



## **Effects of Methanolic Extract of *Citrullus lanatus* Seed on Experimentally Induced Prostatic Hyperplasia**

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### **ABSTRACT**

**Aims:** To investigate the effects of methanolic extract of *Citrullus lanatus* seed (MECLS) on experimentally induced benign prostate hyperplasia.

**Study design:** Animal model of experimentally induced prostatic hyperplasia.

**Place and Duration of Study:** Department of Anatomy, Faculty of Basic Medical Sciences, Ikenne Campus, Ikenne, Ogun State, Nigeria, between May 2010 and August 2010.

**Methodology:** Twenty adult male Wistar rats weighing about 135-180g were randomly divided into four groups of five animals each. Group I, Normal control (NC) was given corn oil as placebo 1g/Kg BW; Group II, Hormone treated control (HTC), Groups III, and IV hormone and extract treated (HTEC), received continuous dosage of 300µg and 80µg of testosterone (T) and estradiol (E<sub>2</sub>) respectively on alternate days for three weeks subcutaneously in the inguinal region while the extract treated received an additional 2g/Kg BW (low dose) and 4g/Kg BW (high dose) of extract orally for 4 weeks after the successful induction of prostate enlargement. Immediately after induction some animals were randomly selected and sacrificed for gross inspection of prostate enlargement and sperm count evaluation, these procedures were repeated again after four weeks of extract treatment. Portion of the prostate were taken and processed routinely for paraffin embedding and stained with H&E.

**Results:** Hormone treatment did not affect the body weight of the animals; however it caused a significant decrease in the weight of the testes and rendered all the rats azoospermia. In addition, treatment with extracts caused a significant decrease in the enlarged prostate, seminal vesicle and testes sizes in a dose related manner (P<0.05)

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compared to the hormone treated control. Histological examination of prostate revealed that the methanolic extract caused significant changes in its histo-architecture. There was an increase in the fibromuscular layer, decrease in prostatic acini size, shrinkage of epithelium, and no infolding of the epithelium into the lumen, rather, it appeared flat compared to the very distinct columnar epithelium of the hormone treated control and conspicuousness of the acini. The extracts further caused a dose dependent reduction in the prostates weight. PSA level was significantly lowered in both HTEC at low and high doses ( $P < 0.05$ ) in dose dependent manner.

**Conclusion:** Administration of MECLS for one month reduced the prostate size significantly ( $P < 0.05$ ), both at high and low dose, but could not restore the initial size of shrunken testes and severe oligospermia caused by the hormones. The histological studies clearly establish MECLS as a potential candidate in management of androgen dependent conditions like benign prostate hyperplasia.

*Keywords: Citrullus lanatus; methanolic extracts; prostate enlargement; testis; sperm count; male contraception; wistar rats;*

## 1. INTRODUCTION

Cucurbitaceae is the largest family containing 120 genera and approximately 825 species (Mabberley, 1987), typically distributed in the tropical countries poorly represented in the temperate regions. Plants are mostly prostrate or climbing herbaceous annuals characterized by 5-angled and coiled tendrils, leaves alternate palmately 5-lobed and divided; exstipulate and flowers are unisexual rarely bisexual. Cucurbitaceae are important source of food like pumpkin (*Cucurbita pepo*), melon (*Cucumis melo*), cucumber (*Cucumis sativa*), water melon (*Citrullus lanatus*), *Lag enaria siceraria* (bottle gourd), and *Luffa cylindrica* (sponge gourd) (Nazimuddin and Naqvi, 1984; Sultan Asyaz et al., 2010).

*Citrullus lanatus* (egusi melon) is the ancestors of the water melon now found all the world, but originated from the West Africa, egusi melon is a member of the family cucurbitaceae. Unlike the common water melon whose flesh is sweet and red, the egusi melon mainly is a pale yellow or green and also taste bitter. A creeping annual herb, the egusi melon is hairy stems, forked tendrils and three lobed hairy leaves, comprising 50% oil, and 35% protein (Jack, 1972). Water melon refers to both fruit and plant of a vine-like (climber and trailer) herb originally from southern Africa and one of the most common types of melon. This flowering plant produces a special type of fruit known by botanists as a pepo, which has a thick rind (exocarp) and fleshy centre (mesocarp and endocarp); pepos are derived from an inferior ovary and are characteristic of the Cucurbitaceae. The watermelon fruit, loosely considered a type of melon has a smooth exterior rind (green and yellow) and a juicy, sweet, usually red, but sometimes orange, yellow, or pink interior flesh (Daniel and Maria, 2000). Water melon seeds have both nutritional and cosmetic importance, the seed contain vitamins B2, minerals, riboflavin, fat carbohydrates and protein (Lazos., 1986).

