



Effects of combined ethanol extract of *Funtumia africana* and *Abutilon mauritianum* leaves on prostate biomarkers and serum mineral levels in prostatic hyperplasia induced male rats

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Abstract

Introduction: Benign prostatic hyperplasia (BPH) is a prostate disorder in ageing males that negatively affects the quality of life and requires multidimensional approaches to ameliorate its adverse health effects.

Objectives: This study evaluated the effects of combined ethanol extract of *Funtumia africana* and *Abutilon mauritianum* leaves on the prostate biomarkers, serum mineral levels and prostate histomorphology of BPH induced rats.

Materials and Methods: Thirty-six male Wistar albino rats randomly distributed into 5 groups containing 6 rats each was used for this study. Group 1 served as the normal control rats without BPH induction while groups 2–5 were BPH induced rats that served as BPH control (untreated), finasteride control, and BPH induced treated with 200 and 600 mg/kg/d of the combined ethanol extract of *F. africana* and *A. mauritianum* leaves respectively. BPH was induced in the rats by subcutaneous injection of 5 mg/kg/d of testosterone propionate injection and treatment followed 1h after the induction for 28 consecutive days. All the biochemical analyses and prostate histological examinations were carried out using standard methods.

Results: BPH induction significantly elevated serum prostatic acid phosphatase activities and serum prostate-specific antigen (PSA) concentrations in the BPH control rats relative to the normal control. The BPH induction caused significant ($P<0.05$) reductions in the serum levels of calcium and selenium levels and significantly increased the serum inorganic phosphate concentration in the BPH control when compared with the normal control. Treatment with the combined extract significantly ($P<0.05$) increased the serum zinc, calcium, copper, iron and inorganic phosphate and significantly reduced serum selenium level when compared with the BPH control. The combined extract further significant ($P<0.0$) reduced the serum prostatic acid phosphatase activities and PSA level relative to the BPH control. The BPH control showed severe prostate histomorphological alterations consistent with BPH which were largely reduced to mild alterations in combined extract-treated BPH induced rats.

Conclusion: This study revealed that the combined ethanol extract of *F. africana* and *A. mauritianum* leaves positively regulate the serum mineral levels, serum prostatic acid phosphatase activities, PSA levels and improves prostate histomorphology BPH induced rats.

Keywords: Benign prostatic hyperplasia, Serum minerals, Prostate-specific antigens, Prostatic acid phosphatase activity, *Funtumia africana*, *Abutilon mauritianum*

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Introduction

Benign prostatic hyperplasia (BPH) is a neoplastic disease common in ageing men that have significantly reduced their quality of life and increased the risk of kidney failure in older men (1,2). It is associated with a progressive proliferation of prostatic epithelial and stromal tissues due to increased dihydrotestosterone stimulation that leads to prostate enlargement and lower urinary tract symptoms (3). Significant increase in the prostatic

acid phosphatase (PAP) activities and prostate-specific antigen (PSA) concentrations have been reported in BPH patients and are usually considered reliable markers of BPH progression (4). There are increasing pieces of evidence that increasing concentration and response to dihydrotestosterone stimulation with ageing are the major factors responsible for increased prostate cell growth and hyperplasia in ageing men (5). Medicinal plant extracts and synthetic drugs that prevent the conversion

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■ Implication for health policy/practice/research/medical education

The investigation of the effects of combined ethanol extract of *Funtumia africana* and *Abutilon mauritianum* leaves on the prostate biomarkers, serum mineral levels and prostate histomorphology of benign prostatic hyperplasia (BPH) induced rats indicated that BPH negatively alters the serum mineral levels in addition to prostate biomarker and histology if not treated. The combined ethanol extract of *F. africana* and *A. mauritianum* leaves ameliorates adverse effects of BPH by maintaining normal serum mineral levels, reductions of serum prostatic acid phosphatase activities and prostate-specific antigen (PSA) levels and prevents prostate enlargement. The findings of this study suggest that regular monitoring and maintenance of normal serum mineral levels could prevent progression of BPH and improves the health status of a BPH patient. Further efforts are required to identify, isolate and characterise the phytochemical constituents of ethanol extract of *Funtumia africana* and *Abutilon mauritianum* leaves respectively and their combined ethanol extract as this could help to maximise their anti-BPH therapeutic potentials.

of testosterone to dihydrotestosterone by inhibiting the action of 5 α -reductase enzyme activity like finasteride could significantly reduce BPH progression and severity (6).

