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Effects of combined ethanol extract of *Anthocleista vogelii* and *Alstonia boonei* stem barks on sex hormonal levels of benign prostatic hyperplasia (BPH) induced rats

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ABSTRACT

Background & Aim: Combined ethanol extract of *Anthocleista vogelii* and *Anthocleista boonei* stem (CEAA) barks is commonly used to treat prostate disorders whereas its effects on benign prostatic hyperplasia (BPH) has not been scientifically validated. This study investigated the effects of CEAA on prostate weight, prostate index and serum androgenic and estrogenic hormonal levels of BPH induced rats.

Experimental: The study had 6 groups containing 5 male Wistar albino rats each. group 1 was the normal control rats without BPH induction while group 2 was the BPH induced rats without any treatment (BPH) control. Groups 3 – 5 were BPH induced rats treated with 5 mg/kg finasteride/day, 200 and 400 mg/kg CEAA/day, respectively. BPH was induced in groups 2– 5 by the subcutaneous administration of testosterone propionate injection in olive oil for 28 consecutive days.

Results: The BPH control indicated significantly ($P<0.05$) increases in prostate weight, prostate index, serum testosterone (TT), dihydrotestosterone (DHT) and estradiol (E_2) concentrations relative to the normal control. Treatment with CEAA caused dose-dependent significant ($P<0.05$) decreases in the prostate weight, prostate index, TT, DHT and E_2 similar to finasteride-treated BPH induced rats when compared with BPH control rats. The CEAA had high inhibitory effects on the prostate weight (55.71 -82.86 %) and prostate index (65.33 – 86.52 %) of the BPH induced rats greater than 71.52 % and 81.63 % inhibition of prostate weight and prostate index by finasteride, respectively.

Recommended applications/ industries: The findings of this study indicated that combined ethanol extract of *A. vogelii* and *A. boonei* stem barks has anti-benign prostatic hyperplasia activities as it down-regulated the serum concentrations of TT, DHT and E_2 implicated in BPH development and progression.

1. Introduction

Benign prostatic hyperplasia (BPH) according to Parsons and Kashefi (2008), is a progressive noncancerous enlargement of the epithelial cells and smooth muscle of the prostate gland which go along

with lower urinary tract symptoms (Parsons and Kashefi, 2008). Hormones, especially androgens and the likes are implicated in the development of BPH. Dihydrotestosterone (DHT), a metabolite of testosterone and the key mediator of prostate development, is formed by degradation of testosterone by 5-alpha reductase enzyme in the prostate cell (McConnell *et al.*, 2003). The 5-alpha reductase inhibitors (5-ARIs) and the alpha-blockers represent the two dominant classes of orthodox therapeutic agents utilized in managing BPH (Gacci *et al.*, 2011). However, 5-ARIs cause certain side effects including gynecomastia, ejaculatory and erectile dysfunctions (Imperato-McGinley *et al.*, 1992). It also affects the diagnosis of BPH by lowering prostate-specific antigens (PSA) by 50% after 6 months on therapy (Imperato-McGinley *et al.*, 1992). The use of minimally invasive therapies (MITs) and surgery also portends some risks including urinary tract infections, perpetual sexual side effects and rarely, urinary incontinence. They are also costly and hospitals discourage BPH patients from undergoing these procedures. These attendant side effects have occasioned BPH patients resorting to phytotherapy for the management of BPH. Due to the adverse health effects of most synthetic drugs used in treating BPH, attention has shifted to the use of medicinal plant products where combined and polyherbal extracts formulated from potent medicinal plants have become useful treatment option because of their high therapeutic potentials and low adverse health effects (Shinde *et al.*, 2016).

Alstonia boonei De Wild is a member of the *Apocynaceae* family commonly found across tropical rain forests in African countries especially in Nigeria where its various parts are used in traditional medicine for treating different diseases (Tepongning *et al.*, 2011). Extract of *A. boonei* is used as traditional medicine for the treatment of diarrhoea, malaria, fever, painful menstruation, snakebite, diabetes, jaundice, inflammatory diseases, rheumatism, hypertension, bacterial infections, and intestinal helminths (Osadebe, 2002; Fakae *et al.*, 2007; Alshawsh *et al.*, 2009; Tepongning *et al.*, 2011). Many researchers have attributed its pharmacological activities to its antioxidant-rich content and other phytochemical constituents (Hadi *et al.*, 2001).

