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Effects of Methanolic Extract of *Trichosanthes Cucumerina* Seed on Experimentally-Induced Prostatic Hyperplasia.

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Abstract:

Benign prostatic hyperplasia, a non-cancerous condition of unknown cause, is the most common prostate disease in men globally. The present study investigated the possible ameliorative effects of methanolic extract of *Trichosanthes cucumerina* seeds (METCS) on experimentally-induced prostatic hyperplasia in Wistar rats.

Twenty adult, male Wistar rats weighing 150-250 g were randomly divided into four groups of five animals each. Group I, normal control, was given corn oil only; Group II, hormone-treated control (HTC), Groups III and IV, hormone- and extract-treated, received continuous doses of 300 µg and 80 µg of testosterone and estradiol, respectively, on alternate days for three weeks subcutaneously in the inguinal region; while the extract-treated rats received additional 400 mg/kg b.w. (low dose) and 800 mg/kg b.w. (high dose) of METCS orally for another three weeks. Immediately after induction of benign prostatic hypertrophy, some animals were randomly selected and sacrificed for gross inspection of prostate enlargement, prostate specific

antigen analysis (PSA) and sperm count evaluation. These procedures were repeated again after three weeks of extract treatment. The prostates were harvested and processed routinely for paraffin embedding, and stained with hematoxylin and eosin (H&E).

Results obtained showed significant ($P<0.05$) reduction in PSA levels following high doses of METCS and also a reversal of histological hyperplasic changes.

Keywords: *Trichosanthes cucumerina*, methanolic extract, testosterone, estradiol, prostate enlargement, prostate specific antigen, male Wistar rats

Introduction:

The global use of herbs in the management and/or treatment of various health problems is rapidly becoming more successful, and also proving to be more cost-effective. This positive observation has rapidly increased scientific investigations for plants that could prove beneficial in the treatment of human diseases. One such plant is *Trichosanthes cucumerina*, the fruit of which is mainly consumed as a vegetable.

T. cucumerina, commonly known as "snake gourd, viper gourd, snake tomato or long tomato", is an annual climber in the cucurbitaceae family. The fruit is usually consumed as a vegetable due to its nutritional value. The plant is rich in chemical constituents such as flavonoids, carotenoids, phenolic compounds, etc [12], which make the plant pharmacologically-and therapeutically-active. It has a prominent place in alternative systems of medicine because of its various pharmacological properties such as; antidiabetic, hepato-protective, gastro-protective, hypoglycemic, anti-infertility, cytotoxic, anti-inflammatory, and larvicidal effects [12, 17].

The whole plant, including roots, leaves, fruits, and seeds, have medicinal properties. The root is used as a cure for bronchitis, headache and boils. Externally, the leaf juice is rubbed over the liver to relieve liver congestion. Both the root and fruit are considered to be cathartic. The fruit is used as an anthelmintic. The seeds are used for stomach disorders and are also considered antifebrile and anthelmintic. The aerial parts of *T. cucumerina* are used along with other plant materials for indigestion, bilious fevers, boils [3], sores, diabetes, ulcers and skin eruptions such as urticaria, eczema, dermatitis, psoriasis [11, 3].

T. cucumerina is a rich source of nutritional constituents. It is very rich in proteins, fat, fibre, carbohydrates, and vitamins A, C and E. The total phenolics and flavonoids content is 46.8% and 78.0% respectively [1]. The crude protein content is 30.18% [20]. The predominant mineral elements are potassium (121.60 mg 100-1 g) and phosphorus (135.0 mg 100-1 g). Other elements found in the plant in fairly high amounts are sodium, magnesium and zinc [14].

Benign prostatic hyperplasia (BPH) or Benign prostatic enlargement (BPE), a non-cancerous condition of unknown cause, is the most common prostate disease in men globally. It occurs in approximately 70% to 80% of men between 70 to

80 years old [7]. The present study investigated the effects of methanolic extract of *T. cucumerina* seeds (METCS) on experimentally-induced prostatic hyperplasia in rats. It is further justified by the paucity of documented studies on the effect of *T. cucumerina* extract on prostatic hyperplasia.

Materials and Methods:

Animal Management:

Twenty adult male Wistar rats weighing 150-250 g obtained from the animal house of the Department of Zoology, University of Ibadan, Ibadan, Nigeria, were used for this study. They were housed and acclimatized for two weeks before the experiment commenced, in the animal holdings of the Department of Anatomy, Olabisi Onabanjo University, Ikenne Campus, Ogun State, Nigeria. Standard rat chow and drinking tap water were given *ad libitum*, and the animals were kept under natural light and dark cycles at room temperature (27 ± 1^{o}C). The rats were randomly divided into four groups of five animals each, housed in separate clean plastic cages. Two controls, normal control (NC) and hormone-treated control (HTC), as well as low- and high-dose METCS-treated animal groups were used.

