelial cells in various condition including normal and hyperplastic conditions although almost all PSA proteins leaks into blood in prostate cancer. Higher level of serum PSA are associated with a high risk of prostate cancer [25]. However, the present study reveals that administration of *D. tripetala* to adult male albino Wistar rat causes non-significant ($P > 0.05$) dose-dependent decrease in the concentration of PSA.

Dennettia tripetala is a rich source of alpha-linoleic acid which has been shown to reduce the risk of prostate cancer in men [26], supporting the decreased PSA level observed in this study. In another study by Jagla (2013), extract of *D. tripetala* was shown to inhibit the growth of prostate cancer cells, suggesting that the extract of *D. tripetala* possess growth inhibitory and cytotoxic effect on the prostate cancer cell lines [27]. The underlying factor linking diet and prostate cancer is probably hormonal. Fats stimulate increased production of testosterone and

other hormones, and testosterone acts to speed the growth of prostate cancer. High testosterone levels may stimulate dormant prostate cancer cells into activity. Some findings suggest that high testosterone levels also influence the initial onset of prostate cancer [28].

The histology of normal prostate gland reveals distinct prostatic features with homogenous acidophilic intraluminal secretions in the control group. Frequent deviations from the normal histology of the prostate have been reported to include post-inflammatory atrophy, basal cell hyperplasia, benign nodular hyperplasia, atypical adenomatous hyperplasia and duct-acinar dysplasia [29]. The administration of extract of ripe fruits of *Dennettia tripetala* showed normal prostatic features though some nodular hyperplasia were observed at low and medium dose. The result corroborates the measurement of PSA concentration indicating the absence of damage to the prostate hence the safety of the ripe fruits extract of *D. tripetala* on the prostate of male albino Wistar rats.

CONCLUSION

The administered doses of ethanol extract of ripe fruits of *Dennettia tripetala* does not have toxic effects on the prostate and testes and may be beneficial in reducing PSA levels as well as improving spermatogenesis in albino Wistar rats.

Conflict of Interest

The authors declare that no of interest exist.

Authors Contribution

This work was carried out in collaboration between all authors. Ekanemesang UM and Bassey UE designed the study. Akpakpan EI and Akaka EUA acquired data and managed statistical analysis. Etim OE interpreted the data. Bassey UE drafted the manuscript while Ekanemesang UM and Etim OE revised the manuscript for intellectual content and gave final approval to the manuscript.

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