Anthocleista vogelii Planch formally a member of the *Loganiaceae* family and now in the *Gentianaceae* family (due to a better understanding of its molecular, morphological, and phytochemical compositions) is a well-known medicinal plant like *A. boonei* used in the treatment of various diseases. Its' stem bark and leaves are effective in the treatment of malaria parasites, diarrhoea, helminths, typhoid fever, ulcer, bacterial infections, infertility, hernia, cancer, diabetes, indigestion, hypertension, inflammations and venereal diseases (Jiofack *et al.*, 2010; Musa *et al.*, 2010; Alaribe *et al.*, 2011, Okon *et al.*, 2014). Chapelle (1976) had reported that it contains high levels of alkaloids, xanthones, terpenes, and phthalides which could be responsible for various biological activities (Chapelle, 1976). The stem barks of *A. vogelii* and *A. boonei* are widely used in the South-East, Nigeria to treat benign prostatic hyperplasia with claims of total cure and recovery from the disease without any scientific evidence in support of their claims. This study was designed to evaluate the effects of combined ethanol extract of *A. vogelii* and *A. boonei* stem barks (CEAA) on prostate weight, prostate index and serum concentrations of hormones implicated in the benign prostatic hyperplasia pathogenesis in BPH induced rats to validate its antibenign prostatic hyperplasia claims in traditional medicine.

2. Materials and Methods

2.1. Collection and identification of plant materials

A. vogelii and *A. boonei* stem barks were collected from the Rubber Forest of the Michael Okpara University of Agriculture, Umudike (MOUAU), Abia State, Nigeria. They were identified and authenticated by Dr Ibe Ndukwe, a Taxonomist at the Herbarium section of the Department of Forestry and Environmental Management, MOUAU.

2.2. Preparation and extraction of plant materials

The plant materials were dried at room temperature for some three weeks. Thereafter, they were further dried in the oven at 45°C. The dried samples were pulverized into a coarse powder using an electric grinding mill. The ground samples were stored in an air-tight plastic bucket and kept until use. The coarsely ground plant materials were mixed in the ratio of 1:1 (i.e. 300 g of coarsely ground sample each, equivalent to 600 g of both *plant* samples) and extracted by

dissolving it in 1.8 L of absolute ethanol for 72 h. Thereafter, it was filtered and concentrated till the ethanol solvent was completely evaporated in a water bath at 45°C and the percentage yield calculated.

2.3. Experimental animals

Thirty male Wistar albino rats weighing 140-150g and 18 male albino mice were purchased from the Animal House, Department of Zoology and Environmental Sciences, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria. The animals were maintained on a standard laboratory diet (Vital Feed®) and water and acclimatized to laboratory condition under 12 hours light/dark cycle for 2 weeks preceding the commencement of the full experiment. The animals for the study were handled in line with the National Institute of Health's guidelines for the care and use of Laboratory Animals and as approved by the Department and College Ethical Committees of MOUAU (Institute of Laboratory Animal Resources, 1986).

2.4. Chemical reagents and drugs

The chemicals and reagents used in this study were of analytical grades were obtained from Standard Laboratory and Chemical Stores at Onitsha, Anambra State, Nigeria.

2.5. Standard drugs

The testosterone propionate was obtained from Laborate Pharmaceuticals Limited, India, and finasteride was sourced from Bafna Pharmaceuticals Limited, India.

2.6. Experimental design

The rats were carefully distributed into 5 groups containing 6 rats each. Group 1 was the normal control without BPH induction, whereas group 2- 5 were BPH induced. Group 2 was the BPH control that was BPH induced without any treatment whereas group 3 was the standard control treated with 5 mg/kg finasteride/day, groups 4 and 5 were treated with 200 and 400 mg/kg CEAA/day, respectively. The rats were benign prostatic hyperplasia (BPH) by subcutaneous injection of 5 mg/kg/day of testosterone propionate in olive oil for 28 days consecutively. Groups 2– 3 received their respective treatments each day 1 h after the testosterone propionate injection for the 28 days consecutively. The body weight (BW) of the rats was recorded every week throughout the study period. After the completion on the 28th day, the rats fasted overnight. On the 29th day, blood samples were withdrawn from the rats and prostate tissues were harvested and their weights recorded.

2.7. Ethical issues

Ethical approval for the study was given by the Ethical Committee of the Department of Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike with a reference number MOUAU/VPP/EC/18/003.

2.8. Acute toxicity study

Lorke's method was used to ascertain the acute toxicity of the plant extract. This method was divided into two phases with the outcome of each phase determining the next step to follow (Lorke, 1983). Eighteen male albino mice randomly grouped into 6 groups comprising 3 rats each were used in phase I and II of the acute toxicity study of the combined ethanol extract of *A. vogelii* and *A. boonei* stem barks (CEAA).

2.9. Determination of prostate weight (PW) and prostate index (PI)

The prostate weight and prostate index were determined using the method described by Hongcai *et al*. (2018).The prostate weight (PW) and body weight (BW) of the rats were recorded in each of the groups. The prostate index was calculated as:

$$PI\ (\%) = \frac{PW}{BW}\ x\ 100$$

Then, mean PI ratio was calculated in each of the groups. The percentage inhibitions of PW and PI were calculated as:

$$100 - T(\{T - C\}|\{B - C\}\ x\ 100)$$

Where C, B, and T were the values of the normal control group, BPH control, and treatment groups, respectively.

2.10. Determination of testosterone (TT), dihydrotestosterone (DHT) and estradiol (E2) concentrations

The serum hormonal levels including testosterone (TT), dihydrotestosterone (DHT) and estradiol (E_2) concentrations in the BPH induced rats were determined using the methods described by Tietz (1995).

