Protective abilities of *Myrianthus arboreus* (Cecropiaceae) extracts on Testosterone-Induced Benign Prostatic Hyperplasia in Male Wistar rats

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Abstract

Benign prostatic hyperplasia (BPH) is one of the common disorders amongst the geriatric male population, affecting their quality of life. Since conventional drugs used for the treatment are sometimes accompanied by serious side effects, the search for alternative treatments remains urgent. This study aimed to evaluate the antioxidant activity and the protective effects of the aqueous and methanolic extracts of *Myrianthus arboreus* (*M. arboreus*) on a BPH model induced in male Wistar rats. The antioxidant activities of the extracts were assayed *in vitro* by free radical scavenging using DPPH and ABTS assays, total antioxidant capacity. For the *in vivo* study, rats were randomly divided into nine groups of six rats each. Groups 1 and 2 received distilled water, group 3 received finasteride (5 mg/kg), while groups 4 to 9 received 50, 100, and 200 mg/kg of *M. arboreus* respectively. The treatment lasted 28 days after which, animals were sacrificed. Relative organ weights, histopathological changes, total protein levels, and oxidative status were determined. In addition, some pro-inflammatory cytokines and prostate-specific antigen levels were measured. Both extracts of *M. arboreus* demonstrated strong radical scavenging and total antioxidant capacity *in vitro*. They counteracted the effects of testosterone by decreasing the relative weight and volume of the prostate, the prostate epithelium height, and the total protein level in the prostate. Likewise, they improved the oxidative status and reduced the concentrations of cytokines in the prostate, and the serum PSA level. *M. arboreus* extracts contain bioactive compounds with antioxidant properties which confers protective abilities against the occurrence of BPH.

Keywords: Benign prostatic hyperplasia; Myrianthus arboreus; Male rats,Antioxydant; Testosterone

Abbreviations

BPH: Benign prostatic hyperplasia; *Myrianthus arboreus*: *M. arboreus*; DHT: dihydrotestosterone; ROS: Reactive Oxygen Species; WHO: World Health Organization; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; ABTS: 2,2′-Azinobis-(3-Ethylbenzthiazolin-6-Sulfonic Acid); SOD: SuperOxyde Dismutase; GSH: Glutathione; IL: Interleukin; MDA, malonedialdehide; NHC: National Herbarium of Cameroon; IC: Inhibitory Concentration.

Introduction

Benign prostatic hyperplasia (BPH), also known as adenomyofibroma or prostatic adenoma, is a common, almost inevitable condition associated with aging in men. It corresponds to an increase in prostate volume due to excessive proliferation of stromal and epithelial cells in the transition zone of the prostate (the area of the prostate that surrounds the urethra) [1]. It can appear as early as the age of 40, and almost one man in two in the 40-60 age bracket is affected; this proportion rises to 75% in the 60-80 age bracket, and even to 90% in the over-80s [2]. This current incidence is only set to increase as a result of demographic growth and longer life expectancy. The etiology and pathogenesis of BPH are not well understood. However, several parameters, including inflammation, hormones, genetics, and dietary factors, are thought to play a role in its development [3].

Benign prostatic hyperplasia (BPH) is closely linked to androgens, in particular testosterone and its active metabolite, dihydrotestosterone (DHT). The latter is a hormone resulting from the conversion of testosterone in the prostate by an enzyme called 5-alpha reductase, and plays a key role in prostate growth and development [4]. In old age, 5-alpha reductase activity in the prostate increases, leading to a greater conversion of testosterone to DHT. DHT binds to androgen receptors in prostate cells and stimulates cell growth, leading to an increase in gland size. Thus, some medical treatments commonly used to reduce BPH symptoms target androgens, namely 5-alpha reductase inhibitors and androgen receptor antagonists which respectively block the conversion of testosterone to DHT and, the action of androgens on the prostate [5-7].

Inflammation also plays an important role in the development

and progression of benign prostatic hyperplasia (BPH), although the exact mechanisms are not fully understood. Several studies have shown that inflammatory markers such as pro-inflammatory cytokines, chemical mediators, and inflammation-specific immune cells are present in enlarged prostate tissue in men with BPH [8,4]. Thus, inflammation can disrupt the balance of hormones and growth factors involved in prostate development and regulation, which can promote the overgrowth of prostate cells and thus lead to the development of benign prostatic hyperplasia [9]. Managing inflammation may therefore be a potential therapeutic approach for the treatment of BPH.

Another important factor in the development of benign prostatic hyperplasia (BPH) is oxidative stress. Indeed, in the context of BPH, oxidative stress can result from a variety of processes, including chronic inflammation of the prostate, accumulation of toxic metabolites, hormonal imbalances, and damage caused by the free radicals themselves. Oxidative stress can also induce increased inflammation in the prostate, creating a vicious circle where inflammation and oxidative stress reinforce each other. Thus, studies have suggested a link between oxidative stress and urinary symptoms associated with BPH. When Reactive Oxygen Species (ROS) levels rise, they can damage prostate cells, leading to increased proliferation, which can contribute to benign prostatic hyperplasia. In addition, antioxidant enzymes, which are responsible for neutralizing free radicals, have been found at reduced levels in the prostate tissue of BPH patients [3,10].

The signs and symptoms of BPH appear when the enlarged prostate puts pressure on the urethra and bladder, which can lead to a reduction in urethral diameter (compression) or obstruction of the urethra, which can cause changes in urinary habits and difficulty in passing urine [2]. Thus, BPH may be accompanied by several signs (also known as lower urinary tract symptoms), including: difficulty in urinating, frequent urination (especially at night), urgent urination (urgent or sudden need to urinate), weak or slow stream of urine, intermittent stream of urine (stop and start), inability to empty the bladder (which can lead to urinary tract infections and bladder stones), difficulty in starting to urinate (straining), difficulty in controlling the bladder (incontinence), which can lead to urine leakage, blood in the urine [11, 12]. It is mainly characterized by low mortality associated with high morbidity, which affects the quality of daily and professional life [13].

The various therapeutic alternatives for the management of BPH such as watchful waiting, drug therapy, and interventional therapy, depend on the severity of symptoms, the severity of the condition, the occurrence of complications, and the patient's preference [14]. However, these conventional methods are associated with numerous side effects, so more and more BPH patients are turning to herbal medicine to treat their condition. The use of phytotherapy in the management of BPH has a long history. In developing countries, particularly in Africa, the use of traditional medicine is part of the cultural heritage. According to the WHO, nearly 80% of the population of developing countries in the African region use traditional medicine [15]. For now, two specialties based on plant extracts are recognized for the treatment of BPH: extracts of *Serenoa repens* or Florida palm in the specialties PERMIXON® 160 mg or PRODINAN® 160 mg, and extracts of *Pygeum africanum* or African plum in the speciality TADENAN® 50 mg. However, these two species represent only a tiny fraction of the options that the plant world continues to offer. Intending to the search for new natural therapeutic options, we have chosen to study *Myrianthus arboreus*, a plant used in Cameroonian folk medicine.

Myrianthus arboreus P. Beauv (M. arboreus), also known as the "indigenous breadfruit tree", is a dioecious tree of the Cecropiaceae or Urticaceae family according to the phylogenetic classification. It is a fruit tree of the genus Myrianthus that grows in the forest zone of tropical Africa. In traditional African pharmacopeia, extracts from the leaves, barks, and roots of *M. arboreus* are used to treat various disorders and diseases including dysentery, diarrhea, vomiting, wounds, infections [16], eyeache, stomach ache, anemia [17,18], malaria, toothache, dysmenorrhea, tumors as an anti-poison and even to improve the quality and quantity of breast milk [19]. However, bark infusions are administered in the treatment of diabetes; some traditional therapists have revealed that they use decoctions of the trunk for the treatment of certain male pathologies such as BPH and prostate cancer. In the safety evaluation of this plant, Awounfack *et al.* (2016), showed that after 28 days of administration to the normal Wistar male rats, the aqueous extract of *M. arboreus* leaves, by contrast, exhibited a reduction of lymphocytes, and a relative low toxicity on accessory sex organs (seminal vesicles, prostate gland), through its effects on the reduction of secretion in the lumen seminal vesicles, the formation of the secondary mucosal folds, and the atrophy of prostatic gland. In addition to its traditional usages, these findings suggest promising indications regarding the potential effects of the plant on benign prostatic hyperplasia (BPH). As a result, further assessment of the plant's effects on BPH is justified. Knowing that the amount of the phytochemical constituents in plant extracts and the biological activities of extracts depend on the polarity of the solvent used for extraction, it was therefore planned to verify the potential beneficial effects of aqueous and methanolic extracts of this plant on BPH.

Materials and Methods

Plant material

The barks of *M. arboreus* were collected in August 2021 in Dschang (West Cameroon Region) at 7 a.m and were identified at the National Herbarium of Cameroon in comparison with the reference specimen number 34045/NHC.