Preparation of Extract:

Six ripe fruits of *T. cucumerina* were purchased from Ayepe, a local town in Ijebu-Remo, Ogun State, Nigeria. The plant material was authenticated by a plant taxonomist from Department of Botany, Faculty of Science, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria. The seeds were taken from the fruits, washed and air-dried at room temperature, and subsequently grinded into fine powder. 40 g of the powdered sample was weighed into a beaker, soaked in 100 ml of methanol for of 48 hours, and filtered. The filtrate was thereafter concentrated in a rotary evaporator.

Animal Treatment:

Testosterone propionate (brand name: Ricostrone; a product of Greenfield pharma, Jiangsu Co Ltd., China) and estradiol valerate (Medipharm Ltd., 108-Kotlakhpat Industrial Est; Lahore, India) were used. Testosterone propionate (T) and estradiol valerate E2 (puregynon depot) were used for the induction of prostate enlargement at a combined dose of 300 µg T and 80 µg E2 [2, 5]. The drugs were administered to the rats for three consecutive weeks subcutaneously in the inguinal region. Low and high-dose METCS-treated animal groups received a combined dose of testosterone 300 µg and estradiol 80 µg per 100 g b.w. diluted with corn oil to the specified dose. Low and high-dose METCS groups were further treated orally with 400 mg/kg and 800 mg/kg b.w. of METCS diluted in corn oil respectively for another three weeks. Normal control animal group received normal saline equivalent of corn oil volume used only.

Histological and Biochemical Analysis:

At the end of the 4-weeks study period, the animals were sacrificed and dissected under chloroform anesthesia. Blood was collected through the apex of the heart, and serum was obtained after centrifuging the blood samples for 5 minutes at 5000 rpm in a Denley BS400 centrifuge (England). The serum was used for biochemical analysis of Prostate Specific Antigen (PSA).

The region around the penile shaft was opened, the bladder located, and the prostate around it was removed. The loop of epididymis was pulled up; the testis was located and taken out. Epididymal sperm was obtained and used for sperm count analysis. The organs (prostate, seminal vesicle, testis and bladder) were dissected out, weighed and fixed in 10% formol-saline. After complete fixation, the blocks were embedded in paraffin wax, and sections cut at 5 μm, and then stained with haematoxylin and eosin method [8]. Microscopic examination of the sections was then carried out under a light microscope.

Statistical Analysis

Results were expressed as Means ± Standard Deviation (SD) for each group. All grouped data were statistically evaluated using SPSS 15.0 software. Hypothesis testing method included the independent – samples – t-test. Statistical significance was set at $P<0.05$.

Results:

Effects of Hormone and METCS treatments on Organ Weights:

There was no statistically significant difference in the weight of the prostate of the HTC, low- and high-dose METCS-treated animal groups when compared to the normal control. Seminal vesicle weight was significantly increased in the HTC group when compared to normal control (NC) group. Treatments with low and high doses of METCS reduced the weight of seminal vesicle, although only high-dose treatments significantly reduce seminal weight when compared with HTC (Table 1).

Table 1: Relative weights of accessory sex gland, testis and bladder of rats treated with methanolic extract of T. cucumerina seed

	Normal control (NC)	Hormone treated control (HTC)	Low dose METCS	High dose METCS
Prostate	0.06±0.01	0.08±0.01	0.06±0.02	$0.05\pm0.01^{\beta}$
Seminal Vessicle	0.09±0.02	$0.21\pm0.05^{\alpha}$	$0.19\pm0.05^{\alpha}$	$0.10\pm0.05^{\beta}$
Testis	0.30±0.05	0.27±0.02	0.28±0.04	0.30±0.04
Bladder	0.05±0.01	0.05±0.01	0.05±0.01	0.04±0.01

α=Significant difference ($P<0.05$) compared to NC. β=Significant difference ($P<0.05$) compared to HTC n=5.

Effects of Hormone and METCS treatments on PSA value:

PSA value was significantly increased in HTC group when compared to NC group. Treatment with low and high doses of METCS reduced PSA value, although only high-dose treatment significantly reduces PSA value when compared to HTC group (Table 2).

