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EFFECTS OF COMBINED METHANOL SEEDS EXTRACTS OF *CITRULLUS LANATUS* AND *ANNONA MURICATA* ON MALE HORMONE PROFILE AND PROSTATE-SPECIFIC ANTIGEN (PSA) LEVEL IN DIHYDROTESTOSTERONE-INDUCED BENIGN PROSTATIC HYPERPLAXIA IN MALE WISTAR ALBINO RATS

***Ihejieto H. A., Kalu M. U., Joel O. M. and Anyanwu C. N.**

Department of Microbiology/Biochemistry, School of Industrial and Applied Sciences, Federal Polytechnic Nekede, Owerri, Imo State.

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***Corresponding Author**
Ihejieto H. A.
Department of Microbiology/Biochemistry, School of Industrial and Applied Sciences, Federal Polytechnic Nekede, Owerri, Imo State.

ABSTRACT

Benign Prostatic Hyperplaxia (BPH) also called prostate gland enlargement is a common condition as men grow old. This study investigated the effects of combined methanol seeds extracts of *Citrullus lanatus* (CLSE) and *Annona muricata* (AMSE) on male hormone profile and Prostate-specific Antigen (PSA) level in dihydrotestosterone-induced benign prostatic hyperplaxia in male Wistar albino rats using standard laboratory techniques. A total of fifty-eight male rats were used for this study; eighteen for acute toxicity test, nine per dose of extract. Doses used were 100, 500, 1000, 1500, 2000, 2500, 3000, 4000 and 5000mg/kg bw. Fourty rats were used for the main study, divided into eight groups of five rats each. The groups were designated as normal control (not induced), positive control (induced and Finasteride), disease (negative) control (induced and untreated), and Test groups 1, 2, 3, 4 and 5. The test groups were induced and treated with 500mg/kg bw of CLSE, 500mg/kg bw of AMSE, 250/250mg/kg bw CLSE/AMSE, 125/375mg/kg bw CLSE/AMSE, 375/125mg/kg bw CLSE/AMSE, respectively. The study lasted for 28 days. Data obtained were analyzed using Duncan multiple range test. The results showed LD_{50} of CLSE to be above 5000mg/kg bw and AMSE, 4000mg/kg bw. Values for average percentage change in weight, relative prostate and testes weight, serum PSA, testosterone and luteinizing hormone levels for the disease control were high. While, the values for these parameters for

test group 3, were close to those obtained for the positive control, but were statistically different ($p<0.05$), except for percentage change in weight at week 4 and serum luteinizing hormone level. This indicates that using a combination of both extracts at 250mg/kg bw for each, showed synergism and holds prospects for being a safe, inexpensive alternative treatment for benign prostatic hyperplaxia.

KEYWORDS: Combined methanol seeds extracts, *Citrullus lanatus*, *Annona muricata*, Dihydrotestosterone-induced, Benign prostatic hyperplaxia.

INTRODUCTION

Benign prostatic hyperplaxia (BPH) is a progressive condition characterized by prostate enlargement accompanied by lower urinary tract symptoms (LUTS) (Emberton *et al*., 2008). It arises in the periurethral and transition zones of the prostatic gland and represents an inescapable phenomenon for the ageing male population (as cited in Lepor, 2005). BPH is not common before age 40, but at 50 years of age, about 50% of men develop BPH-related symptoms. The incidence of BPH increases by 10% per decade as a man ages and reaches 80% at approximately 80 years (Bharti, 2017). An estimated 75% of men above 50 years of age have symptoms arising from BPH, 20–30% of men reaching 80 years of age require surgery to manage BPH. Ageing is a central mechanism implicated in the development and progression of BPH and findings highlighted the key roles of hormonal alterations, metabolic syndrome, and inflammation in the disease (Berger *et al*., 2005).