Experimental Animals

The animals used in this study were sexually inexperienced male WISTAR rats (10 to 12 weeks old), weighing approximately 150 g. They were raised in the animal house of the Laboratory of Animal Physiology of the University of Yaoundé I and were housed in large, well-ventilated plastic cages, with 6 animals per cage. They were kept under standard conditions (room temperature and natural day/night lighting cycle). The animals had free access to tap water and food. They were fed with food of

the following composition: 50% corn, 5% wheat bran, 14% fish, 8% peanuts, 5% bone meal, 7% palm kernel cake, 10% wheat meal, 1% premix (multivitamin complex (Olivitazol® 0.25%)). Rats were treated in accordance with the animal bioethics guidelines and procedures of the National Institutional Ethics Committee of Cameroon, which has adopted all procedures recommended by the European Union for the protection of animals used for scientific purposes (EEC Council 86/609; Reg. no. FWA-IRD 0001954).

Chemicals and kits

The chemical material consisted of Diazepam (Valium® 10 mg/2ml, Roche laboratory, Fontenay-Sous-Bois, France), Testosterone Enantate (Androtardyl® 250 mg, Bayer Pharma AG laboratory, Berlin, Germany), Finasteride (Finasteride® 5 mg, Mylan SAS, allée des parcs, Saint-Priest, France), Ethyl alcohol 95% (BDH Laboratory Reagents), Ketamine (Ketamine hypochloride 50 mg/ml, Rotex Medica, Trittau, Germany). The PSA AccuBind® Elisa Microwells kit was obtained from Monobind Inc. (Lake Forest, USA), the HCUSABIO® TNFα rat ELISA kit was provided by CUSABIO Life Sciences (Wuhan, China), and the IL-8 rat ELISA kit was provided by Kamiya Biomedical Company (Seattle, USA). The reagents used for antioxidant assays were purchased from GIBCO (Grand Island, NY, USA)

Preparation of extracts

After harvesting, the bark from *M. arboreus* trunk, it was dried in the shade, and crushed using a propeller mill. Two extraction methods at low temperature (to prevent the loss of bioactive phytochemical constituants), were used to obtain *M. arboreus* extracts:

- To obtain the aqueous extract, 1 kg of powder was boiled in 10 liters of drinking water, for 30 minutes. After cooling, the solution obtained was filtered using Wattman paper N°4. The filtrate obtained was dried in a ventilation oven at 40°C for 48 hours. With 43.6 g of crude extract, a yield of 4.4% was obtained.

- To obtain the methanolic extract, 2 kg of powder was macerated for 72 hours at room temperature in 10 liters of methanol. The resulting mixture was filtered through Wattman No. 4 paper. After filtration, the filtrate was concentrated under a vacuum with a rotary evaporator (rotavapor Heidolph, Germany) under reduced pressure at 40°C and then air-dried until complete evaporation of the solvent. This process yielded 67.6 g of crude organic extract, representing a yield of 3.4%. After driying, the extracts were stored in the refrigerator (4°C) until use.

Determination of flavonoid content

100µl of extract were added to the microplates, followed by 100µl of 2% $AlCl_3$. The blank consisted of 100µl extract and 100µl $AlCl_3$ dilution solvent. Incubation lasted 15 min and absorbance was read in a microplate reader at 415 nm. Results were expressed as milligrams of quercetin equivalent per gram of dry weight.

Determination of total phenols content

10µl extract, 25µl Ciocalteu folin solution, 25µl Na_2CO_3

solution and 140µl distilled water were added to a 96-well microplate. The blank consisted of 10µl extract plus 25µl Na_2CO_3 and 165µl distilled water. Absorbance was read in a microplate reader at 760 nm. Results were expressed as µg of gallic acid equivalent per gram of dry weight (extract).

Determination of tannins content

In a 96-well microplate, 10µl of extract were introduced, followed by 150µl of vanillin (4% in methanol). Next, 75 µl of HCl solution was added to the mixture. The mixture was incubated for 15 min in the dark and the absorbance read at 500 nm in a microplate reader. Results were expressed as milligrams of berberin equivalent per gram of dry weight.

DPPH scavenging activity

The free radical scavenging capacity of *M. arboreus* extracts was tested by the DPPH radical assay as described by Silihe et al. [20], with a few modifications. Briefly, in a 96-well microplate, 100 µl of sample was introduced and serially diluted from an initial concentration of 1.25 mg/ml. 100µl of freshly prepared DPPH solution was added to each well. The control sample consisted of 100µl methanol and 100µl DPPH methanolic solution. All samples were run three times, incubated in the dark at 25°C, and absorbance was read at 517 nm in a microplate reader (Tecan Pro 200, Tecan Trading AG, Männedorf, Switzerland) every 15 min for 1 h. Gallic acid was used as a positive control, and the percentage of DPPH radical trapping was determined using the equation below: (%) = [(Ac - At)/Ac] × 100, where Ac is the absorbance of the control and At the absorbance of the sample.

ABTS radical scavenging activity

The ABTS radical-neutralizing activity of *M. arboreus* extracts was determined according to the method described by Silihe et al. [20], with slight modifications. Briefly, in a 96-well microplate, 100µl of ABTS solution was added to 100µl of serially diluted samples from an initial concentration of 0,125 mg/ml. Samples were then shaken and incubated at room temperature in the dark, and optical density was read at 734 nm in a microplate reader (Tecan Pro 200, Tecan Trading AG, Männedorf, Switzerland) after 15, 45 and 60 minutes against a blank consisting of 100µL ethanol solution and 100µL samples. The percentage of ABTS radical trapping capacity was determined as indicated above. (%) = [(Ac - At)/Ac] × 100, where Ac is the absorbance of the control reaction and At is the absorbance in the presence of the sample.

Total antioxidant capacity (TAC)

The total antioxidant capacity of *M. arboreus* extracts was determined by the phosphomolybdenum method, as reported by Prieto et al [21]. Briefly, in eppendorf tubes, 100µl of samples were mixed with 1 ml of a solution composed of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The mixture was then incubated for 90 minutes at 95◦C in a water bath, and the optical density was read at 695 nm in a microplate reader against a blank. The operation was performed in triplicate. Results are expressed as equivalents of ascorbic acid per milligram of dry weight.

Solution preparation and doses selection

Testosterone enanthate was used to induce benign prostatic hyperplasia. It was dissolved in corn oil and was administered to rats at a dose of 3 mg/kg, a dose reported to cause prostate hyperplasia in normal rats [22]. Finasteride, a commonly used drug for the treatment of BPH, was used as the reference substance (positive control) in this study. It was suspended in distilled water and was administered to rats at a dose of 5 mg/kg, this is in reference to previous work on BPH [23-25]. Aqueous and methanol extracts of *M. arboreus* were suspended in distilled water and tested at doses of 50, 100, and 200 mg/kg, doses that were extrapolated from previous studies (data not yet published).

Experimental protocol and BPH induction

A total of 54 animals were acclimated for 7 days and then divided into 9 groups of 6 animals each: a normal and a negative (BPH) groups received distilled water; a positive control group received finasteride (5 mg/kg); 3 groups received the aqueous extract at doses of 50, 100 and 200 mg/kg; and 3 other groups received the methanol extract at doses of 50, 100 and 200 mg/kg. BPH was induced in the animals by daily subcutaneous injection of testosterone (3 mg/kg) for 28 days in all groups, except the normal control group which received the vehicle (corn oil). The treatment substances were administered orally and preceded the administration of testosterone and corn oil. All the animals were weighed once a week. At the end of the treatment, the animals were sacrificed by decapitation, after anesthesia with Diazepam (10 mg/kg) and ketamine (50 mg/kg). Arteriovenous blood was immediately collected in dry tubes and centrifuged at 3000 rpm for 15 minutes. The collected supernatant was transferred to Eppendorf tubes and stored at -20°C for biochemical assays. Androgen-dependent organs such as prostate, testes, penis, epididymis and seminal vesicles and other organs such as adrenal glands, bladder, kidney, spleen, liver, lung, heart and brain were collected and weighed. One part of the prostate was used for the determination of some biochemical parameters, while the other part and the testes were fixed in 10% formalin and stained with hematoxylin and eosin for histopathological examinations.

Relative organ weight and volume

The relative organ weight (ROW) in mg/kg of body weight (BW) was determined by the following formula: ROW (mg/kg) = organ weight (mg)/ body weight (kg). The size of the prostate was measured with a 1 mm precision caliper (IGAGING®) and the volume was calculated using the following formula: Prostate volume = $1/2$ $(a \times b^2)$ where a and b refer to longer and shorter dimensions, respectively) [26].

Biochemical analysis

Determination of total protein in the prostate: Total protein levels, a cell growth index, were measured according to the modified assay method described by Gornall *et al.* (1949) [27]. A portion of each collected prostate was ground in a ceramic mortar and homogenized in sodium phosphate buffer (0.1 M; pH 7.5) to obtain a 20% homogenate. The homogenates were centrifuged at 3000 rpm for 15 minutes and the supernatant was collected and stored at -20°C until use.

Measurement of oxidative stress markers

Determination of Superoxide Dismutase Activity: Superoxide dismutase (SOD) activity was determined according to the method of Misra and Fridovish (1972) [28]. Into the test tubes were introduced 134μL of homogenate supernatant and into the blank tube 134μL of carbonate buffer (0.05 M; pH 10.2). Then 1666μL of carbonate buffer (0.05 M; pH 10.2) was introduced into the test tubes and the blank tube. The reaction was initiated by adding 200μL of epinephrine (0.3 mM) to each tube. SOD content was measured using a spectrophotometer at 480 nm. The absorbance of the test tubes was measured at 20 and 80 seconds against the blank.