2.11. Statistical analysis

The data obtained were statistically subjected to one-way analysis of variance (ANOVA) and Duncan multiple range comparison test using Statistical Products and Service Solutions (SPSS) version 22. The levels of statistical significance were established at a 95% confidence level ($P<0.05$) and the results were presented as mean ± standard deviation.

3. Results and discussion

Benign prostatic hyperplasia (BPH) is an androgenic and or estrogenic hormonal related disorders usually experienced by an ageing male that greatly impairs their quality of life. It is commonly treated by active surveillance, chemotherapy and surgery but each of these treatment options has some adverse health effects associated with their use which have necessitated the search for a more potent alternative medicine with fewer adverse effects. High serum level of androgenic hormones like testosterone, dihydrotesterone (DHT) and estradiol (E_2) have been implicated as the major factors responsible for the initiation and progression of BPH and any bioactive agents that down-regulates these androgenic hormones have shown to be effective in the management of BPH (Schatzl *et al.*, 2000).

3.1. Percentage yield

The percentage yield of the combined extract of *A. vogelii* and *A. boonei* stem bark obtained after the extraction was found to be 6.5 % which is equivalent to 39 g of the extract. The moderately high percentage yield obtained from the extraction of the CEAA suggests that ethanol was effective in extracting its polar phytoconstituents to a greater extent.

3.2. Acute toxicity effects of CEAA

The results of the acute toxicity study of CEAA indicated no death or adverse reactions 24 h after the administration of the graded doses of the combined ethanol extract. The acute toxicity study of CEAA showed that combined extract caused no adverse reactions or death in the rats and suggest that it is relatively safe. However, it could have sub-chronic and chronic adverse effects on animal and as such requires further evaluation of its effects on vital organs at sub-chronic and chronic levels in order not to endanger the lives of the animals.

3.3. Effects of CEAA on the body weight of BPH induced rats

The result in Table 1 shows the changes in the body weight (BW) of BPH-induced rats treated with CEAA. From the table, it was observed that the body weight increased significantly ($P<0.05$) in a time-dependent manner (Week 1- 4) across all the treatment groups.

Table 1: Changes in body weight of BPH induced rats treated with CEAA.

Weeks	Changes in body weight (g)				
	Normal control	BPH control	Standard control	200 mg/kgCEAA/day	400 mg/kg CEAA/day
Week 1	110.87±20.31^{a}	105.8667±11.02^{a}	102.37±8.47^{a}	85.38±9.26^{a}	112.40±12.55^{a}
Week 2	156.50±9.38^{b}	146.9500±24.15^{b}	142.62±18.51^{b}	111.48±9.22^{b}	139.88±15.28^{b}
Week 3	150.02±19.13^{b}	153.3167±12.27^{b}	155.77±17.73^{ab}	123.6±8.78^{b}	146.28±14.94^{b}
Week 4	159.73±32.81^{b}	160.0500±13.40^{b}	166.12±17.98^{c}	141.48±9.34^{c}	149.28±15.59^{b}

Values are presented as mean ± standard deviations (n = 6). Values with different superscripts are significantly different from any paired mean at $P < 0.05$.

The increased body weights observed in the normal and BPH induced rats treated with finasteride and graded doses of CEAA indicated that the rats were growing normal due to free access to feed and unaltered appetite and metabolic pattern. The unhindered access to feed could induce the rats to consume more food leading to the storage of the excess lipids in the adipose and increased bodyweight that could make the rats obese.

3.4. Effects of CEAA on the prostate weight (PW) and prostate index (PI) of BPH induced albino rats

The result in Figure 1 shows the prostate weight (PW) of BPH-induced rats treated with CEAA. The

PW observed in BPH control was significantly (P< 0.05) greater when compared with normal control. There was a significant (P<0.05) increase in the prostate weight observed in the standard control and BPH-induced rats treated with 200 and 400 mg/kg /day of CEAA when compared with the normal control. There was a significant (P<0.05) reduction in PW of standard control and BPH-induced rats treated with 400 mg/kg/day of CEAA relative to the untreated BPH control. No significant (P>0.05) variation was observed in the PW of BPH-induced rats treated with 200 and 400 mg/kg/day of CEAA when compared with standard control rats treated with 5 mg/kg/day of finasteride.

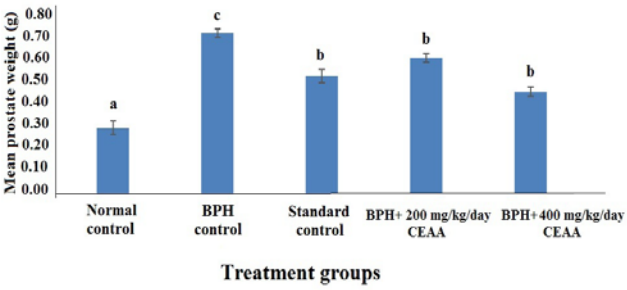

Figure 1. Prostate weight of BPH induced albino rats treated with CEAA. Each bar denotes mean± standard deviation (n=6). Bars with different superscripts are significantly different at p<0.05.