Abutilon mauritianum (Jacq.) Medik a member of Malvaceae family is commonly called African mallow or, country mallow is highly potent medicinal plant with antiproliferative properties and has demonstrated effectiveness in the management of gonorrhoea, diarrhoea, asthma, dysentery, malaria, inflammation and various bacterial infections (7). Further studies have shown its leaves possess hepatoprotective, hypoglycemic, immunomodulatory, diuretic, laxative, antinociceptive, antimicrobial, antimalarial, antifertility, and wound healing properties (8). These pharmacological activities exhibited by *A. mauritianum* leaves are attributed to its rich phytochemical constituents such as alkaloids, saponins, tannins, glycoside, and flavonoids (9,10). *Funtumia africana* (Benth.) Stapf commonly called "false rubber tree" is a member of the Apocynaceae family that has shown to be rich in many pharmacological activities. *F. African* leaves possess wide medicinal properties including antidiarrhoea, hepatoprotectives, antioxidative stress, dysentery, kidneys disorders and as diuretics (11,12).

Objectives

The enormous medicinal properties of *A. mauritianum* and *F. africana* leaves have prompted us to evaluate the effects of their combined ethanol extract on the prostate biomarkers, serum mineral levels and prostate histomorphology of BPH induced rats. This could enable the formulation of a potent herbal drug for the management of BPH and improving the health outcome of BPH patients.

Materials and Methods

Leaves of *F. africana* and *A. mauritianum* were used to

form combined ethanol extract used in this study. The *F. africana* and *A. mauritianum* leaves were obtained from the Forestry Research Institute of Nigeria, Eastern Station, Ahia-Eke Ndume, Umuahia, Abia State. The fresh leaves were properly identified and authenticated as *F. africana* and *A. mauritianum* with voucher numbers 2694-5 (Preuss 1899) and Jones FHI 13749 respectively by Dr Ibe K. Ndukwe, a Taxonomist at the Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State Nigeria.

Chemicals and reagents

The chemicals and reagents used in this study were of analytical grades and were sourced from Sigma Aldrich, USA; AMEKO Inc., China, and other chemical stores located at Nsukka, Enugu State, Nigeria.

Experimental animals

Thirty male Wistar albino rats weighing 120–170 g were obtained from the Animal House, Department of Zoology and Environmental Sciences, Faculty of Biological Sciences, University of Nigeria Nsukka, Nigeria. The rats were subjected to the environmental condition for acclimatization with free access to a standard laboratory diet (Vital feed) and drinking water *ad libitum* and under a 12 h light/dark cycle for 14 days before the experimental study.

Formulation of a combined extract

The combined ethanol extract was obtained by the ethanol extraction *F. africana* and *A. mauritianum* leaves in the combining ratio of 1:1 (i.e. 250 mg of coarsely ground sample each, equivalent to 500 g of both plant samples). The combined plant extract (500 g) was macerated in 1.5 L of cold absolute ethanol for 72 hours and filtered. It was then concentrated in a water bath at 65°C to allow the ethanol solvent evaporate completely and the percentage yield calculated.

Experimental design

The rats were induced BPH was by subcutaneous injection of testosterone propionate in olive oil (5 mg/kg body weight) for 28 days consecutively. The rats were distributed randomly into five groups containing 6 rats each. Group 1 was the normal control rats that were not BPH induced, and group 2 was BPH control that was BPH induced untreated. Group 3 was the standard control that was BPH induced but treated with 5 mg/kg/d of finasteride for 28 days while group 4 and 5 were BPH induced rats treated with 200 and 600 mg/kg of combined ethanol extract of *F. africana* and *A. mauritianum* leaves respectively. Treatments were given to the rats 1 h after testosterone administration every day for 28 days consecutively. After the last administration of testosterone propionate and treatments on the 28th day, the rats fasted overnight. Blood

samples were withdrawn from the rats, prostate tissues, were collected on the 29th day for the biochemical analyses and histological examinations. The rats used in this study were carefully and humanely handled according to the guidelines stipulated by the National Institute of Health's guidelines for the care and use of animals for research (13).

Biochemical analyses

The serum PSA levels were determined according to the immunoenzymometric method using the PSA ELISA Kit and procedure outlined by the manufacturer. The serum prostatic acid phosphatase activities were assayed with the method described by Uotila et al (14).

Determinations of serum mineral levels

The serum zinc level was determined according to the method of Johnsen and Eliasson (15). Serum calcium and magnesium concentrations were determined using the colourimetric method as described by Lorentz, and methods of Faulkner respectively (16,17). Additionally, the serum iron, inorganic phosphate, selenium and copper were determined according to the methods of Henry (18) and Ochei & Kolhatkar (19).