The nutritional quality of watermelon as evaluated by Mathias et al., 2001, shows that it is very rich in vitamins A 3%, some of the B series like Thiamine (Vit. B1), Riboflavin (Vit. B2) , Niacin (Vit. B3), Pantothenic acid (B5), Vitamin B6 and Folate (Vit. B9) which range between 1-3%, Vitamin C 14%. The mineral composition was Calcium 1%, Iron 2%, Magnesium 3%, Phosphorus 2%, Potassium 2%, Zinc 1% Nutrition of Watermelon, raw (edible parts)

Nutritional value per 100 g , percentages are relative to US recommendations for adults. It is also rich in essential amino acids like arginine, glutamine, aspartic and minerals such as zinc and essential fatty acids (Ojeih et al., 2008) which may be of great importance to prostate health. Essential fatty acids (EFA) are nutritionally important in the ensuring good prostate growth and relieving symptoms of prostate ailments (Strategy for Wellness by Source Naturals, Inc., 2000; SEPASAL, 2004).

Watermelon is 92 percent water by weight. Watermelon is also mildly diuretic. Watermelon with red flesh is a significant source of lycopene. Watermelon contains about six percent sugar by weight, the rest being mostly water. As with many other fruits, it is a source of vitamin C. It is not a significant source of other vitamins and minerals unless one eats several kilograms per day. The amino acid citrulline was first extracted from watermelon and analysed. Watermelons contain a significant amount of citrulline and after consumption of several kilogram an elevated concentration is measured in the blood plasma, this could be mistaken for citrullinaemia or other urea cycle disorder (Mandel et al., 2005). There is dearth of publication on the effects of *C. lanatus* on the prostate health. This study therefore sought to know the effects of methanolic extracts of water melon seed on experimentally enlarged prostate.

## 2. MATERIALS AND METHODS

A total of twenty Wistar rats weighing between 135-180g obtained from the animal house of the Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ikenne Campus, Ogun State, Nigeria were used for the experiment. The rats were acclimatized for two weeks before the experiment commenced. The rats were fed rats pellets obtained from SESCO Feed, Ikenne, Ogun State, Nigeria. Food and water was given *ad libitum* and the animals were kept under standard conditions. The animal room was well ventilated with a temperature range of 27-29°C under 12h day/light photoperiod regimen.

### 2.1 Hormones

Testosterone propionate Brand name: Ricostrone; a product of Greenfield pharma, Jiangsu Co Ltd., China. Estradiol valerate (by Medipharm Ltd., 108-Kotlakhpat industrial Est; Lahore, India. Testosterone propionate (T) and estradiol valerate E<sub>2</sub> (puregynon depot) were used for the induction of prostate enlargement at a dose of 300µg T and 80µg E<sub>2</sub> (Bernoulli et al., 2007, 2008). This was administered to the rats for three weeks subcutaneously in the inguinal region after which a few rats were sacrificed and inspected for gross examination of prostate enlargement.

#### 2.1.1 Plant material

Water melon fruit was obtained from Sabo, a local market in Shagamu, Ogun State. The plant was authenticated by plant taxonomist from Department of Botany, Faculty of Science, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria. The flesh was removed and seeds collected were washed, sun-dried and milled into fine powder.

#### 2.1.2 Preparation of extracts

This powdered sample was weighed and divided into two equal halves each weighing 42.4g. The method of extraction employed is percolation. The first portion 42.4g of the powdered sample was weighed into a beaker, soaked into 100ml of Methanol for a period of 48 hours,

filtered and filtrate was concentrated using a rotary evaporator. The second portion 42.4g was also soaked into 150ml of distilled water, filtered after 48 hours and filtrate was concentrated in water bath. The volume of filtrate was 150ml before concentration, weight of residue was 29.9g and the concentration was calculated. The solutions were diluted with corn oil, to produce a solution 100mg/ml and 200mg/ml respectively. The administration of extract was totally by gavage. Proper concentrations were administered by the use of oropharyngeal canula and calibrated hypodermic syringe.