The data in Figure 2 show the effects of CEAA on the prostate index (PI) of BPH-induced rats. The PI observed in the BPH control, and BPH-induced rats treated with 200 and 400 mg/kg/day of CEAA was significantly (P<0.05) higher than the PI of the normal control. Also, the BPH-induced rats treated with 200 mg/kg/day of CEAA had a significant (P<0.05) higher PI comparable to that of standard control and the BPH-induced rats treated with 400 mg/kg/day of CEAA.

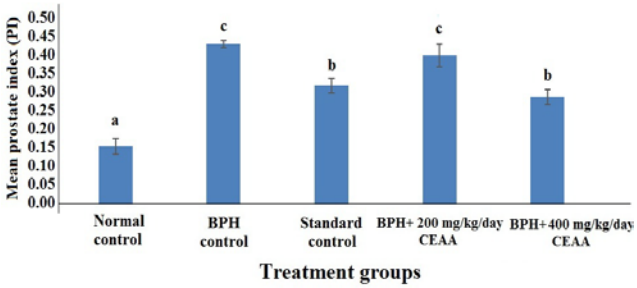

Figure 2. Prostate index of BPH induced albino rats treated with CEAA. Each bar denotes mean± standard deviation (n=6). Bars with different superscripts are significantly different at p<0.05.

No significant (P>0.05) difference in PI was observed in BPH-induced rats treated with 400 mg/kg/day of CEAA when compared with standard control treated with finasteride. However, the PI of the standard and BPH-induced rats treated with 400 mg/kg/day CEAA significantly (P<0.05) decreased when compared with the BPH control. The high level of reductions of prostate weight and prostate index in the BPH induced rats treated with CEAA are indications that the combined extract possesses antibenign prostatic hyperplasia activities. It could have reduced prostate weight and prostate index in the BPH induced rats via inhibitions of the conversion of testosterone to dihydrotestosterone and prevention of excess production of estradiol hormone which prevented stimulation of proliferation of prostate epithelial and stromal tissues implicated in benign prostatic hyperplasia progression. The elevated percentage prostate weight and prostate index observed in the BPH induced rats treated with graded doses of CEAA further showed that the CEAA caused the enlarged prostate to shrink to a greater extent in a dose-dependent manner and indicated recovery from benign prostatic hyperplasia. The reductions in the prostate weight and prostate index observed in this study are in agreement with the findings of Hongcai *et al.* (2018).

3.5. Inhibitory effects of CEAA on Prostate weight and prostate index of BPH induced albino rats

The result in Table 2 shows the degree of the inhibition of prostate weight and prostate index of benign prostatic hyperplasia (BPH) induced rats treated with CEAA. The result indicated a dose-dependent inhibition of the prostate weight and prostate index by the CEAA.

Table 2. Inhibitory effects of CEAA on prostate weight (PW) and prostate index (PI) of BPH induced rats.

Treatment Groups	Degree of inhibition (%)	
	PW	PI
Normal control	-	-
BPH control	-	-
Standard control	71.52	81.63
200 mg/kg/day combined ethanol extract	55.71	65.33
400 mg/kg/day combined ethanol extract	82.86	86.52

PW = Prostate weight; PI = Prostate index

The standard control that received 5 mg/kg finasteride/day had higher inhibitory effects when compared with the BPH induced rats treated with 200

mg/kg CEAA/day of the combined ethanol extract. Contrary, the BPH induced rats treated with 400 mg/kg CEAA/day showed a marked elevated inhibitory effect of the combined at increased dose relative to the standard control (finasteride) and BPH induced rats treated with 200 mg/kg CEAA/day of the extract, respectively. The high percentage dose-dependent inhibitory effects of combined ethanol extract of CEAA on the prostate weight and prostate index of BPH induced rats indicated antibenign prostatic hyperplasia activity of the combined extract. It suggests that CEAA has better antibenign prostatic effects at a higher dose above finasteride and could be used to manage BPH. Since BPH is allied to prostate enlargement, variation in prostate weight is surely a potent indicator of BPH progression and various studies indicated that many drugs and herbal formulations used in the treatment of BPH inhibit prostate weight (Jang *et al.*, 2010; Babu *et al.*, 2020; Pais, 2020). In the current study, the rats with BPH revealed a significant increase in PW as opposed to the normal control rats. However, CEAA-treated rats caused a significant reduction in PW of the rats. This is in agreement with previous studies which indicate a significant decrease in PW after administration of finasteride or other herbal remedies in BPH-induced animals (Bisson *et al.*, 2007; Pais, 2020). Interestingly, 400 mg/kg/day of CEAA appeared to be more effective than 200 mg/kg/day. Therefore, CEAA treatment commendably subdued the BPH occasioned by testosterone propionate injection and restored the enlarged prostate size to normal.