Table 2: PSA value of rats treated with methanolic extract of T. cucumerina seed:

	Normal control (NC)	Hormone treated control (HTC)	Low dose METCS	High dose METCS
PSA value (ng/ml)	3.75±1.71	8.75±0.96 $^{\alpha}$	6.25±2.63	2.75±0.96 $^{\beta}$

α=Significant difference (P<0.05) compared to NC. β=Significant difference (P<0.05) compared to HTC. n=5

Effects of Hormone and METCS treatments on sperm count:

Sperm count was significantly reduced in all treated groups, when compared with NC group. Treatments with low and high doses of METCS did not improve sperm count (Table 3).

Table 3: Sperm count of rats treated with methanolic extract of T. cucumerina seed

	Normal control (NC)	Hormone treated control (HTC)	Low dose METCS	High dose METCS
Sperms (millions/ml)	4.96±1.48	0.27±0.24 $^{\alpha}$	0.25±0.24 $^{\alpha}$	0.34±0.16 $^{\alpha}$

α=Significant difference (P<0.05) compared to NC. β=Significant difference (P<0.05) compared to HTC. n=5

Histological Findings:

As shown in Figure 1, the normal histology of the prostate is seen in NC group. Observe the presence of the prostatic concretions, the excretory duct with secretions in its lumen, the flat glandular epithelium and surrounding layer of finely arranged smooth muscle bundles. Observe also the hormonal effects of estradiol and testosterone in the HTC group. Notice the proliferation of epithelial cells, with the infolding of the epithelium into the lumen of the excretory duct, constricted follicles and minute amount of prostatic secretions. Notice also the approach towards normal histology in the low dose METCS-treated group. There is the presence of prostatic concretions embedded in the layer of glandular epithelium and also the lumen of excretory duct is well appreciated, as well as the surrounding layer of finely arranged smooth muscle bundle. The anti-inflammatory effect of METCS can be better appreciated in the high dose METCS-treated group, where the histology has a closer semblance to normal histology. Observe the presence of the prostatic concretions, flattened glandular epithelium, the excretory duct with

secretions in its lumen, and surrounding layer of finely arranged smooth muscle bundles.