Current conventional treatment regimens for BPH have produced adverse effects such as toxicity and growth inhibition to normal cells. Apart from being expensive, they cause urinary incontinence and erectile dysfunction. Hence, there is pressing need to discover an indigenous plant that has curative effect on BPH and related diseases. The use of medicinal plants is one of the major access avenues of complementary and alternative medicine for various ailments. Due to the widely held perception that phytotherapeutic agents are more cost-effective, safer and have fewer side effects compared with conventional therapy for the management of ailments, there is a growing interest in their development (Wilt *et al*., 1998; Thompson *et al*., 2003).

Watermelon (*Citrullus lanatus*) is a fruit crop of the family Cucurbitaceae, which also includes melon, cucumber, and zucchini. It thrives in the temperate regions of Africa, central Asia and the Mediterranean. Fruit of *Citrullus lanatus* is consumed for its chilling effect and

thirst reduction. The fruit contains so many active phyto-constituents and minerals. The seeds contain fatty acids and beneficial activities. The large edible *Citrullus lanatus* fruits contribute to the diets of consumers throughout the world. Although comprised mainly of water (often over 90%), it also contains important nutritional compounds, including sugars, lycopene and cardiovascular health–promoting amino acids, such as citrulline, arginine and glutathione (Enemor *et al*., 2019; Bello *et al*., 2019).

Annona muricata L., commonly referred to as sour sop, graviola, is a member of the Annonaceae family which comprises approximately 130 genera and 2300 species (Mishra *et al*., 2013). The origin of *A. muricata* can be traced to the warmest tropical areas in South and North America and is now widely distributed throughout the tropical and subtropical zones of the world, India, Malaysia and Nigeria inclusive. All parts of this plant are extensively used as traditional medicines against an array of human ailments and diseases (Adewole & Caxton-Martins, 2009; Gupta *et al*., 2005), Though much work have been done on *C. lanatus* and *A. muricata* (Enemor *et al*., 2019; Bello *et al*., 2019; Orak *et al*., 2019; Fasakin *et al*., 2008), there is paucity of information on the effects of their combined methanol seeds extracts. Therefore, the study aimed at determining their combined effects on male hormone profile and Prostate-specific antigen (PSA) level in dihydrotestosterone-induced benign prostatic hyperplaxia in male Wistar albino rats.

MATERIALS AND METHODS

Acute Toxicity Test

Lethal doses (LD_{50}) was determined on rats using the Lorke (1983) method, and calculated as follows: $LD_{50} = ([M_0+M_1])/2$.

Where, M_0 = highest dose that gave mortality; M_1 = lowest dose that gave mortality.

Hormonal induction of Benign Prostatic Hyperplaxia

Benign prostatic hyperplaxia was induced in rats using dihydrotestosterone (DHT) using a dose of 10 mg/kg body weight given by subcutaneous injection everyday for 28 days. Stock was prepared by dissolving 1g of dihydrotestosterone (DHT) and in 100ml olive oil (Ejike & Ezeanyika, 2010).

Investigation of modulatory effects of methanol seeds extract of *Citrullus lanatus* and *Annona muricata* in Dihydrotestosterone-induced Benign Prostatic Hyperplaxia in albino rats.

Fourty male albino rats of Wistar strain weighing between 80-110g were used. The animals were fed on normal laboratory chow purchased from Vital feeds, Ibadan. Animals were given access to food and water *ad libitum*. They were distributed randomly into eight groups of five animals each.

Group 1 (Normal control): Administered olive oil orally (daily) and Subcutaneous injection of olive oil (every other day) for 28 days, at a dose of 1ml/kg bw.

Group II (positive control): Administered subcutaneous injection of olive oil, 1ml/kg bw, every other day and oral administration of Finasteride (5mg/kg bw) every day for 28 days.

Group III (Negative control): Administered subcutaneous injection of 10mg/kg bw DHT once in two days for 28 days and oral administration of olive oil, 1ml/kg bw, every day 28 days.

Group IV (Test group 1): Administered subcutaneous injection of 10mg/kg bw of DHT once in two days and 500mg/kg of the extract of *Citrullus lanatus* seeds (CLSE) orally every day for 28 days.

