EFFECTS OF COMBINED ETHANOLICIC EXTRACTS OF *FUNTUMIA AFRICANA* AND *ABUTILON MAURITIANUM* LEAVES ON SEX HORMONES AND PROSTATE INDICES OF BENIGN PROSTATIC HYPERPLASIA INDUCED RATS

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ABSTRACT

This study evaluated the effects of a combined ethanolic extract of Funtumia africana and Abutilon mauritianum leaves (CFAAM) on the sex steroid hormone levels and prostate indices of benign prostatic hyperplasia (BPH) induced rats to validate their anti-BPH activities as claimed by traditional medicine healers. 30 male Wistar albino rats were randomly divided into 5 groups. Group 1 served as the normal control that received normal saline and olive oil only, while groups 2 – 5 were BPH induced. Group 2 was the BPH control, group 3 was treated with 5 mg/kg/d Finasteride, while groups 4 and 5 were treated with 200 and 600 mg/kg/d CFAAM respectively for 28 days. BPH induction caused significant ($p<0.05$) increases in the concentrations of testosterone, dihydrotestosterone and estradiol, increased prostate weight and prostate index in the BPH control when compared with the normal control. Treatment with CFAAM significantly ($p<0.05$) reversed the increased testosterone, dihydrotestosterone and estradiol levels, prostate weight, and prostate indices in comparison with the BPH control. This study revealed that the CFAAM ameliorate adverse effects of benign prostatic hyperplasia in rats and may be useful in the management of individuals with benign prostatic hyperplasia.

Keywords: Benign prostatic hyperplasia (BPH), Sex hormones, Prostate indices, *Funtumia africana*, *Abutilon mauritianum*

INTRODUCTION

Benign prostatic hyperplasia (BPH) is a non-malignant prostate disease due to uncontrolled differentiation and proliferation of the prostate epithelial and stromal tissues mostly in senile men above 40 years (Nicholson and Rick, 2011). Although it is generally believed to occur in ageing men, it is common in some family which indicates that there are inheritable genetic factors that predispose some ageing men to the development of BPH. Patients with BPH experience increased frequency of urination, reduced urinary flow, and incomplete emptying of the bladder. Imbalance in the circulating levels of sex hormones including testosterone, dihydrotestosterone (DHT) and oestrogens have been implicated as the risk factors in the pathogenesis of BPH (McPherson *et al.*, 2008; Wilson, 2011). Studies have established that

patients with BPH have a very high level of DHT in serum (Ryl *et al*., 2015). DHT induces prostate growth and inhibits the conversion of testosterone by 5α-reductase, limits the level of DHT, prevents prostate enlargement and reduces prostate weight by at least 30 % (Andriole *et al*., 2004; Nickel *et al*., 2011). The aetiology and mechanisms of pathogenesis of benign prostatic hyperplasia are not fully understood but many researchers in this field believe that increased secretion and accumulation of DHT level in the prostate tissues with ageing is responsible for the initiation of increased prostate cell growth and prostatic hyperplasia (Bartsch *et al*., 2000; Carson and Rittmaster, 2003). Treatment of patients with BPH is mainly by 5α-reductase inhibitors such as Finasteride and dutasteride that block the conversion of testosterone to a more potent DHT. Other proven therapeutics are α-adrenoceptors antagonists including doxazosin and tamsulosin, but there are many adverse side effects associated with their usage (Patel and Chapple, 2008). Many traditional medicines of plant origin have been successfully used to treated BPH patients with total recovery but are rarely documented in the scientific literature.

Funtumia africana (Benth.) is a potent medicinal plant of Apocynaceae family commonly called "Furu" by the Yoruba and "Mbamiri" by the Igbo speaking tribes of Nigeria. It has been used in the treatment of many diseases including cancer, malaria, dysentery, diabetes, bacterial infections, hypertension, inflammation, wounds, urinary incontinence and oedema (Odugbemi *et al*., 2007; Ashidi *et al*., 2010; Ramadwa *et al*., 2017).