3.6. Effects of CEAA on testosterone concentrations of BPH induced rats

The data in Figure 3 show the effects of CEAA on testosterone concentrations of BPH-induced rats. The testosterone concentrations in the BPH control was significantly ($P < 0.05$) elevated when compared with normal, standard control and BPH induced rats treated with 200 and 400 mg/kg CEAA/day, respectively.

Conversely, there was no significant ($P>0.05$) difference in the testosterone concentrations observed in the BPH-induced rats treated with 200 and 400mg/kg CEAA/day, respectively, when compared with the normal control. This trend was also observed related to standard control rats treated with finasteride. The significantly elevated serum testosterone level in the BPH control rats indicated that the high testosterone level could have induced the growth and proliferation of the epithelial and stromal tissues of the prostate which correlate with the increased prostate weight of the BPH control. This finding is consistent with the finding of Sasagawa *et al.* (1990) in which the administration of testosterone to men resulted in the normal prostatic growth and development of BPH.

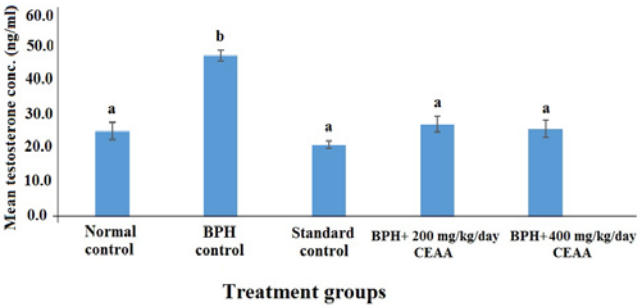

Figure 3. Testosterone concentrations of BPH induced rats treated with CEAA. Each bar denotes mean± standard deviation (n=6). Bars with different superscripts are significantly different at $p<0.05$.

However, the significant reduction was observed in the serum testosterone concentrations of BPH-induced rats treated with graded doses of CEAA relative to the BPH control which shows down-regulation of testosterone by CEAA and its antibenign prostatic hyperplasia activity. The CEAA administration effectively down-regulates the testosterone concentration to a normal level similar to the normal control thereby reducing the testosterone level available to bind to the androgenic receptors to initiate prostate growth and BPH. The reductions of serum testosterone concentrations in BPH induced rats are consistent with the findings of Gasco *et al.* (2007). This also indicated the therapeutic effects of CEAA against BPH progression and pathogenesis as persistent high testosterone level has been implicated in the initiation and development of BPH in animals.

3.7. Effects of CEAA on dihydrotestosterone (DHT) concentrations of BPH induced rats

The data in Figure 4 show the dihydrotestosterone (DHT) concentrations of BPH-induced rats treated with CEAA. The DHT concentration in the BPH control rats showed a significant ($p<0.05$) increase relative to the normal and standard controls, and BPH-induced rats treated with 200 and 400 mg/kg CEAA/day, respectively. Conversely, no significant ($P>0.05$) difference in DHT concentration was observed in the

BPH-induced rats treated with 400mg/kg CEAA/day but a significant (P<0.05) increase in the DHT level observed in the BPH-induced rats treated with 200mg/kg CEAA/day when compared with the normal control. Besides, the BPH-induced rats treated with 200 mg/kg CEAA/day showed no significant (P>0.05) increase in DHT concentration relative to the standard control whereas, BPH-induced rats treated with 400 mg/kg/day of CEAA indicated showed a significant (p<0.05) decrease in the DHT concentration when compared with the normal control.

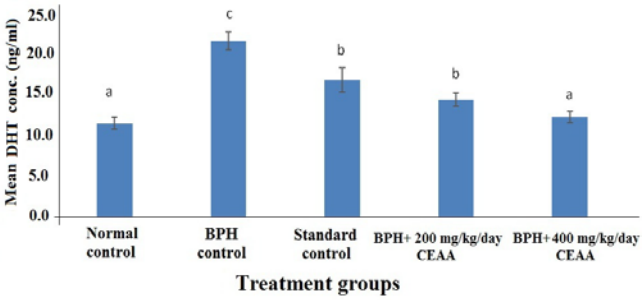

Figure 4. DHT (dihydrotestosterone) concentrations of BPH-induced rats treated with CEAA. Each bar denotes mean± standard deviation (n=6). Bars with different superscripts are significantly different at p<0.05.