Measurement of Catalase activity

Catalase activity was estimated according to the method of Sinha (1972) [29]. Into the test tubes were introduced 50 μL of homogenate (for the sample tubes) and 50μL of distilled water (for the blank tube). Then, 750μL of phosphate buffer (0.1 mM; pH 7.5) was added to it and the timer was started after the addition of 200μL of hydrogen peroxide (50 mM). After 1 min, the reaction was stopped by adding 2 mL of potassium dichromate/acetic acid solution. Each tube was heated at 100°C for 10 minutes. After cooling, the absorbance was read with a spectrophotometer at 570 nm against the blank.

Determination of Reduced Glutathione

The level of glutathione (GSH) was determined by the Ellman method [30]. Into the test tubes, 100μL of homogenates (sample tubes) or 100μL of Tris-HCl buffer (50 mM; pH = 7.4) (control tube) were previously introduced. Then 1500μL of Ellman's reagent was added. After vortexing, the tubes were left for 60 min at room temperature. The absorbance of each tube was then read against the blank at 412 nm with a spectrophotometer.

Estimation of Malondialdehyde level

Malondialdehyde (MDA), an index of lipid peroxidation, was determined according to the method of Wilbur *et al.* (1949) [31]. Into tubes containing 250μL of homogenate (sample tubes) or 250μL of Tris-HCl buffer (50 mM; pH=7.4) (blank tube), 125μL of 20% trichloroacetic acid (TCA) and 250μL of 0.67% thiobarbituric acid (TBA) were introduced. The tubes were capped with glass beads, heated to 90°C in a water bath for 10 minutes, cooled with tap water, and then centrifuged at 3000 rpm at room temperature for 15 minutes. The supernatant was collected and the absorbance was read with a spectrophotometer against the blank at 530 nm.

Determination of Nitrites

Prostate nitrite content was measured according to the method described by Slack (1987) [32]. In each tube was introduced in order, 100μL of homogenate (sample tubes) or 100μL of distilled water (blank tube) and 500μL of GRIESS reagent. After homogenization of the mixture and incubation for 5 to 10 min at room temperature, protected from light, the optical density of each tube was read with a spectrophotometer at 570 nm against the blank.

5Measurement of interleukin-8 (IL-8) and tumor necrosis factor-α (TNF-α) levels.

IL-8 and TNF-α (markers of inflammation) levels were determined in prostate homogenates using enzyme-linked immunosorbent assay (ELISA) kits. All procedures were performed according to the manufacturer's recommendations. The results obtained were expressed as pg/ml.

Determination of serum PSA level

The prostate-specific antigen (PSA) level was measured in the serum samples using a commercial ELISA kit (AccuBind® Elisa Microwells), following the manufacturer's instructions.

Histological analysis

Prostate samples fixed in 10% formalin were dehydrated with a series of alcohol baths of increasing concentration, embedded in paraffin wax, and then cut into 5-μm-thick slices using a microtome. Each slice was fixed on a gelatin-coated slide and then stained with hematoxylin and eosin (H&E). Microphotographs of the tissues were captured using an Axioskop 40 microscope supplied by ZEISS (Hallbermoos, Germany), equipped with an NXM-EP500 CMOS digital camera connected to a Toshiba computer.

Statistical analysis

The results of this experiment are expressed as means ± standard error on the mean (SEM). Comparison between groups was performed using Student's t-test, between the normal and negative control groups. The parametric One Way ANOVA test followed by Dunnett's post hoc test was used for differences between the negative control group and treatment groups (finasteride and *M. arboreus* extract groups). Statistical analyses were performed using GraphPad Prism version 7.0 software (GraphPad Software, Inc, La Jolla, CA, USA). Differences between groups were considered statistically significant at $p < 0.05$.

Results

Content of some secondary metabolites

Table 1 below gives an estimate of the contents of some classes of secondary metabolites present in the aqueous and methanolic extracts of *M. arboreus*. According to this table, total phenols are the most abundant secondary metabolites in both extracts, with contents of 127186.67 ± 310.29 and 121373.29 ± 20.17μg of GAE/g of dry extract weight (respectively for aqueous and methanolic extracts), followed by tannins (63.43 ± 4.94 mg of BE/g of dry weight for the aqueous extract and 18.81 ± 7.41 mg of BE/g of dry weight for methanolic extract). Flavonoids were the least abundant class in both extracts (18.64 ± 2.05 mg of QE/g of dry weight for aqueous extract and 13.75 ± 1.17 mg of QE/g of dry weight for methanolic extract).

Total antioxidant capacity (TAC)

According to the results depicted in table 2 below, the aqueous and methanolic extracts of *M. arboreus* showed total antioxidant activity of 871.55 ± 17.62 and 1072.79 ± 9.58μg AAE/mg MS, respectively.

Table 1: Content of some compounds present in *M. arboreus*.

Phytochemical classes	Contents	
	M. Arboreus aqueous extract	*M. Arboreus* methanolic extract
Flavonoids (mg QE/g DW)	18.64 ± 2.05	13.75 ± 1.17
Total phenols (μg GAE/g DW)	127186.67 ± 310 .29	121373.29 ± 20.17
Tanins (mg BE/g DW)	63.43 ± 4.94	18.81 ± 7.41

mg QE/g DW : milligrams of quercetin equivalent per gram of dry weight ; μg GAE/g DW: μg of gallic acid equivalent per gram of dry weight; mg BE/g DW : milligrams of berberine equivalent per gram of dry weight

DPPH trapping activity

According to the results below, the aqueous and methanolic extracts of *M. arboreus* exhibited DPPH scavenging activity that was not influenced by time, but was concentration-dependent (Figure 1). Percentages inhibition of free radicals obtained with both extracts increased significantly up to nearly 100% at 0,625 mg/ml and was stable still at the highest concentration (1.25 mg/ml) for the aqueous extract. IC50 values representing the 50% scavenging of radicals were calculated and ranged from 0.16 to 0.18 mg/mL for the aqueous extract (Figure 1a). With the methanolic extract, the percentage inhibition did not reach any stabilization point at different concentrations after the 1h experiment but rather, kept increasing at different concentration but was similarly the same at a different time in the stated concentration. The highest percentage inhibition with this extract was about 85% with IC50 ranging from 0.009 to 0.03 mg/mL (Figure 1b). These activities are however significantly lower than those of the standard (gallic acid), whose half-maximal inhibitory concentration (IC50) values range from 1.67 to 1.74μg/mL.

ABTS Scavenging Activity

Aqueous and methanolic extracts of *M. arboreus* showed concentration-dependent ABTS free radical scavenging activities (Figure 2). In the case of the aqueous extract, these activities increased with incubation time, peaking at the highest concentration tested, with 100% trapping. For the methanolic extract, free radical scavenging activities were independent of time up to 0.03 mg/mL and peaked at the highest concentration, where over 100% scavenging was observed. IC50 values confirmed these activities, ranging from 0.34 to 0.80 mg equivalence Trolox per dry weight (DW) for the aqueous extract and from 0.89 to 1.37 mg equivalence Trolox per dry

Table 2: Total antioxidant capacity of *M. arboreus* extracts.

EXTRACTS	TAC (μg AAE/mg DW
M. Arboreus aqueous extract	871.55 ± 17.62
M. Arboreus methanolic extract	1072.79 ± 9.58**

TAC: expressed in μg AAE/mg DW: microgram of ascorbic acid equivalence per milligram dry weight. Values are the mean of three independent experiments and represent the mean ± standard deviation. ** Indicates that this value is significantly different from the other at P < 0.01.
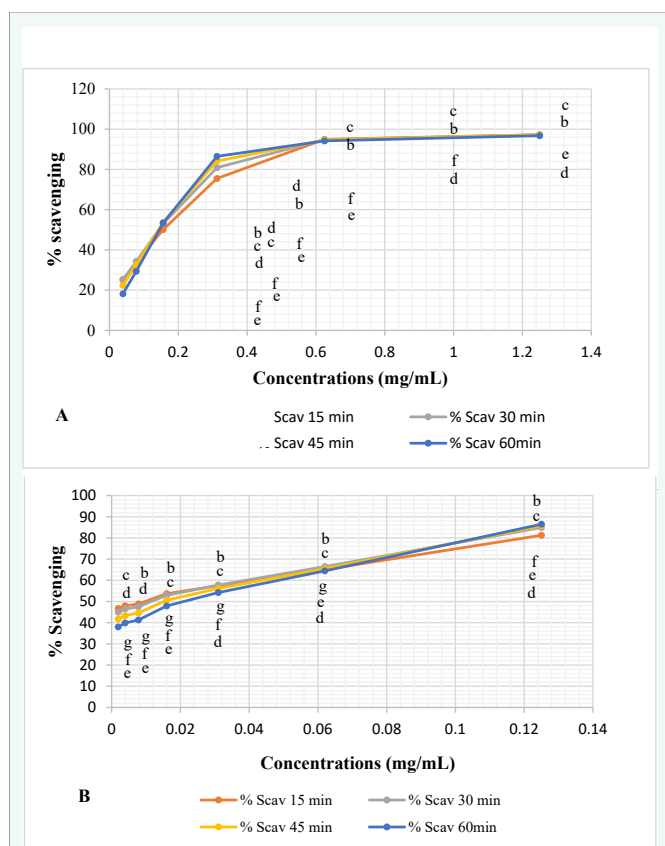
Figure 1 DPPH scavenging activity of *M. arboreus* extracts. (a): MAAq; (b): MAMe. MAAq: aqueous extract of *Myrianthus arboreus*; MAMe: methanolic extract of *Myrianthus arboreus*. Means ± SD with different superscript letters are significant at p < 0.05 using one-way ANOVA (n= 3).

weight (DW) for the methanolic extract. However, these values remain significantly lower than those for gallic acid, whose IC50 =32.33μM/Trolox DW.