#### 2.1.1.1 Animal grouping

The rats were randomly grouped into four groups of five animals each, two controls- normal (NC) and hormone treated (HTC) and low and high dose MECLS treated. The NC received corn oil 1g/1Kg BW alone, HTC received a combined dose of testosterone 300µg and estradiol 80µg per 100gBW (Bernoulli et al., 2007, 2008) diluted with corn to the specified dose and treated for three weeks while MECLS treated received the extracts 2g/kg BW (low dose) and 4g/Kg BW (high dose) in addition to the hormone for another four weeks.

- Histology

The animals were sacrificed and the region around the penile shaft was opened, the bladder was located and the prostate around it removed and loop of epididymal pulled up and testis was located and taken out. The organs were fixed in 10% formol-saline. After complete fixation the blocks was embedded in paraffin and sections cut at 5µm (micron) and then stained with haematoxylin and eosin and mounted in Canada balsam. Microscopic examination of the sections was then carried out under a light microscope.

### 3. RESULTS AND DISCUSSION

The prostate gland provides the semen with vitamins and other nutrients thus, maintaining its vitality during the journey up the female reproductive system. Diseases of the prostate gland, affect millions of people every year, decreasing their quality of life, while in the case of prostate cancer it can prove lethal if not diagnosed in early stages. Identification of genetic and molecular events that could help in the early detection of benign prostatic hyperplasia and prostate cancer, or could be useful as therapeutic targets, is of top priority in research in this scientific field (Wang and Wong, 1998; Partin and Coffey, 1998).

Hormone treatment with T and E<sub>2</sub> caused a significant increase in the PSA value of the HTC (hormone treated control) above the normal range as obtained in NC (normal control), while the methanolic extracts both at high and low doses restored the PSA value to the normal range compared to the NC (Table 1). 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 (USANA Tech. Bulletin, 1997).

Hormone treatment with T and E<sub>2</sub> caused a significant increase in weight of the prostate and seminal vesicle compared to NC (P<0.05) (Table 2). However, treatment with high and low dose of MECLS lowered the weight of the experimentally enlarged prostate and seminal vesicle significantly. In addition, treatment with hormone caused a significant reduction in weight of the testis (P<0.05) but administration of MECLS did not ameliorate the weight of the testis at both low and high doses; Moreover, we observed a further reduction in the testis weight upon administration of MECLS. The effects of MECLS haven observed confirms its

potency against experimentally induced prostatic hyperplasia (BPH) producing an effect almost similar to what obtain when 5-alpha reductase blocking drugs like finasteride and dutasteride were applied in BPH or early Pca. (Rittmaster et al., 1995).

**Table 1: PSA value of rats treated with Methanolic Extract of *Citrullus lanatus* Seed.**

	Normal control (NC)	Hormone treated control (HTC)	HTC + MET- low dose	HTC + MET - high dose
PSA value	3.75 ± 1.71 <sup>b</sup>	8.75 ± 0.96 <sup>a</sup>	4.72 ± 1.65 <sup>a,b</sup>	2.78 ± 0.89 <sup>a,b</sup>

HTC- hormone treated, MET- methanolic seed extract treated; a-significant increase ( $P<0.05$ ) when compared with normal control; b- significant decrease ( $P<0.05$ ) when compared with hormone treated control.

Induction of prostate enlargement experimentally caused shrinkage of the testis and a very drastic reduction in the sperm count which was significant ( $P<0.05$ ) i.e. azoospermia /severe oligospermia were observed in all animals given the hormone regimen of T and E<sub>2</sub>. However, the treatment with *Citrullus lanatus* seed extracts both at low and high doses could not ameliorate hypospermia condition caused by the hormones used in induction of prostate enlargement (Table 3). This finding is in agreement with the work by Vogelsong et al. (2005) who investigated the effects of various doses of testosterone and estrogen/ progestin on the seminal parameters in human, although the report has it that this effect is said to be reversible upon the cessation of treatment, but the present study has not embarked on the reversal study. Progestin are said to work synergistically with an androgen to suppress gonadotrophin synthesis and release, with some level of evidence that progestins can exert their negative effects directly at the level of the testis (Christenesen and Andriole, 2009).