Ethical issues

This experimental protocol was performed in line with the regulations of the Research Ethics Committee of Iranian Ethical Guidelines for the use of animals in research. Additionally, all animal experiments were performed in accordance with protocols approved by the United States National Research Council (NRC, 1985). This study was also approved and supported by the Ethics Committee of Michael Okpara University of Agriculture, Umudike (MOUAU/VPP/EC/18/003).

Statistical analysis

The data obtained were statistically analysed using a one-way analysis of variance (ANOVA) with the aid of Statistical Products and Service Solutions (SPSS) version 22. Means were compared using Duncan's multiple comparison post hoc test (LSD) and results were presented as mean \pm standard deviation ($n = 6$). Statistical significance were established at 95 % confidence level ($P < 0.05$).

Results

The serum zinc levels in the rats showed that the BPH induction caused no significant ($P > 0.05$) decreases in the BPH control, and standard control and increases in the BPH induced rats treated with 200 and 600 mg/kg/d of combined ethanol extract of *F. africana* and *A. mauritianum* leaves respectively relative to the normal control. However, treatment with the combined ethanol extract of *F. africana* and *A. mauritianum* leaves caused significant ($P < 0.05$) elevation of the serum zinc levels in the BPH induced rats treated with 200 and 600 mg/kg/d of combined ethanol extract of *F. africana* and *A. mauritianum* leaves when compared with the BPH control (Table 1).

There were significant ($P < 0.05$) increases in the serum inorganic phosphate (Pi) levels in the BPH control and BPH induced rats treated with 200 and 600 mg/kg/d of combined ethanol extract of *F. africana* and *A. mauritianum* leaves and significant ($P < 0.05$) in the standard control, respectively when compared with the normal control. The standard control treated with 5 mg/kg/d of finasteride showed significant ($P < 0.05$) decrease in serum Pi level relative to the BPH control. The BPH induced rats treated with 200 mg/kg/d of combined ethanol extract of *F. africana* and *A. mauritianum* leaves showed significant ($P < 0.05$) increase in the serum Pi level relative to the BPH control. However, the BPH induced rats treated with 600 mg/kg/d of the combined ethanol extract of *F. africana* and *A. mauritianum* leaves showed no significant ($P > 0.05$) increase in the serum Pi level when compared with the BPH control. All the combined extract-treated BPH induced rats showed significant ($P < 0.05$) increases in the serum Pi level relative to the standard control treated with finasteride (Table 1).

The serum iron (Fe) level in the BPH induced rats showed that the serum Fe level was not significantly ($P > 0.05$) reduced in the BPH control and elevated in the standard control when compared with normal control. the serum Fe level in the BPH induced rats treated with 200 and 600 mg/kg/d of combined ethanol extract of *F. africana* and *A. mauritianum* leaves, respectively showed significant ($P < 0.05$) when compared with the normal control. Also, the standard control, and BPH induced rats

Table 1. Serum mineral level of the benign prostatic hyperplasia induced rats treated with combined ethanol extract of *F. africana* and *A. mauritianum* leaves (CFAAML)

Groups	Zinc (mg/dL)	Ca (mg/dL)	Mg (mg/dL)	Cu (mg/dL)	Sn (mg/dL)	Fe (mg/dL)	Pi (mg/dL)
Normal control	181.29 \pm 8.17 ^a	41.83 \pm 1.71 ^b	11.33 \pm 0.60 ^a	0.40 \pm 0.02 ^a	9.44 \pm 0.32 ^b	38.333 \pm 3.61 ^{ab}	2.13 \pm 0.54 ^a
BPH control	160.47 \pm 7.99 ^a	33.97 \pm 2.07 ^a	11.80 \pm 0.61 ^a	0.44 \pm 0.02 ^{ab}	1.61 \pm 0.37 ^a	33.90 \pm 2.55 ^a	17.39 \pm 0.72 ^d
Standard control	176.15 \pm 8.75 ^a	30.20 \pm 1.40 ^a	12.17 \pm 0.47 ^a	0.43 \pm 0.01 ^{ab}	4.99 \pm 0.28 ^a	41.13 \pm 1.56 ^b	2.45 \pm 0.43 ^a
TP + 200 mg/kg of CFAAML	198.96 \pm 11.32 ^b	42.90 \pm 2.06 ^b	11.03 \pm 0.85 ^a	0.43 \pm 0.02 ^{ab}	6.90 \pm 0.15 ^a	50.27 \pm 3.02 ^c	9.30 \pm 0.89 ^c
TP + 600 mg/kg of CFAAML	200.81 \pm 7.59 ^b	34.10 \pm 2.09 ^a	13.00 \pm 0.57 ^a	0.47 \pm 0.01 ^b	6.95 \pm 0.30 ^a	85.83 \pm 3.57 ^d	6.80 \pm 0.50 ^b

CFAAML, combined ethanol extract of *F. africana* and *A. mauritianum* leaves.