Effects on body weight gain

Figure 3 illustrates the weight evolution in the percentage of the animals of the different groups during the 28 days of treatment. The results of this figure show that the *M. arboreus* extracts did not cause any significant variation in body weight in the treated animals compared to the negative control group (BPH). Similarly, no significant variation in body weight was observed in the normal group compared to the negative group.

Effects on the relative weight and volume of the prostate

Figure 4A shows the relative prostate weight of the animals after 28 days of treatment. Analysis of the results in this figure reveals that testosterone significantly (p<0.001) increased the relative prostate weights of animals in the negative control group (BPH) compared to those in the normal group, an increase of 66.87% (2588.46 ± 129.14 mg/kg BW in the BPH control versus 857.55 ±105.42 mg/kg BW in the normal control). There is a

statistically significant difference between treated groups as determined by one-way ANOVA (F = 7.31; p < 0.001). Dunnett Post Hoc multiple comparisons showed that after 28 days of treatment, finasteride significantly (p < 0.001) decreased relative prostate weight by 42.80% (1480.51 ± 177.77 mg/kg BW in the positive control versus 2588.46 ± 129.14 mg/kg BW in the BPH control). The same effect was observed with *M. arboreus* extracts at the dose of 100 mg/kg BW, which induced a significant decrease (p < 0.05) of 20.25% for the aqueous extract (2064.20 ± 132.83 mg/kg BW) and 20.15% for the methanolic extract (2066.81 ± 75.99 mg/kg BW) compared to the negative control (BPH).

The results in (Figures 4B, 5) show that testosterone induced a significant (p < 0.001) increase in prostate volume after 28 days of induction in the negative control group compared with the normal control group. This increase was about 74.86% (556.63 ± 72.54 cm^3 in the BPH control group versus 139.96 ± 24.92 cm^3 in the normal control group). The result of the prostate volume indicates that there is a statistically significant difference between treated groups as determined by one-way ANOVA (F = 4.69; p < 0.001). Based on multiple comparisons test (Dunnett Post Hoc), Finasteride at a dose of 5 mg/kg BW/D induced a significant (p < 0.001) decrease of 53.93% in this parameter compared with the negative control group (256.47 ± 46.54 cm^3 for the positive control versus 556.63 ± 72.54 cm^3 for the BPH control). Both extracts of *M. arboreus* caused a significant decrease (p < 0.05, p < 0.01, p < 0.001) in this parameter at all tested doses compared to the BPH group, except the dose 50 mg/kg of the aqueous extract.
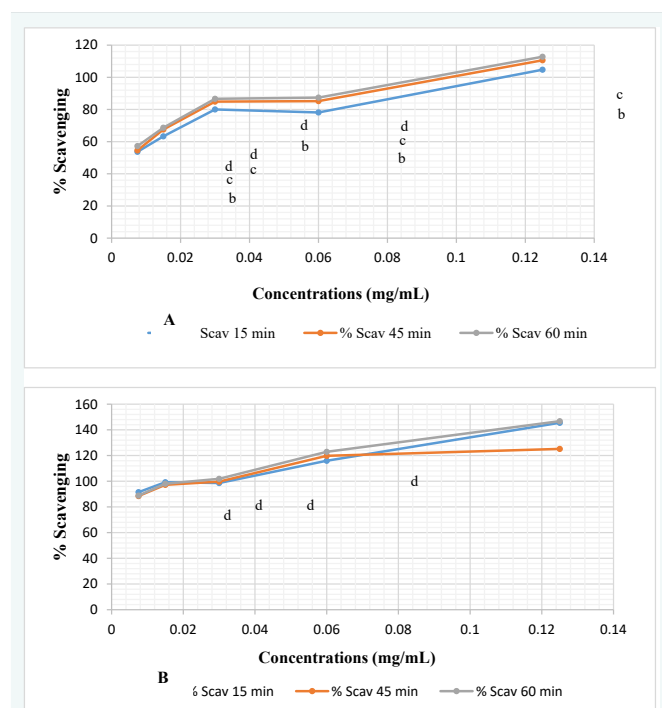

Figure 2 ABTS scavenging activity of *M. arboreus* extracts. (a): MAAq; (b): MAMe. MAAq: aqueous extract of *Myrianthus arboreus*; MAMe: methanolic extract of *Myrianthus arboreus*. Means ± SD with different superscript letters are significant at p < 0.05 using one-way ANOVA (n= 3).
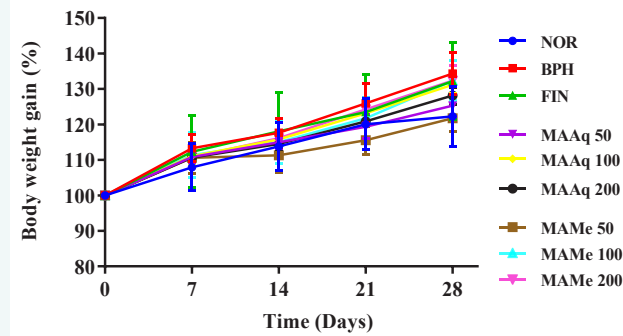
Figure 3 Graphical representations of the effects of aqueous and methanolic extracts of *Myrianthus arboreus* on weight changes of animals after 28 days of treatment.

NOR = normal control animals treated with corn oil and distilled water; BPH = negative control animals treated with testosterone enanthate (3 mg/kg BW/d) and distilled water; FIN = positive control animals treated with testosterone enanthate (3 mg/kg BW/d) and finasteride (5 mg/kg BW/d) ; MAAq = animals treated with testosterone enanthate (3 mg/kg BW/D) and aqueous extract of *Myrianthus arboreus* at 50, 100 and 200 mg/kg BW/d; MAMe = animals treated with testosterone enanthate (3 mg/kg BW/d) and methanolic extract of *Myrianthus arboreus* at 50, 100 and 200 mg/kg BW/d. Each graph represents the mean ± SEM (n =6).
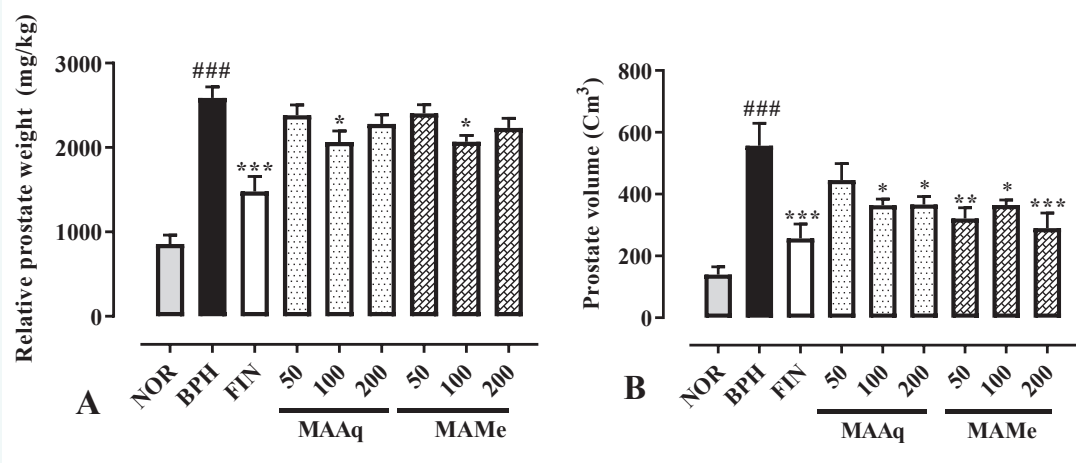

Figure 4 Graphical representations of the effects of aqueous and methanolic extracts of *Myrianthus arboreus* on relative prostate weight (A) and volume (B) after 28 days of treatment.

NOR = normal control animals treated with corn oil and distilled water; BPH = negative control animals treated with testosterone enanthate (3 mg/ kg BW/d) and distilled water; FIN = positive control animals treated with testosterone enanthate (3 mg/kg BW/d) and finasteride (5 mg/kg BW/d) ; MAAq = animals treated with testosterone enanthate (3 mg/kg BW/D) and aqueous extract of *Myrianthus arboreus* at 50, 100 and 200 mg/ kg BW/d; MAMe = animals treated with testosterone enanthate (3 mg/kg BW/d) and methanolic extract of *Myrianthus arboreus* at 50, 100 and 200 mg/kg BW/d. Each graph represents the mean ± SEM (n =6). ### $p < 0.001$: Significance compared with the NOR group. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$: significance compared to the BPH group.