**Table 2: Weight of accessory sex gland and testis of rats treated with methanolic extract of *Citrullus lanatus* seed**

	Normal control (NC)	Hormone treated control (HTC)	HT + MET treated- low dose	HT + MET treated- high dose
Testis	0.59±0.012	0.38±0.351 <sup>c</sup>	0.273±0.063 <sup>b,c</sup>	0.305±0.11 <sup>b,c</sup>
Seminal vesicle	0.06±0.018 <sup>b</sup>	0.24±0.08 <sup>a</sup>	0.113±0.099 <sup>a</sup>	0.058±0.04 <sup>b</sup>
Prostate	0.03±0.02 <sup>b</sup>	0.23±0.081 <sup>a</sup>	0.105±0.01 <sup>b,c</sup>	0.06±0.04 <sup>a,b</sup>

a.-significant increase ( $P<0.05$ ) (compared with normal control); b- significant decrease ( $P<0.05$ ) (compared with hormone treated control); c- significant decrease ( $P<0.05$ ) (compared with normal control).

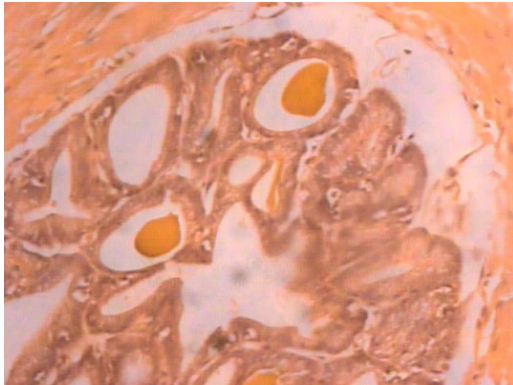
**Table 3: Sperm count of rats treated with methanolic extract of *Citrullus lanatus* seed**

	Normal control (NC)	Hormone treated control (HTC)	HTC + MET- low dose	HTC + MET - high dose
Sperms (millions/ml)	21.7±6.9	1.05±0.27 <sup>a</sup>	1.82±0.59 <sup>a</sup>	1.99±0.95 <sup>a</sup>

a-significant decrease ( $P<0.05$ ) (compared with control).

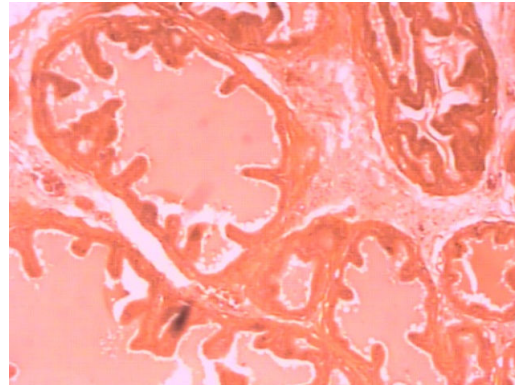
Hyperplastic changes were observed in prostate glands as early as three weeks after the treatment with hormones T and E<sub>2</sub>. The tubules of varying sizes, contained regions were composed of more than one layer of epithelial cells (Plates 3 & 4). Prostatic hyperplasia- is the result of proliferation of epithelial cells and smooth muscle cells, fibroblasts, and other stromal components in variable proportions. Numerous papillary projections were present in the tubules (Bebb et al., 1996). Induction of experimentally enlarged (BPH) is achieved in prostate via a form of tissue damage caused by the hormone regime given to the animal, 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 (Bernoulli et al., 2008; Dhanotiya et al., 2009).

Treatment with methanolic extracts of *Citrullus lanatus* for three weeks caused reduction of the stroma, acini size and shrinking of epithelium, no in-folding of the epithelium; but, it appeared flat with an increase in the fibromuscular layer (Plates 3 &4) both at high and low dose compared to the very distinct columnar epithelium of the hormone treated control and the conspicuousness of the acini in hormone treated control (Plate 2). This result is in agreement with the observation of Dhanotiya et al., 2009 who studied the petroleum ether extract of *Citrullus colocynthis* Schrad (Cucurbitaceae) fruits and a steroidal compound isolated from this extract on Wistar rats for their effect on Prostatic Hyperplasia (PH) induced by testosterone.