Values are presented as mean \pm standard deviation ($n = 6$) and values with different superscripts are statistically significant at $P < 0.05$.

treated 200 and 600 mg/kg/d of combined ethanol extract of *F. africana* and *A. mauritianum* leaves when compared with BPH control. However, the serum Fe levels in all the extract-treated BPH induced rats were significantly ($P < 0.05$) higher than the standard control treated with finasteride (Table 1).

In Table 1, it was observed that the BPH control, standard control treated with finasteride and BPH induced rats treated with 200 mg/kg/d of combined ethanol extract of *F. africana* and *A. mauritianum* leaves showed significant ($P < 0.05$) decreases in the serum calcium (Ca) level when compared with normal control. All the BPH induced rats treated with 200 and 600 mg/kg/d of combined ethanol extract of *F. africana* and *A. mauritianum* leaves showed significant ($P < 0.05$) increase in the serum Ca level relative to the BPH control, unlike the standard control that showed no significant ($P > 0.05$) decrease.

The serum magnesium (Mg) levels in the BPH induced rats treated with combined ethanol extract of *F. africana* and *A. mauritianum* leaves showed no significant ($P > 0.05$) increases in the BPH control and standard control when compared with the normal control. Similarly, the BPH induced rats treated with 600 mg/kg/d of combined ethanol extract of *F. africana* and *A. mauritianum* leaves when compared with the normal control. However, the BPH induced rats treated with 200 mg/kg/d of combined ethanol extract of *F. africana* and *A. mauritianum* leaves showed no significant ($P > 0.05$) decrease in the serum level relative to the normal control (Table 1).

The serum copper (Cu) levels in Table 1 show the BPH induced rats had no significant ($P > 0.05$) increases in the serum Cu levels in the BPH control, standard control and BPH induced rats treated with 200 mg/kg/d of combined ethanol extract of *F. africana* and *A. mauritianum* leaves when the normal control. The BPH induced rats treated with 600 mg/kg/d of combined ethanol extract of *F. africana* and *A. mauritianum* leaves showed significant ($P < 0.05$) in the serum Cu level relative to the normal control.

The serum selenium (Sn) level in the BPH induced rats showed that there were significant ($P < 0.05$) decreases in the serum Sn levels in the BPH control and standard control relative to the normal control. Also, the BPH induced rats treated with 200 and 600 mg/kg/d of the combined ethanol extract of *F. africana* and *A. mauritianum* leaves when compared with the normal control. However, the standard control and BPH induced rats treated with graded doses of combined ethanol extract of *F. africana* and *A. mauritianum* leaves respectively showed no significant ($P > 0.05$) increases in the serum Sn levels when compared with the BPH control.

The serum acid phosphatase activities of the BPH induced rats in Figure 1 indicated significant ($P < 0.05$) increases in the acid phosphatase activities of the BPH control, and the BPH induced rats treated with the combined ethanol extract of *F. africana* and *A. mauritianum* leaves, respectively relative to the normal control. However, the standard control induced BPH and treated with 5 mg/kg/d of finasteride showed no significant ($P > 0.05$) increase in the acid phosphatase activity when compared with the normal control. The serum acid phosphatase activities in the standard control and all the extract-treated BPH induced rats showed significant ($P < 0.05$) reductions in the serum acid phosphatase activities relative to the BPH control.

The PSA concentrations in the BPH induced in Figure 2 showed that the PSA level of the BPH control, standard control, and BPH induced rats treated with 200 and 600 mg/kg/d of the combined ethanol extract of *F. africana* and *A. mauritianum* leaves respectively significantly ($P < 0.05$) increased relative to the normal control. It was also observed that there was significant ($P < 0.05$) decrease in the PSA level of the standard control and the BPH induced rats treated with 200 and 600 mg/kg/d of the combined ethanol extract when compared with the BPH control.