The decrease was respectively 26.97%, 25.93% for the aqueous extract and 42.37%, 26.70%, 47.88% for the 100 and 200 mg/kg methanolic extract doses.

Effects on total prostate protein levels and prostate epithelial size

The results in Figure 6A show that administration of testosterone for 28 days resulted in a significant ($p < 0.05$) increase in total protein levels in the BPH group (0.22 ± 0.01µg/mL), in the range of 25.80%, compared to the normal group

(0.17 ± 0.02 µg/mL). One-way ANOVA revealed that there is a statistically significant difference between groups (F = 4.55; p = 0.001) on the prostate total protein. Multiple comparisons test (Dunnett Post Hoc), showed that Finasteride, as well as *M. arboreus* extracts at all doses, significantly ($p < 0.01$; $p < 0.001$) reduced this rate compared to the negative control group (BPH). This decrease was 34.26% for the FIN group, 34.03%, 32.67%, and 33.25% for the aqueous extract, and 28.87%, 32.67%, and 37.57% for the methanolic extract at 50, 100, and 200 mg/kg, respectively.
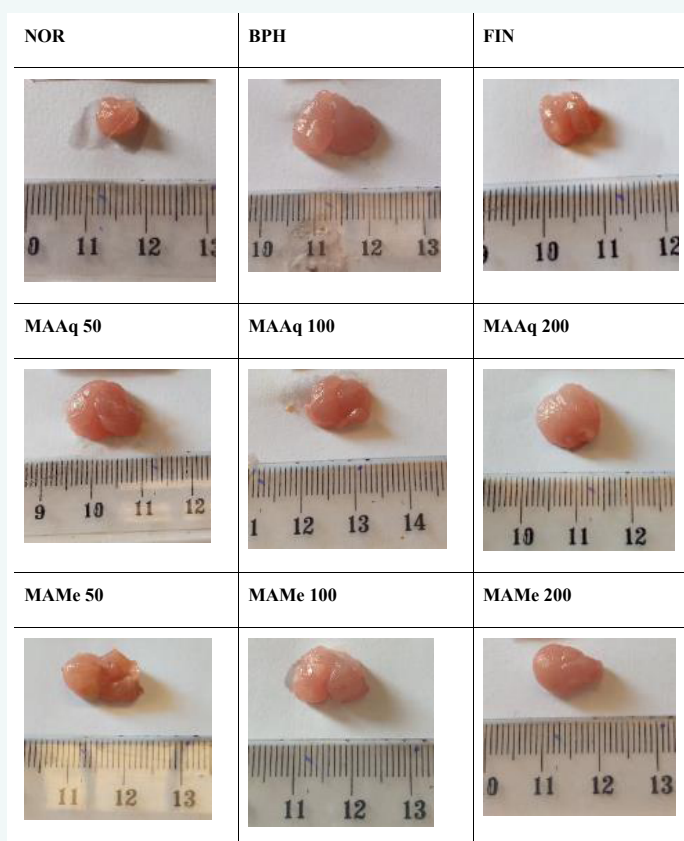
Figure 5 Photographs showing the effects of aqueous and methanolic extracts of *Myrianthus arboreus* on the prostate of animals after 28 days of treatment.

NOR = normal control animals treated with corn oil and distilled water; BPH = negative control animals treated with testosterone enanthate (3 mg/ kg BW/d) and distilled water; FIN = positive control animals treated with testosterone enanthate (3 mg/kg BW/d) and finasteride (5 mg/kg BW/d) ; MAAq = animals treated with testosterone enanthate (3 mg/kg BW/D) and aqueous extract of *Myrianthus arboreus* at 50, 100 and 200 mg/ kg BW/d; MAMe = animals treated with testosterone enanthate (3 mg/kg BW/d) and methanolic extract of *Myrianthus arboreus* at 50, 100 and 200 mg/kg BW/d.

After 28 days of treatment, the rats' prostatic epithelium size is shown in Figures 6B 5. According to this result, daily administration of testosterone resulted in a significant (p<0.001) increase in the size of the prostatic epithelium in the negative group (BPH) compared to the normal group; an increase of about 38.29% (25.59 ± 0.65μm for the BPH group compared to 15.79 ± 0.65 μm for the normal group). On this parameter, one-way ANOVA indicated that there is a statistically significant difference between groups (F = 3.95; p = 0.002). Dunnett Post Hoc multiple comparisons test showed that administration of finasteride induced a significant (p < 0.001; 19.76 ± 0.89μm) decrease (22.80%) in the size of the prostatic epithelium, compared to the BPH group (25.59 ± 0.65 μm). Compared to the BPH group, aqueous and methanolic extracts of *M. arboreus* also significantly (p < 0.01; p < 0.05) reduced this parameter (respectively by 13.47%, 22.21%, and 17.77% for the aqueous extract, and by 14.02%, 15.17%, and 12.83% for the methanolic extract), at the doses of 50, 100 and 200 mg/kg respectively from each extract.

Effects on the relative mass of some organs

Table 3 summarizes the effects of 28 days of treatment on the relative mass of some organs. According to the results reported in this table, treatment of animals in the negative control group (BPH) for 28 days with testosterone resulted in a significant increase in penile mass (p < 0.01) and seminal vesicle mass (p < 0.001) and a significant decrease (p < 0.01) in testicular mass compared to animals in the normal control group. Compared to the BPH group, one-way ANOVA showed that there is a statistically significant difference between groups (F =14.7; p < 0.001), only on the mass of seminal vesicles amongst all the relative mass of all the organs presented in the table. Multiple comparisons test (Dunnett Post Hoc), revealed that compared to the negative control group (BPH), finasteride induced a significant decrease (p < 0.001), of the order of 40.91% on the relative mass of seminal vesicles, and did not induce any significant effect on the relative mass of all the other organs. Similarly, the treatment of animals with *M. arboreus* extracts did not induce any significant effect on these parameters (Figure 7).

Effects on oxidative stress markers: superoxide dismutase (SOD) and catalase activities

Figure 8A shows the SOD activity in the prostate homogenates

Table 3: Effects of aqueous and methanolic extracts of *Myrianthus arboreus* on the relative mass of some organs.

	NOR	BPH	FIN	MAAq			MAMe		
				50	100	200	50	100	200
TESTIS	13844.46 ± 506.98	11172.44 ± 516.49##	9103.51 ± 730.37	10345.17 ± 481.06	10773.57 ± 393.95	10693.56 ± 592.45	11618.50 ± 1046.97	9793.27 ± 1024.72	9847.97 ± 955.83
PENIS	1102.13 ± 60.15	1388.39 ± 60.09##	1271.32 ± 68.87	1555.57 ± 101.79	1417.98 ± 46.28	1508.14 ± 63.76	1555.49 ± 39.15	1481.28 ± 48.29	1503.79 ± 37.90
EPIDIDYMUS	4127.01 ± 133.44	4368.29 ± 188.15	3462.09 ± 392.85	4594.15 ± 239.63	4625.26 ± 174.05	4851.53 ± 308.16	5134.38 ± 216.89	4158.42 ± 424.42	4004.63 ± 461.93
SEMINAL VESICLES	4235.79 ± 722.68	10954.84 ± 453.41###	6473.28 ± 369.09***	11037.68 ± 493.07	10624.47 ± 458.60	11581.97 ± 121.21	12372.18 ± 788.92	10282.28 ± 333.01	10531.91 ± 352.97
BLADDER	427.20 ± 69.84	506.05 ± 26.80	403.95 ± 41.67	469.91 ± 36.43	489.36 ± 41.10	453.53 ± 61.08	553.88 ± 56.82	480.45 ± 50.50	678.88 ± 51.64
LIVER	28307.70 ± 1105.98	31522.70 ± 1183.95	31144.19 ± 1430.34	31349.02 ± 1366.79	29299.20 ± 1403.19	29948.08 ± 1301.59	31389.01 ± 1873.06	28336.68 ± 930.04	29671.44 ± 1059.30
KIDNEYS	5452.31 ± 258.60	5925.71 ± 212.15	5928.87 ± 331.52	6452.36 ± 147.80	5794.43 ± 102.66	6451.24 ± 115.20	5969.93 ± 163.16	5997.73 ± 171.89	5812.99 ± 196.51
LUNG	6412.03 ± 178.84	6727.58 ± 350.49	7119.85 ± 558.89	7082.59 ± 562.75	6034.04 ± 306.39	6900.72 ± 351.04	7910.65 ± 637.56	7700.47 ± 500.11	7931.29 ± 830.60
ADRENAL GLANDS	191.28 ± 13.80	219.90 ± 23.38	191.55 ± 16.25	202.28 ± 8.08	202.91 ± 10.89	209.45 ± 9.05	242.50 ± 18.331	233.62 ± 9.64	238.03 ± 11.88
SPLEEN	5660.27 ± 540.91	4777.71 ± 442.98	5767.62 ± 609.95	5497.92 ± 514.60	5100.59 ± 626.10	5145.78 ± 623.77	5386.69 ± 715.02	5093.29 ± 443.20	4789.81 ± 557.07
HEART	3336.70 ± 85.84	3466.67 ± 88.97	3491.00 ± 103.74	3395.90 ± 149.40	3336.64 ± 64.32	3344.96 ± 68.13	3518.00 ± 111.60	3391.48 ± 94.57	3427.47 ± 108.17