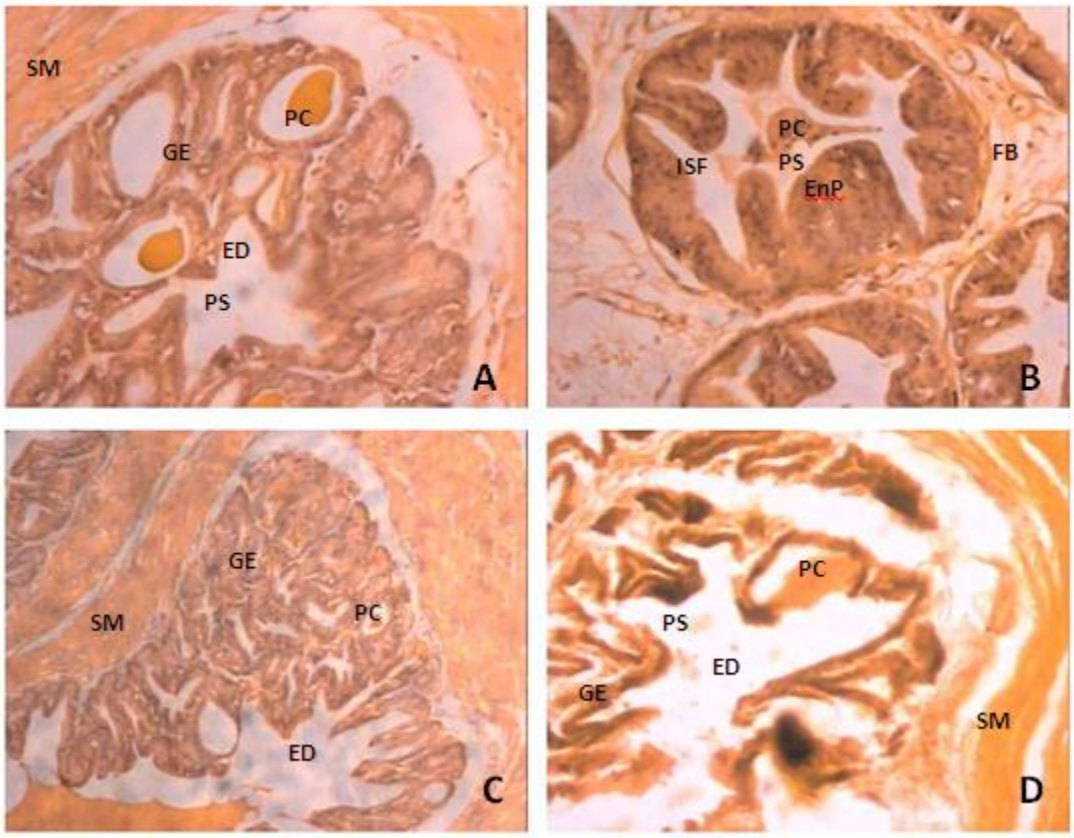

Figure 1: Light photomicrographs of prostate of NC (A), HTC (B), low-dose METCS-treated (C) and high-dose METCS-treated (D) rats groups (H&E x1000). SM = Smooth Muscle Bundle; PC = Prostatic Concretion; GE = Glandular Epithelium; ED = Excretory Duct of Prostate; PS = Prostate Secretions; ISF = Irregular Shaped Follicles; FB = Fibromuscular tissue, EnP = Enlarged Prostatic cells.

Discussion

The epidemiology of BPH is complex and not fully understood. The androgenic hormones, testosterone and dihydrotestosterone, play at least a permissive and important role. Growth factors and other hormones, including estrogens, may play a role. BPH is a truly hyperplasic process resulting in growth of glandular epithelial and stroma/muscular tissues in prostate, leading to often measurable growth, taking on different shapes and configuration which may impact symptoms such as difficulty in micturation [16].In the present study, PSA level was significantly increased. A raised PSA level can be a sign of prostate cancer (Pca). However, a raised PSA level can also occur in other prostate conditions, such as some cases of benign enlargement (BPH) and inflammation of the prostate prostatitis [19], as observed in this study. Although low dose administration of METCS lowered the PSA level, it was only at high METCS treatment level that the level was brought within normal range.The decrease in sperm count may be due to the negative feedback sent to the anterior

pituitary to stop the production of luteinizing hormone-LH (due to excess testosterone in the system), which normally would have stimulated the Leydig cells in the testis interstitium [10]. METCS administration caused no significant change in the sperm count. This may be because the plant has antifertility attribute which have been reported in female rats, but yet to be confirmed in males [6].

Following high-dose administration of METCS, there was a significant reduction ($p<0.05$) in the weight of the prostate which had increased following hormone treatment. Administration of androgens, testosterone and estradiol, induces increases in the prostate and seminal vesicle weights, and this may be due to cell proliferation and stromal growth. The stromal provides the infrastructure for development of epithelial cells. The present findings reflect the anabolic effects of androgens, and support the work reported by [9]. The hypertrophy of the epithelial cells of HTC-treated animals suggests that addition of estradiol potentiates the action of testosterone, causing an increase in the thickness of epithelial cells and can directly stimulate the activity of secretory epithelial cells [13; 15]. The reduction in weights of accessory sex glands following high dose METCS treatment may be attributed to the degradation of basement membrane and interstitial tissues, and absence of cell proliferation due to depletion of androgens [18]. Histological findings in the present study showed morphological alterations of the prostate in the form of hyperplasic changes, following hormone treatment, which were reversed following METCS administration, especially at high dose. Prostatic hyperplasia is the result of proliferation of epithelial cells and smooth muscle cells, fibroblasts, and other stromal components in variable proportions [4]. Induction of experimentally-enlarged (BPH) is achieved in prostate via a form of tissue damage caused by the hormone regime given to the animals. This enlargement is characterized by increased size which has been reported to be accompanied by small number of neutrophils which were found in the secretory cell layer within the gland and duct lumen. The secretory cell layer is broken with increasing severity, and glands become filled with inflammatory exudates containing intact and fragmented neutrophils which may indicate some form of free radical damage in the cells [2; 5]. High-dose administration of MECTS was most effective in reversing the hyperplasic changes, as it brought about a reduction in stroma size as well as shrinking of the epithelium, and also reducing infolding of epithelium.

T. cucumerina is a rich source of flavonoids and phenolic compounds [12; 17]. Thus, the plant extract possesses the ability to scavenge free radicals and inhibit hydrolytic and oxidative enzymes and anti-inflammatory action. The mechanisms by which this plant's extract protects against experimentally-induced BPH may be as a result of the good source of vitamin C and other essential minerals present in the plant [14; 20]. High concentration of vitamin C in *T. cucumerina* provides a highly effective anti-oxidant property, reversing the effects of androgen induced BPH following administration of the extract to the animals.

In conclusion, the present study has shown that high-dose METCS administration could reverse hyperplasic changes in the prostate, suggesting that *T. cucumerina* is a potential candidate for the management of benign prostatic hyperplasia.