The section of prostate gland from the normal control rats showed the normal histomorphology of the rodent

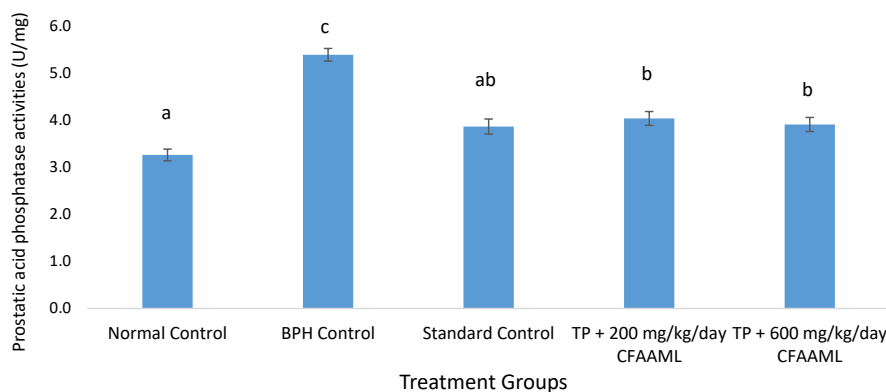


Figure 1. Acid phosphatase activities of BPH induced rats treated with combined ethanol extract of *F. africana* and *A. mauritianum* leaves (CFAAML). Each bar represent mean \pm standard deviation ($n = 6$). Bars with different superscripts are significantly different ($P < 0.05$). TP = testosterone propionate.

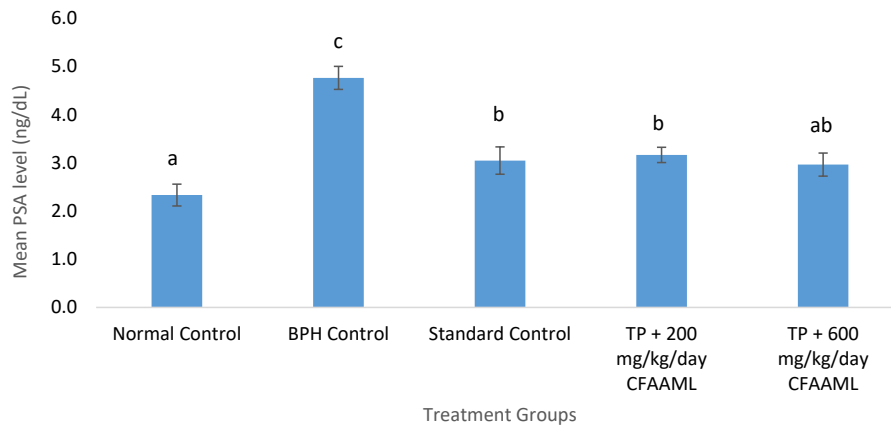


Figure 2. Prostate-specific antigen concentrations of BPH induced rats treated with combined ethanol extract of *F. africana* and *A. mauritianum* leaves (CFAAML).

prostate gland (Figure 3A). The prostate section showed mostly normal prostatic acini (A) of varying sizes, lined by simple cuboidal to low columnar epithelial cells with basally located heterochromatic nuclei surrounded by thin fibromuscular stroma (S). The acini contained varying amounts of acidophilic intra-luminal secretions and a few epithelial infoldings (arrow) were observed.

The prostate gland from the BPH (BPH control) induced untreated showed histomorphological alterations consistent with benign prostate hyperplasia (Figure 3B). There was an obvious increase in height of the mostly columnar epithelia with marked multiple infolding (black arrow) and sloughing (white arrow) of the acinar epithelium. Focal areas of intraepithelial stratification with preserved basement membrane and atypical cells with loss of polarity and euchromatic nuclei were frequently observed (red arrowhead). The fibromuscular stroma surrounding the prostatic acini were variably thickened (blue arrow).

Sections of the prostate gland from the standard control rats induced BPH and treated with finasteride indicated a heterogenous histomorphology with normal acini co-localizing with altered ones (Figure 3C). Multiple acini with epithelial infolding as well as multiple foci of intraepithelial stratification were observed. However, these alterations were most evident on the peripheral acini while the more central acini appeared normal. Normal acini containing acidophilic secretions (A); Intraepithelial stratification (arrow).

The prostate gland obtained from the benign prostatic induced rats treated with 200 mg/kg/d of combined ethanol extract of *F. africana* and *A. mauritianum* leaves (CFAAML) indicated an almost homogenous histomorphological alteration, consistent with benign prostatic hypoplasia (Figure 3D). It showed a few relatively normal central acini and obvious increase in the number and height of acinar epithelial cells, with numerous infolding of the epithelium (black arrow), intraepithelial stratifications (white arrow) and formation

cribriform growths in some of the acini (red arrow). Variable thickening of the fibromuscular stroma (S) was also observed.