NOR = normal control animals treated with corn oil and distilled water; BPH = negative control animals treated with testosterone enanthate (3 mg/ kg BW/d) and distilled water; FIN = positive control animals treated with testosterone enanthate (3 mg/kg BW/d) and finasteride (5 mg/kg BW/d) ; MAAq = animals treated with testosterone enanthate (3 mg /kg BW/D) and aqueous extract of *Myrianthus arboreus* at 50, 100 and 200 mg/kg BW/d; MAMe = animals treated with testosterone enanthate (3 mg/kg BW/d) and methanolic extract of *Myrianthus arboreus* at 50, 100 and 200 mg/kg BW/d. Each graph represents the mean ± SEM (n =6). ## p < 0.01; ### p < 0.001: significance compared with NOR group. *** p < 0.001: significance compared with the BPH group.
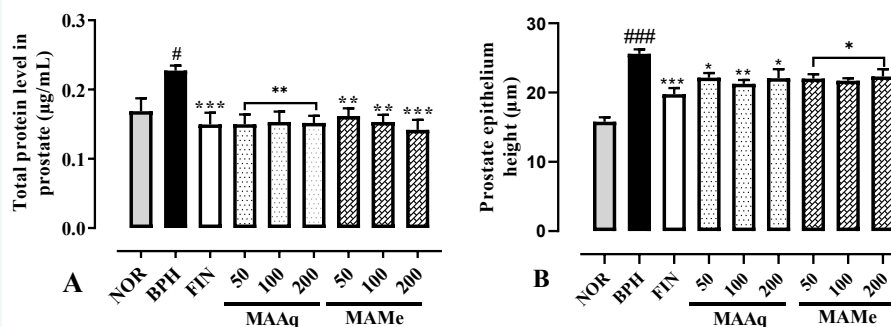
Figure 6 Graphical representations of the effects of aqueous and methanolic extracts of *Myrianthus arboreus* on prostate protein level (A) and prostate epithelial size (B) after 28 days of treatment.

NOR = normal control animals treated with corn oil and distilled water; BPH = negative control animals treated with testosterone enanthate (3 mg/kg BW/d) and distilled water; FIN = positive control animals treated with testosterone enanthate (3 mg/kg BW/d) and finasteride (5 mg/kg BW/d) ; MAAq = animals treated with testosterone enanthate (3 mg/kg BW/D) and aqueous extract of *Myrianthus arboreus* at 50, 100 and 200 mg/kg BW/d; MAMe = animals treated with testosterone enanthate (3 mg/kg BW/d) and methanolic extract of *Myrianthus arboreus* at 50, 100 and 200 mg/kg BW/d. Each graph represents the mean ± SEM (n =6). # p < 0.05; ### p < 0.001: significance compared with NOR group. * p < 0.05; ** p < 0.01; *** p < 0.001: significance compared with the BPH grou

of animals from the different groups. According to the results of this figure, after 28 days of treatment, testosterone caused a significant (p < 0.001) decrease of 471.57% in SOD activity in the negative control group (BPH) compared to the normal group (346.76 ± 39.87 units for BPH versus 1981.99 ± 184.71 units for NOR). One-way ANOVA revealed that there is a statistically significant difference between groups (F =6.69; p < 0.001) on the superoxide dismutase (SOD) activity. Multiple comparisons test (Dunnett Post Hoc) showed that, finasteride, as well as aqueous and methanolic extracts of *M. arboreus* at all doses, resulted in a significant (p < 0.05; p < 0.01; p < 0.001) increase of this parameter compared to the BPH group, respectively in the order of 333.41% for finasteride, 270.34%, 268.68% and 211.92% for the aqueous extract, and 168.20%, 211.53% and 203.22% for the methanolic extract, respectively for the doses 50, 100 and 200 mg/kg BW.

Figure 8B shows the effects of 28 days of treatment on catalase activity in the prostate. Analysis of the results in this figure reveals that administration of testosterone for 28 days resulted in a significant (p < 0.001) decrease in catalase activity in the BPH group compared with the normal control group, a decrease of 244.70% (126.65 ± 12.98 for NOR versus 36.74 ± 5.91 for BPH). The result on catalase activity showed that, there is a statistically significant difference between groups as determined by one-way ANOVA (F = 2.43; p = 0.041). Dunnett Post Hoc multiple comparisons test showed a significant (p < 0.05, 95.68%) increase in the finasteride group as compared to the BPH group (36.74 ± 5.91 for BPH vs. 71.90 ± 14.57 for FIN). Just like finasteride, both extracts of *M. arboreus* induced a significant increase (p < 0.05) in this activity at all doses compared to the BPH group, except at the doses of 100 and 50 mg/kg of the aqueous and methanolic extracts respectively. This increase was 97.92%, 112.07% for the aqueous extract, and 110.24%, 93.22% for the 100 and 200 mg/kg methanolic extract doses.

Effects on prostatic Reduced Glutathione levels, prostate MDA, and prostate nitrite concentrations

Analysis of the results presented in Figure 9A reveals that daily administration of testosterone for 28 days induced a significant (p < 0.01) decrease in prostatic reduced glutathione levels in the negative control group (BPH) compared with the normal group, a decrease of 206.82% (178.34 ± 11.26µmol/g for the BPH group, compared with 547.19 ± 74.04µmol/g for the NOR group). On prostatic reduced glutathione level, the result showed a statistically significant difference between groups as revealed by one-way ANOVA in different treated groups (F = 2.74; p = 0.024). Dunnett Post Hoc multiple comparisons test showed that finasteride significantly (p < 0.05) increased this concentration by 53.56% (273.85 ± 27.41) compared to the BPH group. The aqueous extract of *M. arboreus* at 50 and 100 mg/kg and the methanolic extract at 100 mg/kg showed the same effect, they significantly (p < 0.05) increased this concentration compared to the BPH control group. This increase was 57.25% and 57.14% for the aqueous extract and 49.12% for the methanolic extract, respectively.

The effects of 28-day treatment on prostatic MDA concentration are shown in Figure 9B. From the results in this figure, testosterone induced in the negative control animals (BPH) a significant (p < 0.001) increase in MDA concentration compared with the normal group animals. This increase was about 65.56% (24.24 ± 1.12µmol/g for the NOR group, and 70.40 ± 7.68µmol/g for the BPH group). On this parameter, one-way ANOVA indicated that there is a statistically significant difference between groups (F = 27.3; p < 0.001). Dunnett Post Hoc multiple comparisons text showed that, finasteride, as well as aqueous and methanolic extracts of *M. arboreus* at all doses, significantly (p < 0.001) decreased this concentration by 76.66% for finasteride, 77.17%, 81.02%, and 84.84% for aqueous extract, 82.35%,

Figure 7 Microphotographs (x25 and x100 magnification, hematoxylin-eosin staining) showing the effects of *Myrianthus arboreus* extracts on the prostate microarchitecture of rats after 28 days of treatment.

NOR = normal control animals treated with corn oil and distilled water; BPH = negative control animals treated with testosterone enanthate (3 mg/kg BW/d) and distilled water; FIN = positive control animals treated with testosterone enanthate (3 mg/kg BW/d) and finasteride (5 mg/kg BW/d) ; MAAq = animals treated with testosterone enanthate (3 mg/kg BW/D) and aqueous extract of *Myrianthus arboreus* at 50, 100 and 200 mg/kg BW/d; MAMe = animals treated with testosterone enanthate (3 mg/kg BW/d) and methanolic extract of *Myrianthus arboreus* at 50, 100 and 200 mg/kg BW/d. Ep: epithelium; Iv: involution; Lg: glandular lumen.
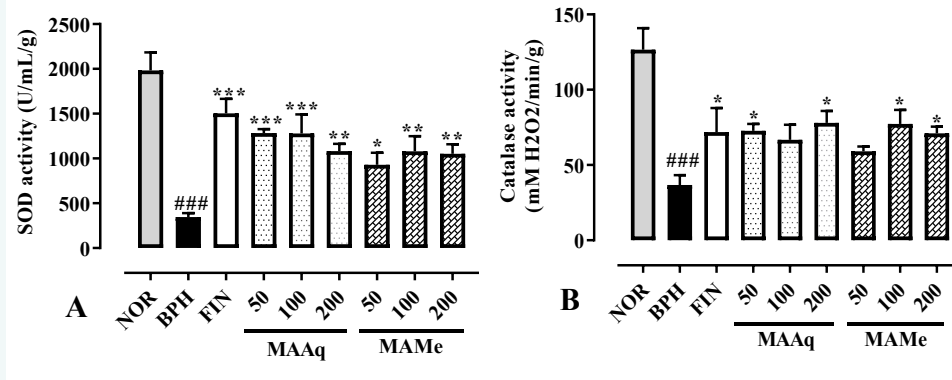
Figure 8 Graphical representations showing the effects of aqueous and methanolic extracts of *Myrianthus arboreus* on SOD (A) and catalase (B) activities, after 28 days of treatment.