Abutilon mauritianum (Jacq.) is commonly known as African mallow in English, "Akoire" in Yoruba and "Kawo" in Igbo is a member of the Malvaceae family that has demonstrated various medicinal potentials and constitute part of major herbal formulation in West African traditional medicine especially in the south-eastern part of Nigeria. It has been reported that various herbal formulations with *A. mauritianum* parts are used in the treatment of diarrhoea, gonorrhoea, cough, pile and as antipyretic (Olowokudejo *et al*., 2008). The members of the genus *Abutilon* are used to treat malaria (Beha *et al*., 2004), snakebite (Shrikanth *et al.,* 2011), Viral diseases (Mohamed *et al*., 2010), ulcer (Ponnudurai *et al*., 2011) and wound healing (Suresh *et al*., 2011).

Extracts of *F. africana* leaves have been reported to be rich in saponins, flavonoids, tannins, polyterpenes, polyphenols terpenoids, steroids, cardiac glycosides and alkaloids and many of their pharmacological activities are attributed to these phytochemical constituents (Kouadio *et al*., 2017; Nwandu *et al*., 2019). Similarly, the pharmacological activities of *A. mauritianum* leaf extract are attributed to its rich contents of steroids, saponins, tannins, phenols, alkaloids and flavonoids (Banso and Adeyemo, 2004). The combined ethanol extract of *F. africana* and *A. mauritianum* leaves is relatively safe for consumption with LD_{50} value of above 5,000 mg/kg (Uroko *et al*., 2020).

The various medicinal properties of these plant extracts prompted this study to evaluate the effects of CFAAM on the sex steroid hormone levels and prostate indices in benign prostatic hyperplasia induced rat.

MATERIALS AND METHODS

Collection and Identification of Plant Materials: *Funtumia africana* and *Abutilon mauritianum* leaves were used in this study. The fresh leaves of *F. africana* and *A. mauritianum* were sourced from the Forestry Research Institute of Nigeria, Eastern Station, Ahia-Eke Ndume, Umuahia, Abia State. The plants were identified and authenticated as *F. africana* and *A. mauritianum* with voucher numbers 2694-5 [Preuss 1899] and Jones FHI 13749 respectively by a plant taxonomist at the Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The plant samples were handpicked, rinsed in clean running water and dried under shade till constant weights were obtained. The dried plant samples were pulverised into coarse powders using a mechanical grinder and stored in clean plastic containers.

Ethical Issues: The experimental procedures employed in the study carefully adhered to the guidelines stipulated by the National Institute of Health's guidelines for the care and use of animals for research (ILAR, 1986) and the guidelines of the Research Ethics Committee of Michael Okpara University of Agriculture, Umudike for experiments with animals. The ethical clearance was duly obtained from the Ethical Committee of the Department of Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike with the Ethical Clearance Number - MOUAU/VPP/EC/18/003.

Experimental Animals: Thirty (30) male Wistar albino rats aged between 15 – 16 weeks and weighing 100 – 120 g were purchased from the Animal House, Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria Nsukka, Nigeria. The rats were kept on a standard laboratory diet (Vital feed) and drinking water *ad libitum* and acclimatized under a 12 hour light/dark cycle for 14 days before the commencement of the experimental study.

Formulation of a Combined Extract: The combined extract was formulated using coarsely ground *Funtumia africana* and *Abutilon mauritianum* leaves in the ratio of 1:1 (i.e. 250 g each, equivalent to 500 g of both plant samples). The decision to combine the plants in a ratio of 1:1 for extraction was to mimic their local use in traditional medicine though there was no literature to support it. Each dosage was 20 times reduction of the LC_{50} earlier reported as safe (Uroko *et al*., 2020). The combined plant powders were extracted by dissolving it 1.5 L of absolute ethanolic for 72 hours. After which it was filtered and concentrated till the ethanolic solvent was completely evaporated in the water bath at 45℃ and the percentage yield calculated.