The sections of the prostate gland from benign prostatic induced rats treated with 600 mg/kg/d of CFAAML showed an almost homogenous histomorphological alteration, consistent with benign prostatic hypoplasia (Figure 3E). There was an obvious increase in the number and height of acinar epithelial cells, with numerous infolding of the epithelium and intraepithelial stratifications (white arrow).

Discussion

This study investigated the effects of CFAAML on the prostate biomarkers and serum mineral levels in prostatic hyperplasia (BPH) induced rats. BPH occurs in ageing males because of accelerated differentiation and proliferation of prostate epithelial and stromal tissues due to increased stimulatory effects of dihydrotestosterone generated from the metabolism of excess testosterone in the prostate gland while the small concentration of it can be found men with healthy prostate (20). In this study, testosterone propionate injection was used for the induction of BPH by the subcutaneous injection of 5 mg/kg/d of it for 28 days.

The reductions in the serum zinc levels in the BPH control rats can be attributed to the effects of induction of BPH with testosterone propionate on serum zinc level and suggests that reductions in the serum zinc levels could increase the development and progression of BPH which could adversely impair the health outcome of BPH patients. The BPH induced rats treated with 200 and 600 mg/kg/d of the combine ethanol extract of *F. africana* and *A. mauritianum* leaves caused significant elevation of serum zinc levels which indicated that the combined extract effectively reversed the adverse effects of BPH on the serum zinc level. It showed that the combined ethanol extract could be rich in zinc content that replenished the inadequate zinc level in the BPH control and may prevent

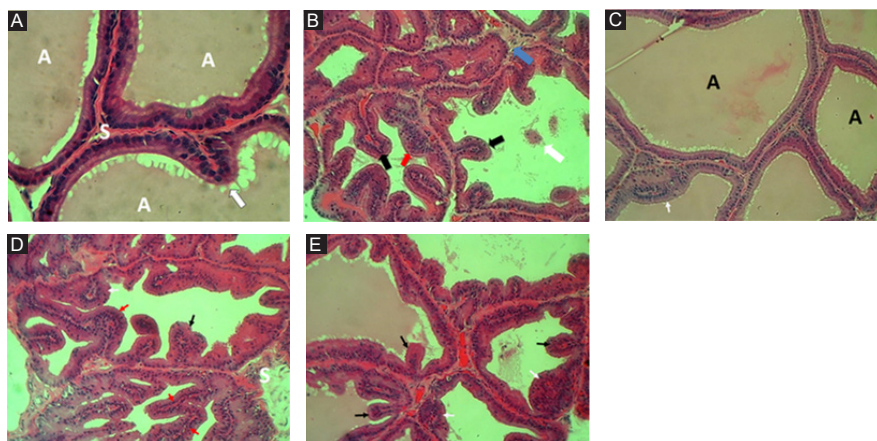


Figure 3. (A) Histomorphology of prostate gland from the normal control rats that were not induced benign prostatic hyperplasia. (B) Histomorphology of prostate gland from the benign prostatic hyperplasia induced untreated rats (BPH control). (C) Histomorphology of prostate gland from the benign prostatic hyperplasia induced rats treated with 5 mg/kg/d of finasteride (standard drug). (D) Histomorphology of prostate glands from benign prostatic hyperplasia induced rats treated with 200 mg/kg/d of CFAAML. (E) Histomorphology of prostate gland from the benign prostatic hyperplasia induced rats treated with 600 mg/kg/d of CFAAML.

zinc deficiency in BPH patients treated with the extract.

The significant reductions in the serum calcium level in the BPH induced rats indicated that the rats suffered impaired intestinal and kidney calcium reabsorption which could induce increased neuromuscular excitation in the patients with BPH. It has been reported that kidney failure, vitamin deficiency, low magnesium level and decreased thyroid functions are the major causes of low serum calcium level. The serum phosphorus level regulates the concentration of serum calcium. A reduction in the serum phosphate level will lead to an increase in the serum calcium level and vice versa (21). Low serum level can be manifest impaired blood coagulation, intestinal malabsorption, and defective bone mineralization and in extreme case may lead to convulsive seizures, and cardiovascular disorders (21,22). The increase in the serum calcium level in the BPH induced rats treated with the combined ethanol extract of *F. africana* and *A. mauritianum* leaves suggests that the extract contains a high level of calcium that replenished serum lost due to poor intestinal and kidney reabsorption of calcium. The extract could also have increased the ability of the proximal kidney tubule to reabsorb calcium and decreased its loss via urinary excretion and this could be responsible for the high serum calcium level in the BPH induced rats treated with a lower dose of the combined extract and prevents the rats from any possible adverse effects of calcium deficiency.