Dihydrotestosterone (DHT) is a potent metabolite of testosterone breakdown by the action of 5-*α*-reductase which implicated in the initiation and progression of BPH and its down regulation greatly inhibits and reverses in BPH development (Steers, 2001). At increased concentration, DHT easily binds to its androgenic receptors and stimulates the growth and proliferation of the prostate epithelial and stromal cells and because of this, prostatic epithelial and stromal cell hyperplasia is attributed to the high level of DHT and its affinity to androgenic receptors (Steers, 2001; Carson and Rittmaster, 2003; Hongcai *et al.*, 2018). The high level of DHT observed in the serum of BPH control in this study indicated its up regulation and could have effects in stimulating proliferation of the prostate epithelial and stromal tissues unhindered and causing prostatic hyperplasia in the rats. However, the standard control had low serum DHT level is similar to the CEAA treated BPH induced rats because of the selective inhibition of 5*α*-reductase by finasteride which agrees with previous findings (Aggarwal *et al.*, 2010; Traish *et al.*, 2011). The reduced DHT in standard control rats could be responsible for the significant reduction in the prostate weight relative to the BPH control rats. In the present study, CEAA significantly suppressed the DHT levels in the prostate serum of BPH-induced rats probably by inhibiting the conversion of testosterone by the 5*α*-reductase enzyme. The reduced DHT levels in the CEAA treated BPH-induced rats could be responsible for the reductions in prostate weight by reducing the growth and proliferation of prostate epithelial and stromal tissues that are sensitive to DHT stimulation as reported by Mizokami *et al*. (2009). This is suggestive of inhibition of BPH development by CEAA via down regulation of DHT and encourages the use of CEAA in the management of BPH instead of finasteride as it poses no or minimal adverse health effects compared with the finasteride and other 5*α*- reductase inhibitors (Traish *et al.*, 2011).

3.8. Effects CEAA on estradiol (E_2) concentrations of BPH induced rats

The data in Figure 5 show the estradiol (E_2) concentrations of BPH-induced rats treated with CEAA. The results indicated a significant (P>0.05) increase in the E_2 of BPH control when compared with the normal control, standard control and BPH induced rats treated with 200 and 400 mg/kg CEAA/day, respectively. However, there was a significant (P>0.05) decrease in the E_2 of the standard control and BPH-induced rats treated with 200 and 400 mg/kg CEAA/day, respectively, when compared with the normal control. There was no significant (p>0.05) difference observed in the E_2 of the BPH-induced rats treated with 200 and 400 mg/kg/day of CEAA when compared with standard control rats.

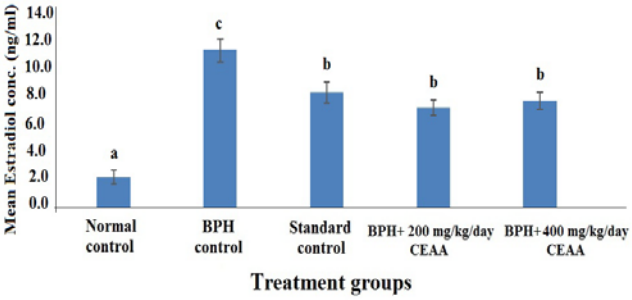

Figure 5. Estradiol concentrations of BPH-induced rats treated with CEAA. Each bar denotes mean± standard deviation (n=6). Bars with different superscripts are significantly different at p<0.05.

Estradiol is one of the estrogen hormones that regulate prostate development and studies have shown that an abnormal level of estradiol (E_2) induces excess prostatic growth and development and contributes largely to the increased number of BPH in ageing men. The effects of estrogens in prostate tissues are mediated by the estrogen receptors (ERα and ERβ) as the binding of effector molecules or ligands to any of the receptors modulates the expression of prostate genes. The binding of the effector molecule to the ERα stimulate prostate inflammation resulting in hyperplasia and binding of the effector molecule to the ERβ reverses hyperplasia because it induces antiproliferative proapoptotic effects in the prostate tissue as earlier reported by Tsurusaki *et al*. (2003) and McPherson *et al*. (2010), respectively. The reductions in the serum estradiol concentrations in the BPH induced rats treated with CEAA are probably due to the interaction of its bioactive constituents with the ERβ which induced apoptosis and inhibition of proliferation prostate tissues and reverse prostate hyperplasia induced testosterone propionate. However, the high level of estradiol concentration in the BPH control had a high affinity to ERα and its binding to the ERα initiate and accelerate inflammation and prostate hyperplasia as indicated by large prostate weight and prostate index of the BPH control rats. The increased estradiol concentrations in the BPH control indicated that the testosterone propionate injections induced BPH in the rats, which is in agreement with the findings of Irwin*et al*. (2009). The CEAA probably prevented aromatization of testosterone into estradiol and preferentially interacted with ERβ in BPH induced rats which reduced prostate hyperplasia in the rats. Present results are in agreement with the findings of Grant and Ramasamy (2012). The reduced estradiol concentration in the CEAA treated BPH induced rats reduced the prostate weight in line with the findings of Schatzl *et al*. (2000).

4. Conclusion

The findings of this study suggest that the combined ethanol extract of *A. vogelii* and *A. boonei* (CEAA) stem barks have antibenign prostatic hyperplasia activity comparable to finasteride. Treatment of BPH induced rats with CEAA greatly reduced prostate weight and prostate index and down regulated testosterone, dihydrotestosterone (DHT) and estradiol (E2) serum concentrations to normal levels suggesting these could be its mechanism of action against BPH. A supplementary study is necessary to explore the effects of the key active components of *A. vogelii* and *A. boonei* and the molecular mechanisms underlying their actions.