Experimental Design: The study used completely randomised design (CRD) of five treatment groups, replicated thrice with each replicate having two rats. The 30 rats were randomly grouped into five groups, Group 1 served as the normal control without BPH induction, whereas group 2 served as BPH control that was BPH induced untreated. Group 3 served as the standard control that BPH induced but treated with 5 mg/kg/d Finasteride for 28 days while groups 4 and 5 were BPH induced rats treated with 200 and 600 mg/kg/d CFAAM respectively. Benign prostatic hyperplasia (BPH) was induced in the rats by subcutaneous injection of testosterone propionate in olive oil (2:1 v/v) (5 mg/kg/d) for 28 days consecutively. Treatments were given to the rats one hour after testosterone administration every day for 28 days consecutively. The body weights (BW) of the rats were recorded every week throughout the study period. After the last administration of testosterone propionate and treatments on the 28^{th} day, the rats fasted overnight. The rats were anaesthetized by the intraperitoneal administration of a low dose of pentobarbital (25 mg/kg) and allowed to stay for 10 minutes before blood samples were collected from the rats through cardiac puncture and prostate tissues harvested and weighed on the 29^{th} day.

Determination of Prostate Weight (PW) and Prostate Index (PI): The prostate weight and prostate index were determined according to the methods of Cai *et al*. (2018). Prostate weight (PW) and body weight (BW) of the rats were recorded in each group. The prostate index was calculated as PI (%) = PW/BW x 100 and the mean PI ratio was calculated for each group. 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 group respectively.

Determination of Testosterone (TT), Dihydrotestosterone (DHT) and Estradiol (E2) Concentrations: The serum testosterone, dihydrotestosterone and estradiol concentrations were determined using the methods described by Tietz (1995). The principle of this assay is based on competitive binding between testosterone in the test specimen and testosterone-horseradish peroxidase (HRP) conjugate for a constant amount of rabbit anti-

testosterone in the incubation. Goat anti-rabbit IgG-coated wells were incubated with testosterone standards and sample, testosterone-HRP conjugate reagent and rabbit anti-testosterone reagent for 90 minutes. The serum concentrations of dihydrotestosterone (DHT) were determined with an enzyme-linked immunosorbent assay (ELISA) kit using the instructions outlined by the manufacturer (AMEKO Incorporated, China) and the concentrations obtained expressed as ng/mL (Tietz, 1995).

The estradiol was assayed using immunoassay which required an antibody, enzyme-antigen conjugate and the native antigen as essential reagents. The principle of assaying for estradiol using immunoassay is because when a biotinylated antibody is mixed with a serum containing the antigen, a reaction occurs between the antigen and the antibody. After a short incubation, the enzyme conjugate is added, competition reaction results between the enzyme analogue and the antigen in the sample for a limited number of antibody binding sites (not consumed in the first reaction). A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody-bound fraction after decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration (Tietz, 1995).

Statistical Analysis: The data generated from this study were analysed with one-way analysis of variance (ANOVA) and Duncan's multiple comparison post hoc test using Statistical Package for Social Sciences (SPSS) Version 22. The level of statistical significance was established at $p<0.05$.

RESULTS

Percentage Yield of the Combined Extract: The extraction of 500 g of the combined plant samples gave a percentage yield of 4.23 % equivalent to 21.15 g of the CFAAM.

Effects of Combined Ethanolic Extract of *F. africana* and *A. mauritianum* Leaves (CFAAM) on the Body Weight of Benign Prostatic Hyperplasia (BPH) Induced Rats: It was observed that the body weights of the normal control and BPH control rats showed no significant ($p>0.05$) increases after week 2, and significant ($p<0.05$) after weeks 3 and 4, respectively when compared with their respective body weight at week 1 (Table 1). However, standard control, and BPH induced rats treated 200 mg/kg/d CFAAM, respectively showed no significant ($p<0.05$) increase in body weight after weeks 2, 3, and 4 when compared with their respective body weight at week 1. Contrary, BPH induced rats treated with 600 mg/kg/d CFAAM showed significant ($p<0.05$) increase in their body weight after weeks 2, 3, and 4, respectively when compared with its body weight after week 1.