Group V (Test group 2): Administered subcutaneous injection of 10mg/kg bw of DHT once in two days and 500mg/kg of the extract of *Annona muricata* seeds (AMSE) orally every day for 28 days.

Group VI (Test group 3): Administered subcutaneous injection of 10mg/kg bw of DHT once in two days and combined extract of 250mg/kg of *Citrullus lanatus* seeds and 250mg/kg of *Annona muricata* seeds orally everyday for 28 days.

Group VII (Test group 4): Administered subcutaneous injection of 10mg/kg bw of DHT once in two days and combined extract of 125mg/kg of *Citrullus lanatus* seeds and 375mg/kg of *Annona muricata* seeds orally everyday for 28 days.

Group VIII (Test group 5): Administered subcutaneous injection of 10mg/kg bw of DHT once in two days and combined extract of 375mg/kg of *Citrullus lanatus* seeds and 125mg/kg of *Annona muricata* seeds orally everyday for 28 days.

At the end of 28 days, the rats were fasted overnight and euthanized by cervical dislocation and blood collected by cardiac puncture into plain sample tubes and allowed to clot. Serum was separated by centrifugation at 3000xg for 20min after and collected using Pasteur pipette for analysis. The serum samples were used for assay of prostate-specific antigen, testosterone and luteinizing hormone levels. The organs (prostate, testis) were excised, rinsed in ice-cold 1.15% KCl, blotted and weighed using an electronic weighing balance (Ohaus corporation, USA).

Determination of Body Weight

The body weights of the rats were taken and recorded on daily basis for 28 days. Average percentage change in weight was calculated thus:

$$\frac{\text{Final body weight} - \text{Initial body weight}}{\text{Final body weight}} \times 100$$

Relative Organ Weights

The relative prostate weight was expressed in g/1000g while other relative organ weights were expressed in g/100g as follows:

Calculation of Relative Prostate weight

$$\text{Relative Prostate weight (g/1000g)} = \frac{\text{Total Prostate weight}}{\text{Final body weight}} \times 1000$$

Calculation of Relative Testis weight

$$\text{Relative Testis weight (g/100g)} = \frac{\text{Total Testis weight}}{\text{Final body weight}} \times 100$$

Assay of Prostate Specific Antigen (PSA) (Biocheck, Enzyme Immunoassay (EIA) test kit method)

The PSA levels in the serum were measured using the method described by Stowell *et al*. (1991).

Assay of Testosterone (Biocheck, Enzyme Immunoassay (EIA) test kit method)

The serum testosterone level determination protocol is based on the method of Turkes *et al*. (1979).

Determination of Luteinizing Hormone Concentration

The serum luteinizing hormone (LH) level test is based on a solid phase enzyme linked immunoabsorbent assay (ELISA).

Statistical Analysis

Data was analyzed using one way analysis of variance (ANOVA) with repeated measures using Statistical Pakages for Service Solutions (SPSS) version 20. Results were expressed as mean ±SD. Differences between means were considered to be significant at P=0.05 using Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Table 1: Percentage change in body weight.

GROUP	Week one	Week two	Week three	Week four
Normal control	1.00±0.16^{b}	6.00±0.12^{e}	8.00±0.16^{e}	10.00±0.19^{d}
Positive control	3.00±0.08^{d}	5.00±0.27^{d}	6.00±0.12^{d}	8.00±0.12^{c}
Negative control	-1.00±0.17^{a}	-3.00±0.10^{a}	-5.00±0.16^{a}	-6.00±0.07^{a}
Test group 1	3.00±0.12^{d}	3.00±0.10^{b}	4.00±0.16^{b}	4.00±0.12^{b}
Test group 2	3.00±0.08^{d}	4.00±0.10^{c}	5.00±0.36^{c}	5.00±0.22^{b}
Test group 3	4.00±0.19^{e}	7.00±0.27^{f}	8.00±0.07^{e}	8.00±0.19^{c}
Test group 4	3.00±0.12^{d}	3.00±0.07^{b}	4.00±0.19^{b}	5.00±0.20^{b}
Test group 5	2.00±0.12^{c}	3.00±0.12^{b}	4.00±0.10^{b}	4.00±0.16^{b}

Results are presented as mean±sd, sd is the standard deviation, superscripts in a row with different alphabets are significantly different.