5. References

Aggarwal, S., Thareja, S., Verma, A., Bhardwaj, T.R. and Kumar, M. 2010. An overview of 5α-reductase inhibitors. *Steroids*, 75(2): 109–153.

Alaribe, C.S.A., Corker H.A.B, Shode, F.O., Ayoola, G., Adesegun, S.A., Bamiro, J., Anyim, E.I. and Anyakora, C. 2011. Antiplasmodial and phytochemical investigation of leaf extract of Anthocleista vogelii. *Journal of Natural Products*, 5: 60–67.

Alshawsh, M.A., Mothana, R.A., AlShamahy, H.A., Alsllami, S.F. and Lindequist, U. 2009. Assessment of antimalarial activity against Plasmodium falciparum and phytochemical screening of some Yemeni medicinal plants. *Evidence-Based Complementary and Alternative*, 6: 453–456.

Babu, S.V., Veeresh, B., Patil, A.A. and Warke, Y.B. 2020. Lauric acid and myristic acid prevent testosterone-induced prostatic hyperplasia in rats. *European Journal of Pharmacology*, 626(2-3): 262-265.

Bisson, J.F., Hidalgo, S., Rozan, P. and Messaoudi, M. 2007. The therapeutic effect of ACTICOA powder, a cocoa polyphenolic extract, on experimentally induced prostate hyperplasia in Wistar-Unilever rats. *Journal of Medicinal Food*, 10(4): 628-635.

Carson, C. and Rittmaster, R. 2003. The role of dihydrotestosterone in benign prostatic hyperplasia. *Urology*, 61(4): 2-7.

Chapelle, J.P. 1976. Vogeloside et acide secologanique, glucosides seco-iridoids d' Anthocleista vogelii. *Planta Medica*, 29: 268–274.

Fakae, B.B., Campbell, A.M., Barrett, J., Scott,I.M., Teesdale-Spittle, P.H., Liebau, E., and Brophy, P.M. 2000. Inhibition of glutathione S-transferases (GSTs) from parasitic nematodes by extracts from traditional Nigerian medicinal plants. *Phytotherapy Research*, 14(8):630–634.

Gacci, M., Eardley, I., Giuliano, F., Hatzichristou, D., Kaplan, S.A., Maggi, M. and Roehrborn, C.G. 2011. Critical analysis of the relationship between sexual dysfunctions and lower urinary tract symptoms due

to benign prostatic hyperplasia. *European Urology*, 60(4): 809-825.

Gasco, M., Villegas. L., Yucra. S., Rubio, J. and Gonzales, G.F. 2007. Dose-response effect of red maca (*Lepidium meyenii*) on benign prostatic hyperplasia induced by testosterone enanthate. *Phytomedicine*, 14(7-8): 460-464.

Grant, P. and Ramasamy, S. 2012. An update on plant-derived anti-androgens. *International Journal of Endocrinology and Metabolism*, 10(2): 497–502.

Hadi, S. and Bremner, J.B. 2001. Initial studies on alkaloids from Lombok medicinal plants. *Molecules*, 6(2):117–129

Hongcai, C., Guowei, Z., Zechen, Y. and Xuejun, S. 2018. The effect of Xialiqi capsule on testosterone-induced benign prostatic hyperplasia in rats. Evidence-Based Complementary and Alternative, 2018: 1-9

Imperato-McGinley, J., Walsh, P.C., McConnell, J.D. and Vaughan, E.D. 1992. The effect of finasteride in men with benign prostatic hyperplasia. The New *England Journal of Medicine*, 327(17): 1185-1191.

Institute of Laboratory Animal Resources 1986. Committee on Care, Use of Laboratory Animals. Guide for the care and use of laboratory animals (No. 86). US Department of Health and Human Services, Public Health Service, *National Institutes of Health*.

Irwin, D.E., Milsom, I., Kopp, Z., Abrams, P., Artibani, W. and Herschorn, S. 2009. Prevalence, severity, and symptom bother of lower urinary tract symptoms among men in the EPIC study: impact of overactive bladder. *European Urology*, 56(1):14-20.

Jang, H., Ha, U.S., Kim, S.J., Yoon, B., Han, D., Yuk, S. and Kim, S.W. 2010. Anthocyanin extracted from black soybean reduces prostate weight and promotes apoptosis in the prostatic hyperplasia-induced rat model. *Journal of Agricultural and Food Chemistry*, 58(24):12686-12691.

Jiofack, T., Fokunang, C., Guedje, N., Kemeuze, V., Fongnzossie, E., Nkongmeneck, B.A., Mapongmetsem, P.M. and Tsabang, N. 2010. Ethnobotanical uses of medicinal plants of two ethnoecological regions of Cameroon. *International Journal of Medical Sciences*, 2: 60-79.

Lorke, D. 1983. A new approach to practical acute toxicity testing. *Archives of Toxicology*, 54(4): 275-287.