Effects of Combined Ethanolic Extract of *F. africana* and *A. mauritianum* Leaves (CFAAM) on the Prostate Weight and Prostate Index of Benign Prostatic Hyperplasia (BPH) Rats: It was observed that there were significant ($p<0.05$) increases in the prostate weight of the BPH control, standard control (Finasteride) and BPH induced and BPH induced rats treated with 200 and 600 mg/kg/d CFAAM, respectively when compared with the normal control rats (Table 2). BPH induced rats treated with 5 mg/kg/d Finasteride (standard control) and 600 mg/kg/d CFAAM, respectively showed significant ($p<0.05$) decrease in prostate weight relative to the BPH control rats. Whereas, BPH induced rats treated with 200 mg/kg/d CFAAM showed no significant ($p>0.05$) decrease in prostate weight when compared with BPH control. Also, it was observed that BPH induced rats treated with 600 mg/kg/d CFAAM had significantly ($p<0.05$) lower prostate weight when compared with the BPH induced rats treated with 5 mg/kg/d Finasteride (standard control). Also, the prostate index of benign prostate hyperplasia (BPH) induced rats treated with CFAAM showed that the induction caused significant ($p<0.05$) increase in the prostate index relative to the normal control rats with that of the BPH control having highest increase.

Table 1: Body weight of benign prostatic hyperplasia induced rats treated with combined ethanolic extract of *F. africana* and *A. mauritianum* leaves (CFAAM)

Weeks	Changes in body weight (g)				
	Normal Control	BPH Control	Standard Control	200 mg/kg/d CFAAM	600 mg/kg/d CFAAM
Week 1	147.37 ± 8.17^{a}	138.8 ± 9.04^{a}	144.23 ± 8.50^{a}	143.62 ± 8.32^{a}	136.67 ± 9.52^{a}
Week 2	151.30 ± 155^{ab}	143.3 ± 8.72^{ab}	147.15 ± 9.66^{a}	159.37 ± 10.49^{a}	152.25 ± 8.18^{b}
Week 3	172.88 ± 9.09^{b}	160.38 ± 6.53^{b}	166.12 ± 7.98^{a}	166.52 ± 7.65^{a}	161.68 ± 7.26^{b}
Week 4	171.42 ± 7.08^{b}	157.88 ± 7.90^{b}	160.93 ± 8.53^{a}	164.50 ± 9.14^{a}	161.18 ± 8.40^{b}

Values are presented as mean ± standard deviation (n = 6), Values with different superscripts are significantly different (p<0.05)

Table 2: Prostate weight and prostate index of benign prostatic hyperplasia (BPH) induced rats treated with combined ethanolic extract of *F. africana* and *A. mauritianum* leaves (CFAAM)

Treatment groups	Prostate weight (g)	Prostate index (PI)
Normal Control	0.233 ± 0.022^{a}	0.136 ± 0.020^{a}
BPH Control	0.667 ± 0.037^{c}	0.422 ± 0.030^{c}
Standard Control	0.500 ± 0.034^{b}	0.311 ± 0.012^{b}
200 mg/kg/d CFAAM	0.533 ± 0.025^{bc}	0.324 ± 0.020^{b}
600 mg/kg/d CFAAM	0.300 ± 0.023^{a}	0.186 ± 0.013^{a}

Values are presented as mean ± standard deviation (n = 6), Values with different superscripts are significantly different (P<0.05)

However, treatment with the CFAAM caused significant (p<0.05) reduction of the prostate index of the standard control treated with Finasteride and BPH induced rats treated with 200 and 600 mg/kg/d CFAAM relative to the BPH control rats. It was also observed that the BPH induced rats treated with 600 mg/kg/d CFAAM showed no significant (p>0.05) increase in the prostate index when compared with normal control rats.