The serum magnesium level is primarily regulated by its rate of excretion in the urine by the kidneys and when there is a failure of the proximal tubule to reabsorb enough magnesium low serum level results (23). Low blood magnesium level could cause loss of appetite, nausea, vomiting, fatigue and weakness, and the condition progresses, the patient may experience tremor, depression, numbness, tingling, muscle contractions, cramps, and seizures (24). The no significant differences

observed in the serum magnesium levels in the BPH induced rats treated with combined ethanol extract of *F. africana* and *A. mauritianum* leaves relative to the normal and positive controls, respectively showed that the combined extract and BPH have no adverse effects on the serum magnesium levels. The combined extract may not contain a sufficient amount of magnesium that could have significantly elevated the serum magnesium level in the BPH induced rats and thus has similar effects on serum magnesium level like finasteride. The normal serum magnesium level observed in the BPH induced rats would ensure that the functions of Mg which is to serve as a cofactor for some enzymes involved in carbohydrate breakdown and protein synthesis including regulation of blood pressure, transport of potassium and calcium across cell membrane and maintenance of glutathione structure are effectively carried out (25).

The elevated serum copper level in the BPH control and BPH induced rats treated with finasteride and combined ethanol extract of *F. africana* and *A. mauritianum* leaves respectively could be one of the defence mechanisms adopted by the body to withstand the adverse effects of BPH in the rats. Copper together with zinc and glutathione containing antioxidant enzymes including superoxide dismutase and catalase play key catalytic roles in the conversion of reactive free radicals such as reactive oxygen species (ROS) and hydrogen peroxide (H_2O_2) into harmless products in the body (26,27). It has been reported that the increase in the estrogen level like estradiol implicated in the pathogenesis of BPH and also increases the serum and plasma copper level besides dietary intake of food rich in copper. The elevated serum copper level in the BPH induced treated with a high dose of the combined ethanol extract suggest that the extract contributed partly to the increased copper level in the rats.

Selenium is a trace element and a component of most antioxidant enzymes including glutathione peroxidase

that ameliorate oxidative stress via scavenging of free radicals such as reactive oxygen species and hydrogen peroxide thereby detoxifying them into harmless products and prevents lipid peroxidation and oxidative damage to critical biomolecules (28,29). The significantly reduced serum selenium level in the BPH control relative to the normal control suggests that lower serum selenium level promotes development and progression of BPH. The elevated serum selenium concentrations in the BPH induced rats treated with finasteride and combined ethanol extract of *F. africana* and *A. mauritianum* leaves, respectively relative to the BPH control are indicative that selenium plays a key therapeutic role in ameliorating the adverse effects of BPH. The selenium could have exerted its therapeutic effect by stimulating glutathione and antioxidant enzymes activities to reduce oxidative stress which is in agreement with findings of Mohamed *et al*, that increased antioxidant activities reduce progression and health burdens of BPH (30).

Iron (Fe) is a key element required in small quantity in the body for the synthesis of haemoglobin needed for transportation of oxygen in the body and diminished blood level could lead to anaemia while its excess blood level could trigger iron toxicity and induction of oxidative stress due to Fenton reactions (31). Moreover, poor nutrition, parasitic infections like malaria that destroy red blood cells, and inflammatory diseases such as BPH could lead to iron deficiency (32). The significantly reduced serum iron level in the BPH control relative to the normal control indicated that the BPH induced rats suffered a loss of iron which could have impaired haemoglobin synthesis and transport of oxygen in the body. The decrease in the serum iron level would have had additional adverse health effects on the BPH induced rats and thus suggests that BPH patients should receive iron supplementation to prevent iron deficiency and improve their quality of life. However, the significantly elevated serum iron levels in the BPH induced rats treated with finasteride and the combined ethanol extract, respectively showed that maintenance of normal serum iron level is critical to slowing the growth and proliferation of prostate epithelial and stromal cells involved in BPH progression. The combined ethanol extract could have prevented the loss of iron possibly from destruction red blood cells. The extract may be highly rich in iron content that replenished the lost iron as demonstrated by its dose-dependent effects on the BPH induced rats. Besides the therapeutic effects of the combined ethanol extract of *F. africana* and *A. mauritianum* leaves, its excessive consumption should be avoided to prevent iron overdose and its attendant adverse health effects(32-34).