McConnell, J.D., Roehrborn, C.G., Bautista, O.M., Dixon, C.M., Kusek, J.W., Lepor, H., McVary, K.T., Nyberg, K.T., Leroy, M., Harry S. C., Crawford, E.D., Diokno., Diokno, A., Foley, J.P., Foster., H.E., VS.C., KaplanS.A., Kreder, K.J., Lieber, M.M., Lucia., M.S., Miller, G.J., Menon, M., Milam, D.F., Ramsdell, J.W., Schenkman, N.S., Slawin, K.M. and Smith, J.A. 2003. The long-term effect of doxazosin, finasteride, and combination therapy on the clinical progression of benign prostatic hyperplasia. *The New England Journal of Medicine,* 349(25): 2387-2398.

McPherson, J.S., Hussaina, S., Balanathana, P., Hedwards, S.L., Niranjan, B., Grant, M., Chandrasiri, U.P., Toivanen, R., Wang, Y., Taylor, R.A. and Risbridger, G.P. 2010. Estrogen receptor-activated apoptosis in benign hyperplasia and cancer of the prostate is androgen-independent and TNF mediated. *Proceedings of the National Academy of Sciences*, 107(7): 3123–3128.

Mizokami, A., Koh. E., Izumi, K., Narimoto., K., Takeda, M., Honma S., Dai, J., Keller , E.T., and Namiki, M. 2009. Prostate cancer stromal cells and LNCaP cells coordinately activate the androgen receptor through a synthesis of testosterone and dihydrotestosterone from dehydroepiandrosterone. *Endocrine-Related Cancer*, 16(4): 1139–1155.

Musa, A.D., Yusuf, G.O., Ojogbane, E.B. and Nwodo, O.F.C. 2010. Screening of eight plants used in folkloric medicine for the treatment of typhoid fever. *Journal of Chemical and Pharmaceutical Research*, 2(4): 7-15.

Okon, E.O., Gboeloh, L.B. and Udoh, S.E. 2014. Antimalarial effect of combined extracts of the leaf of Ficus exasperata and stem bark of Anthocleista vogelii on mice experimentally infected with plasmodium Berghei berghei (Nk 65). *Research Journal of Medicinal Plants*, 8: 99-111.

Osadebe, P.O. 2002. Anti-inflammatory properties of the root bark of A. boonei. *Nigerian Journal of Natural Products and Medicine*, 6: 39–41.

Pais, P. 2020. Potency of a novel saw palmetto extract, SPET-085, for inhibition of 5 alpha-reductase II. *Advances in Therapy*, 27: 555-563.

Parsons, J.K. and Kashefi, C. 2008. Physical activity, benign prostatic hyperplasia, and lower urinary tract symptoms. *European Urology*, 53(6): 1228-1235.

Sasagawa, I., Nakada, T., Kazama, T., Satomi, S., Terada, T. and Katayama, T. 1990. Volume change of the prostate and seminal vesicles in male hypogonadism after androgen replacement

therapy. *International Urology and Nephrology*, 22: 279–284.

Schatzl, G., Brössner, C., Schmid, S., Kugler, W., Roehrich, M., Treu, T., Szalay, A., Djavan, B., Schmidbauer, C.P., Söregi, S. and Madersbacher, S. 2000. Endocrine status in elderly men with lower urinary tract symptoms: correlation of age, hormonal status, and lower urinary tract function. The Prostate Study Group of the Austrian Society of Urology. *Urology*, 55: 397–402.

Shinde, J.S., Khurde, S.S., Suchita, S.L., Chavan, S.S. and Hulmajge, S.B. 2016. Need of polyherbal formulations and its standardization: A Review. *World Journal of Pharmaceutical Sciences*, 5(11): 526-533.

Steers, W.D. 2001. 5 alpha-reductase activity in the prostate. Urology, 58 (1 Suppl, 6): 17–24.

Tepongning, R.N., Lucantoni, L., Nasuti, C.C., Dori, G.U., Yerbanga, S.R., Lupidi, G., Marini, C., Rossi, G., Esposito, F., Habluetzel, A. 2011. Potential of a *Khaya ivorensis*- *Alstonia boonei* extract combination as antimalarial prophylactic remedy. *Journal of Ethnopharmacology*, 137: 743-751.

Tietz, N.W.1995. Clinical Guide to Laboratory Tests, 3rd Ed, W.B Sanders Co, Philadelphia. pp. 578-580.

Traish, A.M., Hassani, J., Guay, A.T., Zitzmann, M. and Hansen, M.L. 2011. Adverse side effects of 5α -reductase inhibitors therapy: persistent diminished libido and erectile dysfunction and depression in a subset of patients. *Journal of Sexual Medicine*, 8(3): 872–884.

Tsurusaki, T., Aoki, D., Kanetake, H., Inoue, S., Muramatsu, M., Hishikawa, Y. and Koji, T. 2003. Zone-dependent expression of estrogen receptors and in human benign prostatic hyperplasia. *Journal of Clinical Endocrinology and Metabolism*, 88(3):1333–1340.