Inhibitory Effects of Combined Ethanolic Extract of *F. africana* and *A. mauritianum* Leaves on Prostate Weight and Prostate Index of Benign Prostatic Hyperplasia Induced Rats: The results in Table 3 showed that CFAAM possesses dose-dependent inhibitory effects on prostate weight and prostate index of benign prostatic hyperplasia induced rats. The CFAAM at 200 mg/kg/d had lower inhibitory effects on the prostate weight and prostate index when compared with standard control treated with 5 mg/kg/d of Finasteride. However, BPH induced rats treated with 600 mg/kg/d CFAAM had greater reductions in prostate weight (84.54 %) and prostate index (82.52 %) relative to the standard control treated with Finasteride.

Table 3: Inhibitory effects of a combined extract of *F. africana* and *A. mauritianum* leaves (CFAAM) on prostate weight (PW) and prostate index (PI) of benign prostatic hyperplasia (BPH) induced rats

Treatment Groups	Degree of inhibition (%)	
	PW	PI
Normal control	-	-
BPH control	-	-
Standard control	38.48	38.81
200 mg/kg/d CFAAM	35.8	34.27
600 mg/kg/d CFAAM	84.54	82.52

PW = Prostate weight; PI = Prostate index

Effects of Combined Ethanolic Extract of *F. africana* and *A. mauritianum* Leaves (on the Serum Testosterone (TT) Levels of Benign Prostatic Hyperplasia Induced Rats: The testosterone concentrations in BPH induced rats treated with CFAAM showed that the BPH of the control rats had significantly (p<0.05) elevated testosterone concentrations relative to the normal control (Figure 1). The standard control that received 5 mg/kg/d Finasteride had no significant (p>0.05)

reduction in testosterone concentration, while all the CFAAM-treated groups showed no significant ($p>0.05$) increase in testosterone concentrations when compared with the normal control. BPH induced rats treated with Finasteride, 200 and 600 mg/kg/d CFAAM-treated rats had significantly ($p<0.05$) reduced testosterone concentrations when compared with the BPH control. The testosterone concentrations the CFAAM-treated rats were significantly ($p<0.05$) elevated relative to the standard control rats.

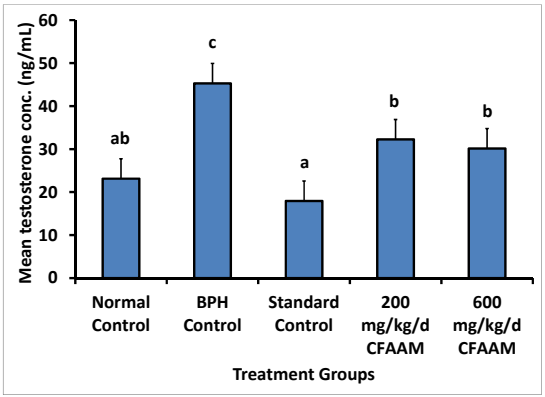

Figure 1: Testosterone (TT) concentrations in benign prostatic hyperplasia (BPH) induced rats treated with combined ethanolic extract of *F. africana* and *A. mauritianum* leaves (CFAAM) ***Key:*** *Each bar represent mean ± standard deviation (n = 6), Bars with different superscripts are significantly different at p<0.05*

Effects of Combined Ethanolic Extract of *F. africana* and *A. Mauritianum* Leaves (CFAAM) on the Serum Dihydrotestosterone (DHT) Levels of Benign Prostatic Hyperplasia Induced Rats: The dihydrotestosterone (DHT) concentrations indicated a significant ($p<0.05$) increase in DHT concentrations of BPH control, standard control (Finasteride) and BPH induced rats treated with 200 mg/kg/d CFAAM, respectively when compared with the DHT concentration of the normal control (Figure 2). However, there were significant ($p<0.05$) decreases in the DHT concentrations of the standard control, and BPH induced rats treated with 200 and 600 mg/kg/d CFAAM relative to the BPH control rats. The DHT concentrations observed in the BPH induced rats treated with the CFAAM were lower than the DHT concentration of BPH induced rats treated with 5 mg/kg/d Finasteride, with the DHT concentration of the BPH induced rats treated with 600 mg/kg/d CFAAM been significantly ($p<0.05$).