The significantly elevated serum level of inorganic phosphate (Pi) in the BPH control could be attributed to the effect of the BPH on the kidney functions that decreased its ability to excrete sufficient amount of phosphate from the body. The kidney proximal tubule in

the BPH control could have reabsorbed a large amount of phosphate which reduced the serum calcium level the BPH induced rats in this study in line with findings of Slatopolsky (33). However, the significantly reduced serum phosphate levels in the BPH induced rats treated with the combined ethanol extract of *F. africana* and *A. mauritianum* leaves relative to the BPH control showed that the combined extract possesses lower regulatory effects on the serum phosphate level as it could protect the BPH induced rats from hyperphosphatemia. Finasteride had better effects in regulating the serum phosphate level as seen in the standard control than the combined extract as it could have maintained a normal level of circulating parathyroid hormones implicated in hyperphosphatemia in addition to the stimulation of the kidney to excrete enough phosphate from the body (30-34).

The significantly elevated serum prostatic acid phosphatase activities in the BPH control and BPH induced rats treated with combined ethanol extract of *F. africana* and *A. mauritianum* leaves indicated prostate hyperplasia that stimulated increased secretion of prostatic acid phosphatase into the blood and this agrees with the findings of Mohamed *et al* (30). The combined ethanol extract of *F. africana* and *A. mauritianum* leaves treated BPH induced rats showed dose-dependent reductions in the prostatic acid phosphatase activities relative to the BPH control and could be attributed to the therapeutic effects of the combined ethanol extract on BPH which prevented increased growth of prostate cells and secretion of prostatic acid phosphatase. The reductions in the prostatic acid phosphatase activities in the combined ethanol extract of *F. africana* and *A. mauritianum* leaves treated BPH induced rats is in agreement with the findings of Ejike and Ezeanyika; and Ezugwu *et al*, that reductions in prostate volume significantly reduces prostatic acid phosphatase secretion and activities and indicated recovery from BPH (4,34).

The PSA is generally considered a reliably selective marker of BPH and prostate cancer as the expression of PSA significantly increases under this conditions and besides PSA could increase under high prostate activity (35). It is also known as gamma-seminoprotein which is secreted by the prostate epithelial cells and its increases in the serum concentration and activities in BPH is due to the increased number of prostate epithelial cells that increases its secretions. The significantly elevated PSA level in the BPH control relative to the normal control indicated an increased number of prostate epithelial cells and prostate volume due to effects of testosterone propionate injection and resultant increased PSA secretions by the epithelial cells. The significant reductions in the PSA level of the BPH induced rats treated with the combined ethanol extract of *F. africana* and *A. mauritianum* leaves relative to the BPH control could be attributed to the anti-BPH activities of the combined extract that caused the reductions in the number of the prostate epithelial cells and decreased the

PSA secretions. The combined extract lowered the serum PSA level in a dose-dependent manner and suggested that a high dose of the combined ethanol extract of *F. africana* and *A. mauritianum* leaves possess therapeutic effects than a lower dose of it. This is in agreement with findings of Iweala and Ogidigbo that plant extract that reduces PSA level and improve prostate histomorphology of BPH induced rats possesses anti-BPH activity that prevents the development and progression of BPH (5).

The prostate histomorphology of the BPH control and BPH induced rats treated with the combined ethanol extract of *F. africana* and *A. mauritianum* leaves were consistent with the serum mineral levels, serum prostatic acid phosphatase activities and serum PSA level obtained in this study. The BPH induced rats treated with combined ethanol extract had significantly reduced number and height of acinar epithelial cells and improved prostate histomorphology relative to the BPH control which suggests therapeutic effects of the combined ethanol extract against BPH which is line with findings of Iweala and Ogidigbo (5).

Conclusion

The findings of this study indicated that the combined ethanol extract of *F. africana* and *A. mauritianum* leaves restores normal serum prostatic acid phosphatase activities and serum PSA levels and improves prostate histomorphology of BPH induced rat. Thus, the combined ethanol extract of *F. africana* and *A. mauritianum* leaves possesses anti-BPH activity and further research should be conducted on it to identify its bioactive constituents and mechanism of action.

Authors' contribution

URI, AFA and ESI were the principal investigators of the study. URI, AFA, ESI, ACL and AP were included in preparing the concept and design. NCP, URI, AFA and ESI revised the manuscript and critically evaluated the intellectual contents. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

Conflicts of interest

The authors declared no conflict of interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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