References:

1. Adebooye OC (2008) Phytoconstituents and anti-oxidant activity of the pulp of 'snake tomato' (*Tricosanthes cucumerina*). Afr J of Trad Compl and Alt Med, 5 (2): 173-179.
2. Adesanya AO, Olaseinde OO, Oguntayo OD, Otulana JO, Adefule AK (2011) Effects of Methanolic Extract of *Citrullus lanatus* Seed on Experimentally-Induced Prostatic Hyperplasia. Eur J of Med Plants, 1(4): 171-179.
3. Arawwawala LDAM, Thabrew MI, Arambewela LSR (2010) Gastroprotective activity of *Trichosanthes cucumerina* in rats. J of Ethnopharm, 127: 750–754.
4. Bebb RA, Anawalt BD, Christensen RB, Paulson CA, Brenner WJ, Matsumoto AN, (1996) Combined administration of levonorgestrel and testosterone induces more rapid and effective suppression of spermatogenesis than testosterone alone; a promising male contraceptive approach. J of Clin Endocr and Metab, 81(2), 757-62.
5. Bernoulli J, Yatkin E, Konko lY, Talvitie EM, Santti R, Streng T (2008) Prostatic inflammation and obstructive voiding in the adult rat: impact of the testosterone to estradiol ratio in serum. Prostate, 68(12): 1296-1306.
6. Devendra NK, Vijay KB, Mala S (2009) Effect of ethanol extract of whole plant of *Tricosanthes cucumerina* (Var. Cucumerina) on gonadotropins, ovarian follicular kinetics and estrous cycle for screening of anti-fertility activity in albino rats. Int J Morphol, 27(1): 173-182.
7. Dixon JS, Chow PH, Gosling JA (1999) Anatomy and function of the prostate gland. In Nickel JC(ed) Textbook of prostatis. Iris Medical Media Ltd, Oxford, UK. pp 39-46.
8. Drury RAB, Wallington EA (1980). Carleton's Histological Techniques. 4th Ed., Oxford University Press, NY. USA, Pp: 279-280.
9. Eero H, Timo H, Heikki JH (1982) Testosterone Action on the Ventral Prostate Lobe of the Castrated Rat as Assessed with a Stereo-logic Morphometric Method. Am J of Anat, 165: 199-20.
10. Guyton AC and Hall JE (2006) Textbook of Medical Physiology. 11 Ed. Elsevier Saunders, Philadelphia, USA. Pp 996-1010
11. Jayaweera DMA (1980) Medicinal Plants (Indigenous and Exotic) used in Ceylon (Part 2). A Publication of the National Science Council of Sri Lanka, pp. 162–163.
12. Jose B, Reddy JL, Anjana JC Ruveena TN (2010) Evaluation of antibacterial activity of *Trichosanthes cucumerina* L. and Cassia didymobotrya Fresh leaves. Int J Pharm and Pharmcal Sci Vol 2, Suppl 4: 153-155.
13. Leav I, Merk FB, Ofner P, Goodrich G, Kwan PW, Stein BM, Sar M, Stupft WE

(1978) Biopotentialty of response to sex hormones by the prostate of castrated or hyposectomised dogs, direct effects of estrogen. Am J Pathol. 93: 69-85.

14. Ojiako OA, Igwe CU (2008) The Nutritive, Anti-Nutritive and Hepatotoxic Properties of *Trichosanthes anguina* (Snake Tomato) Fruits from Nigeria. Pakistan J of Nutrition. 7 (1): 85-89.
15. Pelletier G (2002) Effects of estradiol on prostate epithelial cells in the castrated rats. J Histochem Cytochem, 50: 1517-1523.
16. Roehrborn CG (2008). Pathology of benign prostatic hyperplasia. Int J Impot Res. 20: 11-18
17. Sandhya S, Vinod KR, Chandra SJ, Aradhana R, Vamshi SN (2010) An updated review on *Tricosanthes cucumerina* L. Int J. Pharmacal Sci Rev and Res. 1(2): 56-60
18. Srinivasan N, Micheal Aruldhas M, Govindarajulu P (1986) Sex steroid induced changes in collagen of the prostate gland and seminal vesicle of rats. J Andrrol, pp. 55-58.
19. USANA. (1997)Technical Bulletin on prostate 7/97: Pp 1-3.
20. Yusuf AA, Folarin OM, Bamiro FO (2007) Chemical composition and functional properties of snake gourd (*Trichosanthes cucumerina*) seed flour. Nigerian Food J 25 (1): 36-45.