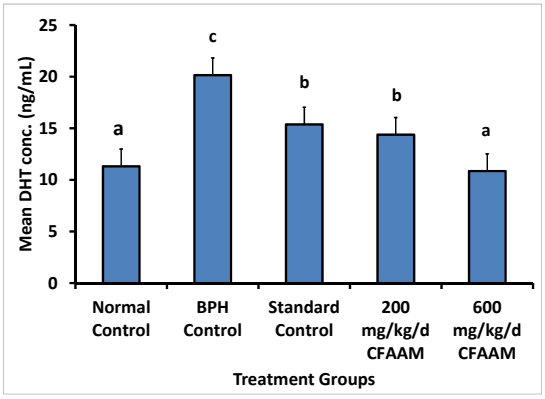

Figure 2: Dihydrotestosterone (DHT) concentrations in benign prostatic hyperplasia (BPH) induced rats treated with combined ethanolic extract of *F. africana* and *A. mauritianum* leaves (CFAAM) ***Key:*** *Each bar represent mean ± standard deviation (n = 6), Bars with different superscripts are significantly different at p<0.05*

Effects of Combined Ethanolic Extract of *F. africana* and *A. mauritianum* Leaves (CFAAM) on the Serum Estradiol (E_2) Levels of Benign Prostatic Hyperplasia Induced Rats: It was evidenced that the BPH induction caused significant ($p<0.05$) increases in the estradiol concentrations in all the induced rats when compared with the normal control (Figure 3).

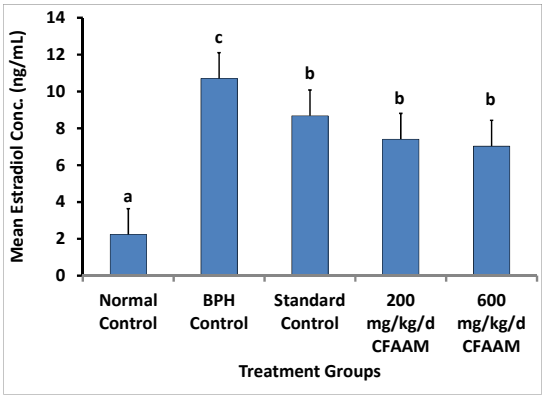

Figure 3: Estradiol concentrations in benign prostatic hyperplasia (BPH) induced rats treated with combined ethanolic extract of *F. africana* and *A. mauritianum* leaves (CFAAM) ***Key:*** *Each bar represent mean ± standard deviation (n = 6), Bars with different superscripts are significantly different at p<0.05*

Treatment of the standard control rats with 5 mg/kg body weight of Finasteride and the groups treated with 200 and 600 mg/kg/d of CFAAM, respectively reduced estradiol concentrations significantly ($p<0.05$) relative to the BPH control. The CFAAM-treated BPH induced rats had no significantly ($p>0.05$) lower estradiol concentrations when compared with standard control rats.

DISCUSSION

This study evaluated the effects of CFAAM on sex steroid hormone levels and prostate indices of benign prostatic hyperplasia induced rats using testosterone propionate injection. Benign prostatic hyperplasia (BPH) is a common prostate disorder in ageing men that greatly impair quality of life, and shorten their life expectancy due to lack of a cure, high cost of treatments and limited available effective treatment options. Abnormal levels of sex steroid hormones including testosterone, (DHT) and estradiol (E2) have been implicated in the initiation and progression of BPH in ageing men via the induction of prostate epithelial cell growth and proliferation. The conversion of testosterone to DHT by 5α-reductase enzymes and interaction of the DHT with its androgen receptors promotes protein synthesis, epithelial cell growth of the prostate and prostate enlargement (Carson and Rittmaster, 2003; Cai *et al*., 2018).