Table 2: Average percentage change in weight.

GROUP	Average percentage change in weight
Normal control	5.25±2.99^{bc}
Positive control	5.50±2.08^{bc}
Negative control	-3.75±2.22^{a}
Test group 1	3.50±0.58^{b}
Test group 2	4.25±0.96^{bc}
Test group 3	6.75±1.89^{c}
Test group 4	3.50±0.58^{b}
Test group 5	3.25±0.58^{b}

Results are presented as mean±sd, sd is the standard deviation, superscripts in a column with different alphabets are significantly different.

Table 3: Relative prostate weight.

GROUP	Relative prostrate weight (g/100g)
Normal control	1.69±0.15^{a}
Positive control	3.29±0.28^{b}
Negative control	9.75±0.25^{f}
Test group 1	8.47±0.46^{e}
Test group 2	6.28±0.47^{d}
Test group 3	4.95±0.30^{bc}
Test group 4	5.70±0.53^{cd}
Test group 5	5.37±0.28^{c}

Results are presented as mean±sd, sd is the standard deviation, superscripts in a column with different alphabets are significantly different.

Table 4: Relative testes weight.

GROUP	Relative testis weight (g/100g)
Normal control	0.64 ± 0.03^{e}
Positive control	0.67 ± 0.02^{e}
Negative control	0.43 ± 0.01^{a}
Test group 1	0.52 ± 0.02^{bc}
Test group 2	0.54 ± 0.03^{c}
Test group 3	0.61 ± 0.01^{d}
Test group 4	0.50 ± 0.02^{b}
Test group 5	0.50 ± 0.23^{b}

Results are presented as mean±sd, sd is the standard deviation. Superscripts in a column with different alphabets are significantly different.

Table 5: Prostate Specific Antigen level.

GROUP	Prostate specific antigen level (ng/ml)
Normal control	3.99 ± 0.06^{a}
Positive control	$3.85\pm\text{-}0.09^{a}$
Negative control	8.00 ± 0.21^{f}
Test group 1	6.70 ± 0.28^{d}
Test group 2	7.10 ± 0.10^{e}
Test group 3	4.05 ± 0.14^{b}
Test group 4	5.88 ± 0.18^{c}
Test group 5	7.29 ± 0.24^{e}

Results are presented as mean±sd, sd is the standard deviation. Superscripts in a column with different alphabets are significantly different.

Table 6: Serum Testosterone level.

GROUP	Serum testosterone level (ng/ml)
Normal control	3.77 ± 0.63^{a}
Positive control	4.09 ± 0.05^{b}
Negative control	6.14 ± 0.82^{f}
Test group 1	5.55 ± 0.05^{d}
Test group 2	5.80 ± 0.05^{e}
Test group 3	5.04 ± 0.05^{c}
Test group 4	6.12 ± 0.10^{f}
Test group 5	5.73 ± 0.03^{e}

Results are presented as mean±sd, sd is the standard deviation. Superscripts in a column with different alphabets are significantly different.

Table 7: Lutenizing hormone assay.

GROUP	Lutenizing hormone assay (mIU/ml)
Normal control	5.97 ± 0.06^{a}
Positive control	6.83 ± 0.05^{ab}
Negative control	9.20 ± 0.04^{d}
Test group 1	8.33 ± 0.01^{cd}
Test group 2	8.77 ± 0.02^{cd}
Test group 3	7.57 ± 0.06^{bc}
Test group 4	9.00 ± 0.06^{d}
Test group 5	8.23 ± 0.07^{cd}

Results are presented as (n±sd). N is the mean and sd is the standard deviation, superscripts in a row with different alphabets are significantly different.