NOR = normal control animals treated with corn oil and distilled water; BPH = negative control animals treated with testosterone enanthate (3 mg/kg BW/d) and distilled water; FIN = positive control animals treated with testosterone enanthate (3 mg/kg BW/d) and finasteride (5 mg/kg BW/d) ; MAAq = animals treated with testosterone enanthate (3 mg/kg BW/D) and aqueous extract of *Myrianthus arboreus* at 50, 100 and 200 mg/kg BW/d; MAMe = animals treated with testosterone enanthate (3 mg/kg BW/d) and methanolic extract of *Myrianthus arboreus* at 50, 100 and 200 mg/kg BW/d. Each graph represents the mean ± SEM (n =6). ### p < 0.001: significance compared with NOR group. * p < 0.05; ** p < 0.01; *** p < 0.001: significance compared with the BPH group.

82.78%, and 90.58% for methanolic extract, respectively, compared to the BPH group.

Figure 9C shows the effects of *M. arboreus* extracts on prostatic nitrite concentration. The results in this figure show that administration of testosterone for 28 days resulted in a significant (p < 0.01) increase of 23.52% in nitrite concentration in the negative control animals (BPH group) compared to the normal group (0.36 ± 0.02 mol/g for the NOR group, and 0.47 ± 0.01 mol/g for the BPH group). Compared to the BPH group, one-way ANOVA indicated that there is a statistically significant difference between groups (F = 15.1; p < 0.001). Dunnett Post Hoc multiple comparisons test showed that finasteride induced a significant (p < 0.001) decrease of about 32.55% in nitrite concentration; the same effect was observed with *M. arboreus* extracts at all doses tested (p < 0.001), which induced a decrease of 53.82%, 32.12%, 45.83% with *M. arboreus* aqueous extract, and of 54.91%, 58.39%, 49.00% with *M. arboreus* methanolic extract, for the doses of 50, 100 and 200 mg/kg BW respectively.

Effects on the prostatic concentration of TNF-α

Figure 10A illustrates the effects of *M. arboreus* extracts on prostatic TNF-α concentration, after 28 days of treatment. From the results depicted in this figure, it appears that daily administration of testosterone resulted in a significant (p<0.001) increase in the concentration of TNF-α in animals prostates' in the negative group (BPH) compared to those in the normal group. This increase was about 43.82% (161.15 ± 8.33 pg/mL for the NOR group and 286.85 ± 14.13 pg/mL for the BPH group). One-way ANOVA indicated that there is a statistically significant difference between groups (F = 12.8; p < 0.001). Multiple comparisons test called Dunnett Post Hoc showed that finasteride significantly (p < 0.001) reduced this concentration, by about 19.1%; the same was true for aqueous extract of *M. arboreus* (p < 0.01) at 200 mg/kg

(17.07%) and methanolic extract (p < 0.05; p < 0.01; p < 0.001) at all doses (about 16.75%, 13.22%, and 24.07% respectively).

Effects on the prostatic concentration of IL-8

The effects of treatments on prostatic IL-8 concentration are shown in Figure 10B below. According to the results in this figure, the IL-8 concentration is significantly (p < 0.001) higher in the animals in the BPH group compared with the normal group; this difference is about 66.57% (95.01 ± 6.48 pg/mL for the NOR group and 284.22 ± 18.98 pg/mL for the BPH group). The difference between groups is statistically significant as shown by one-way ANOVA (F = 8.61; p <0.001). Dunnett Post Hoc multiple comparisons test showed that, like finasteride, the aqueous and methanolic extracts of *M. arboreus* at doses of 100 and 200 mg/kg induced a significant (p < 0.01; p < 0.001) decrease in this concentration, respectively by 23.76% for finasteride, 23.18% and 22.73% for the aqueous extract, and 15.15% and 21.36% for the methanol extract, respectively.

Effects on serum PSA levels

Figure 11 shows the serum PSA level in the different groups after 28 days of treatment. Analysis of the results in this figure reveals that administration of testosterone for 28 days resulted in a significant (p < 0.05) increase in the PSA level in the BPH group compared to the normal group, i.e. an increase of about 42.67% (0.26 ± 0.03 ng/mL in the normal control versus 0.45 ± 0.03 ng/mL in the BPH control). As revealed by one-way ANOVA (F = 0.864; p = 0.548), the *M. arboreus* extracts at all doses, and finasteride caused a non-significant decrease in this concentration. However, this decrease was 12% for finasteride, 25.33%, 20%, 29%, for the aqueous extract of *M. arboreus*, and 19%, 27%, and 21.33% for the methanol extract.
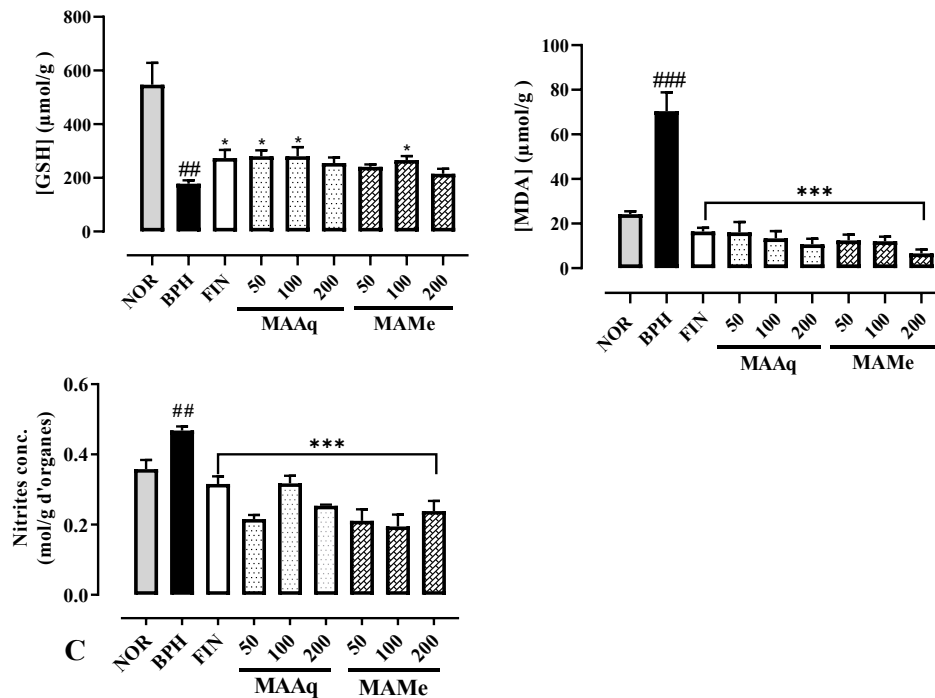
Figure 9 Graphical representations showing the effects of aqueous and methanolic extracts of *Myrianthus arboreus* on prostatic concentrations of reduced glutathione (A), malondialdehyde (B) and nitrites (C) after 28 days of treatment.

NOR = normal control animals treated with corn oil and distilled water; BPH = negative control animals treated with testosterone enanthate (3 mg/kg BW/d) and distilled water; FIN = positive control animals treated with testosterone enanthate (3 mg/kg BW/d) and finasteride (5 mg/kg BW/d) ; MAAq = animals treated with testosterone enanthate (3 mg/kg BW/D) and aqueous extract of *Myrianthus arboreus* at 50, 100 and 200 mg/kg BW/d; MAMe = animals treated with testosterone enanthate (3 mg/kg BW/d) and methanolic extract of *Myrianthus arboreus* at 50, 100 and 200 mg/kg BW/d. Each graph represents the mean ± SEM (n =6). ## $p < 0.01$; ### $p < 0.001$: significance compared with NOR group. * $p < 0.05$; *** $p < 0.001$: significance compared with the BPH group.

Discussion

Benign prostatic hyperplasia (BPH) is one of the most common conditions that affects geriatric men. It is characterized by an abnormal increase in the size and weight of the prostate gland [33]. In BPH, the prostate becomes larger, compressing the urethra while putting pressure on the bladder, which impedes normal urine output. As a consequence, it progressively impairs the ease and comfort of urination, as well as sexual function, and can cause severe urologic or nephrologic complications [34,35]. However, existing conventional treatments are associated with many undesirable side effects. In addition, most elderly people have co-morbidities such as hypertension and diabetes, which do not allow them to undergo surgical procedures. Therefore, there is a need to focus a lot of research on this disease [36,37].

The results of this study show that after 28 days of treatment, testosterone significantly increased the relative weight and volume of the prostate in the negative control group compared with the normal group. According to several studies, animals with BPH show an increase in prostate weight and volume, a characteristic sign of this pathology [38-40]. Indeed, testosterone has been shown to be involved in the development of BPH by triggering abnormal proliferation of epithelial and stromal cells in the prostate, leading to hypertrophy of this organ [41,42].

In the prostate, free testosterone is irreversibly converted to dihydrotestosterone (DHT) by the steroidal enzyme 5a-reductase, located on the nuclear membrane of epithelial and stromal cells. DHT then binds to the androgen receptor with high affinity, and the complex formed, mediated by coregulators, is transported into the nucleus. Once inside, this complex binds to specific DNA binding sites, stimulating the transcription of growth factors which, in turn, affect the expression of genes that control cell proliferation and death [5-7].