The high percentage yield of the CFAAM recorded in this indicates that *F. africana* and *A. mauritianum* leaves are rich in polar phytoconstituents extractable with ethanol.

The persistent increase in the body weight of the BPH induced rats similar to the normal control showed that BPH has no effects on the body weight of the rats. The BPH did not alter appetite of the rats for food, loss of body weight, and similarly, CFAAM had no effects on the body weight. The extract was unable to induce excessive weight gain through increased food intake and storage in the adipose tissues and vice versa and agreed with the findings of Cai *et al.* (2018).

The elevated concentration of testosterone in the benign prostatic hyperplasia induced rats showed that the administration of testosterone propionate injection resulted in the high serum level of testosterone responsible for the initiation and progression of BPH. This elevated serum level of testosterone predisposed the rats to the development of BPH because it can be converted to a more active DHT when acted upon by 5α-reductase enzymes which may induce increased protein synthesis, growth, and proliferation of epithelial and stromal tissues. However, the significant reduction in the testosterone levels in the BPH induced rats treated with CFAAM was similar to Finasteride-treated rats may be attributed to the anti-benign prostatic hyperplasia activities of its phytoconstituents such as steroids, phenols and flavonoids (Banso and Adeyemo, 2004; Kouadio *et al*., 2017). The effect of CFAAM was not dose-dependent and can substitute for Finasteride as testosterone lowering agent in patients with benign prostatic hyperplasia. The reductions of testosterone level by CFAAM prevented BPH development and progression and it was consistent with the findings that drugs that down-regulate testosterone and DHT prevent BPH development (Patil *et al*., 2016).

The significantly elevated DHT concentration in the BPH control rats showed that 5α-reductase enzymes were able to convert the excess testosterone to DHT unhindered. The excess DHT concentrations in the rats promoted benign prostatic hyperplasia and decreased the quality of life. This is because excess DHT binds to androgen receptors and stimulate cellular proliferation factors and progression of BPH synthesis was in line with the findings of Da Silva and De Souza (2019). Whereas, the significant reduction in DHT concentrations in the Finasteride and the CFAAM- treated rats, respectively showed that they were able the activities of 5α-reductase enzymes and impaired conversion of testosterone to DHT. The CFAAM exhibited a dose-dependent reduction of DHT with the CFAAM possessing high activities far above Finasteride at high concentration and can be more effective in the management of benign prostatic hyperplasia than Finasteride. The low DHT concentrations in the CFAAM-treated rats did not promote prostate growth by induction of differentiation and proliferation of epithelial

tissues of the prostates. This was in agreement with previous findings of that the conversion of testosterone to dihydrotestosterone decreases the levels of dihydrotestosterone, prevents enlargement of the prostatic epithelium and reduction in the prostate size (Nickel *et al*., 2011; Wu *et al*., 2014).