The use of medicinal plants is one of the major avenues of complementary and alternative medicine for various ailments due to the belief that they are cost-effective, safer, and with fewer side effects. The results of this study are presented in tables 1 to 7. The LD_{50} of CLSE was observed to be greater than 5,000mg/kg body weight, while that of AMSE is 4,000mg/kg bw.

Table 1 shows the percentage change in body weight. The normal and positive controls showed steady increases. Test groups 1 and 4 showed equal percentage change in weight at weeks 1, 2 and 3. While test groups 2, 3 and 5 showed steady increase in percentage weight up to week 3, with the percentage weight at week 3 remaining same at week 4. The negative control showed steady decline. At week 1, apart from the positive control and test groups 1, 2 and 4 whose percentage change in weight were not significantly different ($p>0.05$) from each other, the rest were significantly different ($p<0.05$) from each other and the four groups. At week 2, all values were significantly ($p<0.05$) different from each other except test groups 4 and 5. At week 3, except for test groups 1, 4 and 5, the rest of the values were significantly ($p<0.05$) different from each other and the control groups. At week 4, values for the positive control and test group 3 were not significantly ($p>0.05$) different, same for those observed for test groups 1, 2, 4 and 5. The average percentage change in weight as presented in table 2, indicates no significant ($p<0.05$) differences in all the groups except the negative control.

Table 3 presents relative prostate weight. Compared with the negative control, the test groups induced varying degrees of reduction in prostate weight which were significant, with test group 3 showing the highest reduction. Relative testis weight (table 4) results showed that the values obtained for the normal and positive controls were not significantly ($p>0.05$) different

while the test groups were significantly different from all the controls. Although, the value observed with test group 3 was the closest to the positive and normal controls among the test groups. The negative control was observed to be the lowest when compared with the other groups, indicating reduced testes.

Table 5 presents the serum PSA level which revealed that only values obtained for the normal and positive controls were within the normal range, <4.00ng/ml (American Cancer Society, 2021). The rest were observed to be high with the negative control being the highest indicating enlargement of the prostate. While among the test groups, test group 3 showed reduced serum PSA level closer to the positive control treated with standard drug, indicating a reversal in the prostate size. The normal and positive controls were not significantly ($p>0.05$) different from each other, same was the case with test groups 2 and 5, but were when compared with other groups. Okuja *et al*. (2021) went ahead to establish that serum PSA levels and PV (prostate volume) progressively increased with age, but direct correlation between serum PSA levels and PV was weak. Serum PSA levels may be used to screen men at risk of cancer and determine choice of medical treatment in BPH and evaluation of patients with prostatitis.

The serum testosterone level as presented in table 6 showed that the negative control was observed to be the highest while the normal control showed the least value followed by the positive control. Among the test groups, test group 3 showed the least value. All values were significantly ($p<0.05$) different from each other except test groups 2 and 5, with all the other groups, indicating presence of prostate enlargement. While among the test groups, test group 3 showed reduced serum testosterone level closer to the positive control treated with standard drug. Tripathy *et al*. (2015) recorded that the normal range for serum testosterone level in men is 3 to 12ng/ml. Hence, despite differences observed with the values, they were still within normal range. This result agrees with the work of Liu *et al*. (2007) that stated that the serum testosterone levels in aging men did not correlate with the measures of BPH, but age correlated with the measures of BPH.

According to table 7, serum level of luteinizing hormone of the negative control was observed to be the highest when compared with all the other groups, indicating testicular failure. Although, all the values were not exactly significantly ($p>0.05$) different from each other. According to Sullivan (2019) normal range for luteinizing hormone in males, values for the normal control, positive control and test group 3 were normal. This indicates that test

group 3 induced reversal in the ailing condition of the testicles. This agrees with a previous study which reported that increase in serum LH and FSH levels indicates increase in measures of BPH (Gray *et al*., 1991).

CONCLUSION

Based on the findings of this study, the test samples showed varying capacities in the reversal of dihydrotestosterone-induced BPH. However, test group 3 with equal concentrations (250mg/kg bw) of both extracts holds better prospects in the reversal of BPH. It is therefore recommended that prevention studies should be carried out using these extracts.

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