**Plate 1: Prostate of Normal untreated control** Mag X 400

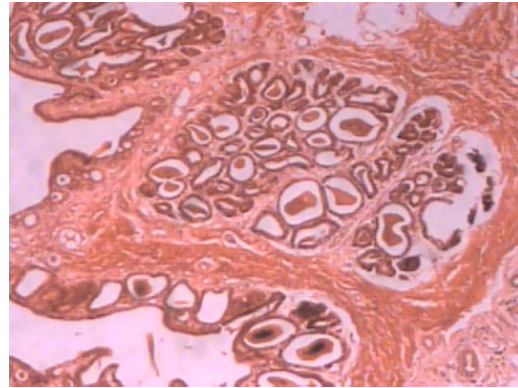
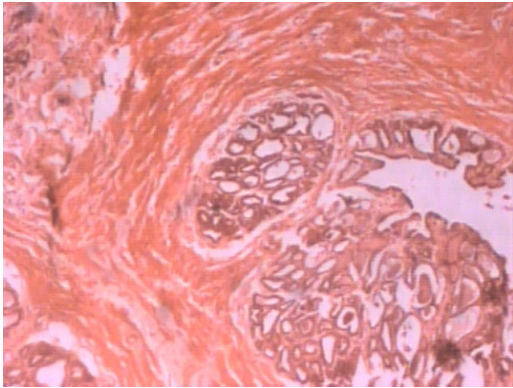
Note that tubules are lined with a single layer of cuboidal cells, with secretion in the lumen. Epithelial tubules, surrounded by a thin layer of smooth muscle cells, are loosely distributed within the loosely organized stromal tissue. Acini are small sized, the epithelium is flat, and the fibro-muscular stroma is obvious, with presence of prostatic concretion (corporal amyloca).



**Plate 2: Prostate of Hormone treated control** Mag X 400

There was an enlargement of the gland and stroma size, increase in the epithelial size with infolding of the epithelium into the lumen, prostatic concretion (corporal amyloca are not obvious).





**Plate 3: Prostate of Methanolic Extract treated -Low Dose Mag X 400**

There was reduction in the size of gland and stroma, reduction in epithelial size which appeared very thin, and prostatic concretion (corporal amyloids are obvious) in few of the gland. In addition, there was an increase in size of the fibromuscular stroma (connective tissue) layer.

**Plate 3: Prostate of Methanolic Extract treated -Low Dose Mag X 400**

There was reduction in the size of gland and stroma, reduction in epithelial size which appeared very thin, and prostatic concretion (corporal amyloids are obvious) in few of the gland. In addition, there was an increase in size of the fibromuscular stroma (connective tissue) layer.

*C. lanatus* is a rich source of flavonoids and phenol, with ability to scavenge free radical and inhibit hydrolytic and oxidative enzymes and anti-inflammatory action (Frank, 1995). It is also a rich source of phytochemical minerals and vitamins. *C. lanatus* can be a potential source of useful drug. The mechanisms by which this plant extracts protect against experimentally induced BPH may be as a result of the good source of vitamin C, thiamine and including riboflavin it contains, and high level of polyphenolic compounds present in the plant. High concentration of vitamin C in *C. lanatus* provides a highly effective anti-oxidant, reversing the effects of induced BPH following the administration of the extracts to the animal. Even in small amount vitamin C can protect indispensable molecules in the body such as proteins, carbohydrates, lipids and nucleic acids (DNA & RNA) from damage by free radicals and reactive oxygen species that can be generated during normal metabolism as well as through exposure to toxins and pollutants (such as during smoking and alcohol ingestion) (Akashi et al., 2004).

#### 4. CONCLUSION

Administration of MECLS for a month reduced the size of the prostate significantly ( $P < 0.05$ ) both at high and low dose but could not restore the size of the shrunken testes and severe oligospermia caused by the hormones. The histological studies clearly established MECLS as a potential candidate in management of androgen dependent conditions like benign prostate hyperplasia.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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