Estradiol is mainly produced in the prostate gland by the action of aromatase which converts much of the circulating testosterone to estradiol for spermatogenesis and maintenance of sperm quality (Mäkelä *et al*., 2000). It has two receptors denoted by ERα expressed in a few stromal cells, and ERβ, largely expressed in the epithelium and some stromal cells. Normally, when estradiol binds to ERα, it stimulates cell proliferation and inflammation of the prostate, while binding of estradiol to ERβ counters the effects of ERα to estradiol by exhibiting anti-proliferative and anti-inflammatory activities (Ellem and Risbridger, 2009; Da Silva and De Souza, 2019). However, in ageing men, under high estradiol concentration and in BPH condition, ERα is highly expressed and actions of ERα – estradiol predominate over ERβ – estradiol and promotes differentiation and proliferation of prostate tissues and prostate inflammation leading prostate enlargement (Hewitt *et al*., 2010). The elevated estradiol concentrations in the BPH control rats relative to the normal control rats showed that much of the testosterone produced in the rats due to testosterone propionate injection were converted to estradiol by the actions of aromatase enzymes in the prostate. The increased estradiol concentration may have interacted with estrogen receptor (ERα) and induced prostate cell differentiation and proliferation and eventual prostate enlargement. On the contrary, the dose-dependent decrease in the estradiol concentration of Finasteride and CFAAM, respectively relative to the BPH control may be attributed to the therapeutic effects of the bioactive constituents like steroids, phenolic and flavonoids present in each of the plants. Plant extracts rich in steroids like beta-sitosterol have been demonstrated to prevent benign prostatic hyperplasia progression by reducing the circulating level of dihydrotestosterone (DHT) which inhibits prostate tissue growth (Bishnoi *et al*., 2017).

The reductions in the serum estradiol levels are indicative of a reduction in prostate volume which is in line with the report of Vermeulen *et al*. (2002) that most patients with larger volumes of BPH have increased levels of serum estradiol and vice versa. The combined ethanolic extract reduced the testosterone concentrations available as a substrate for conversion to estradiol by aromatase enzymes. Sequel to this, CFAAM-treated BPH induced rats had reduced level of adverse health effects associated with benign prostatic hyperplasia. However, none of the treatments was able to maintain estradiol concentration equivalent to the normal control and predisposed the rats to some levels of adverse health effects of increased serum estradiol concentrations.

The highly elevated prostate weight and prostate index in the BPH control rats showed that the testosterone propionate caused benign prostatic hyperplasia in the rats. These rats with an enlarged prostate suffered urinary difficulties such as increased urinary urgency, and incomplete emptying of the bladder and impaired their quality of life. This was in agreement with the finding of Sayed *et al*. (2016) that rats received testosterone injection for 28 consecutive days without treatment showed increased prostate weight. The reductions in the prostate weight and prostate index in the standard control treated with Finasteride and CFAAM-treated rats relative to the BPH control may be attributed to anti-benign prostatic hyperplasia activities of each of the treatments in accordance with the findings of Ishola *et al*. (2018). CFAAM effectively shrunk the enlarged prostate most especially at high dose (600 mg/kg/d) and suggested that 600 mg/kg/d was the effective dose of CFAAM that shrink enlarged prostate to normal size as indicated in the normal control. CFAAM possess better anti-benign prostatic hyperplasia activity at a high dose than Finasteride and represent a more viable option for the treatment of BPH. However, further research is needed to identify and characterise the actual bioactive phytoconstituents such as steroids, phenols and flavonoids responsible for the observed anti-

benign prostatic hyperplasia activities. CFAAM achieved greater reductions in prostate weight and prostate index possibly by inhibiting the differentiation and proliferation of epithelial and stromal tissues of the prostate. The high inhibitory effects of CFAAM on the prostate weight and prostate index at a high dose further confirm that CFAAM exhibited better anti-benign prostatic hyperplasia than Finasteride with low anti-benign prostatic hyperplasia activities. The reduction of prostate weight and prostate index is indicative that the combined ethanolic extract possesses anti-benign prostatic hyperplasia activity in line with the previous report by Cai *et al*. (2018) that reduction of prostate weight and prostate index is attributed inhibition of benign prostatic hyperplasia.

Conclusion: The findings of this study revealed that the combined ethanolic extract of *F. africana* and *A. mauritianum* leaves reduce excessive concentrations of steroid hormones such as testosterone, dihydrotestosterone, and estradiol implicated in the pathogenesis of benign prostatic hyperplasia. The combined ethanolic extract lowers prostate weight and prostate index and showed very high inhibitory effects on the prostate weight and prostate index at high concentration. Further research should be conducted on this combined ethanolic extract to identify, isolate, and characterise its anti-benign prostatic hyperplasia principles.

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