Testosterone treatment also significantly increased prostatic protein levels, as well as the size of the prostatic epithelium. These results are similar to those obtained by several authors who observed histologically significant stromal and epithelial proliferation, associated with partial obliteration of the glandular lumen, in the prostate of rats treated for 28 days with testosterone [1,41,42]. Thus, an increase in the number of cells (hyperplasia) in the prostate will lead to a secondary increase in its weight [43,44], confirming the results obtained on prostate weight and volume. As proteins are the essential constituents of cells, any cell proliferation within a tissue is accompanied by an increase in the protein content of that tissue [45,46].

Aqueous and methanolic extracts of *M. arboreus* remarkably inhibited the development of testosterone-induced BPH, with
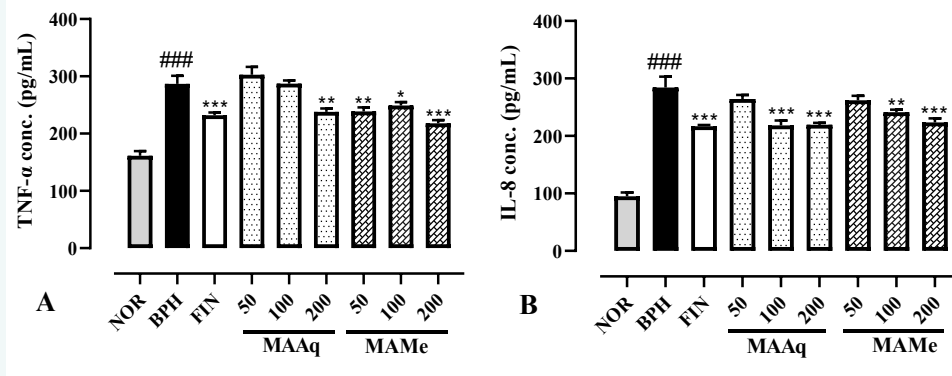
Figure 10 Graphical representations of the effects of aqueous and methanolic extracts of *Myrianthus arboreus* on prostatic TNF-α and IL-8 concentrations after 28 days of treatment.

NOR = normal control animals treated with corn oil and distilled water; BPH = negative control animals treated with testosterone enanthate (3 mg/kg BW/d) and distilled water; FIN = positive control animals treated with testosterone enanthate (3 mg/kg BW/d) and finasteride (5 mg/kg BW/d) ; MAAq = animals treated with testosterone enanthate (3 mg/kg BW/D) and aqueous extract of *Myrianthus arboreus* at 50, 100 and 200 mg/kg BW/d; MAMe = animals treated with testosterone enanthate (3 mg/kg BW/d) and methanolic extract of *Myrianthus arboreus* at 50, 100 and 200 mg/kg BW/d. Each graph represents the mean ± SEM (n =6). ### $p < 0.001$: significance compared with NOR group. ** $p <0.01$; *** $p < 0.001$: significance compared with the BPH group.
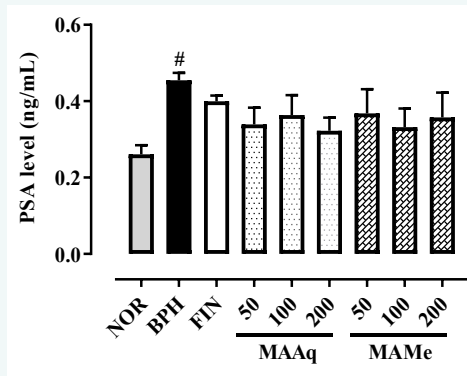

Figure 11 Graphical representations of the effects of aqueous and methanolic extracts of *Myrianthus arboreus* on serum PSA levels after 28 days of treatment.

NOR = normal control animals treated with corn oil and distilled water; BPH = negative control animals treated with testosterone enanthate (3 mg/ kg BW/d) and distilled water; FIN = positive control animals treated with testosterone enanthate (3 mg/kg BW/d) and finasteride (5 mg/kg BW/d) ; MAAq = animals treated with testosterone enanthate (3 mg/kg BW/D) and aqueous extract of *Myrianthus arboreus* at 50, 100 and 200 mg/kg BW/d; MAMe = animals treated with testosterone enanthate (3 mg/kg BW/d) and methanolic extract of *Myrianthus arboreus* at 50, 100 and 200 mg/kg BW/d. Each graph represents the mean ± SEM (n =6). # $p < 0.05$: significance compared with NOR group.

a significant reduction in prostate weight (both extracts at 100 mg/kg) and volume (methanolic extract only at 50 and 200 mg/kg) compared with the negative group (BPH). These results corroborate those of Awounfack et al. [16], who showed that the aqueous extract of *M. arboreus* leaves, after a 28-day treatment induces a dose-dependent loss of amyloid bodies, accompanied

by marked atrophy of prostate glands in normal males. These effects were confirmed biochemically by a significant decrease in total prostatic protein levels induced by both *M. arboreus* extracts (at all doses), and histologically by a significant reduction in the size of the prostatic epithelium. These results suggest that *M. arboreus* extracts are capable of counteracting or opposing the proliferative effects of testosterone on the prostate such as observed with *Lespedeza cuneata* aqueous extract on prostatic hyperplasia induced by testosterone [47].

Oxidative stress is considered to be one of the factors playing an important role in the chain of reactions involved in the development and progression of BPH [48,49]. It results from an imbalance between the production of free radicals and their elimination by antioxidants and can damage important tissue components such as DNA, lipids and proteins [50]. Indeed, it is well established that androgens effectively promote an increase in cellular metabolism in the prostate, resulting in a substantial production of free radicals, coupled with a significant utilization of antioxidant enzymes. When this intrinsic production of reactive oxygen/nitrogen species (ROS/RNS) exceeds cellular antioxidant protection, it results in damage to the cellular component, leading to abnormal proliferation of prostatic cells. These alterations are accompanied by histological changes and cellular dysfunction [51]. In this study, testosterone caused a significant increase in MDA and nitrite concentrations, as well as a significant reduction in SOD, catalase, and GSH, suggesting oxidative stress. Previous studies have demonstrated a reduction in antioxidant levels in the serum and prostate of animals with BPH, while oxidant levels (nitrite, MDA) are increased [52,53]. Similarly, some studies have reported increased lipid peroxidation, marked by elevated levels of MDA and nitrites in serum samples from BPH patients, as well as a decrease in antioxidant factors responsible for ROS removal and thus prevention of oxidative damage, compared with controls [54,55].

Treatment of animals with aqueous and methanolic extracts of *M. arboreus* attenuated testosterone-induced lipid peroxidation by lowering MDA levels, improved nitrite levels, and significantly restored SOD and catalase activities, as well as GSH levels. The various extracts tested thus enhanced and strengthened antioxidant defense mechanisms in the prostate. These results were confirmed by in vitro antioxidant tests. The scavenging of DPPH and ABTS free radicals serves as a recognized mechanism for assessing the antioxidant activity of plant extracts, a crucial aspect in mitigating the deleterious impact of free radicals in the pathogenesis of various diseases [56]. In this investigation, the outcomes of DPPH and ABTS radical scavenging assays indicated pronounced activity of the plant; both extracts demonstrated significant free radical scavenging capacities. This scavenging efficacy was prompt, as observed in both extracts, with nearly 100% inhibition of free radicals within 15 minutes of incubation. Similarly, the results revealed an increase in radical scavenging activity (DPPH and ABTS) proportionate to the concentration of plant extracts. The DPPH radical scavenging potential was markedly superior in the methanolic extract compared to the aqueous extract, as evidenced by lower IC50 values. Conversely, the aqueous extract exhibited the most potent ABTS radical scavenging activities. Indeed, a lower IC50 value signifies heightened free radical scavenging activity [57]. Collectively, these findings suggest that the plant extracts contain compounds endowed with redox properties, facilitating electron transfer or hydrogen donation to free radicals, thereby eliminating the unpaired electron responsible for radical reactivity. The assessment of the total antioxidant capacity of *M. arboreus* extracts demonstrated a superior capacity in the methanolic extract compared to the aqueous extract. Additionally, it is established that the total polyphenol content exhibits a robust correlation with free radical scavenging activities [20]. Likewise, prior investigations into distinct components of *M. arboreus* (leaves, bark, and roots) underscored the abundance of phenolic compounds in the tested extracts, along with their antiradical activities [58-60]. This study is in line with previous studies carried out on various parts of *M. arboreus* (leaves, bark, and roots), which have shown that this plant is endowed with good antioxidant properties [55-57]. The plant's antioxidant potential could be attributed to its wealth in secondary metabolites [61]. Indeed, the phytochemical screening of *M. arboreus* carried out in this study revealed the presence of large quantities of, tannins, flavonoids, and total polyphenols (phenols), compounds with proven antioxidant activity as well as metal-chelating properties [62-64]. These compounds could therefore be responsible for protecting the prostate against oxidative damage and potentially suppressing the progression of BPH. In addition, previous studies revealed that many bioactive compounds have been isolated to *M. arboreus* extracts [65], specially triterpenoids such as arjunolic acid and ursolic acid, known for their antioxidant properties. They are known to play an important role in the protection of tissues and cells against side effects of reactive oxygen species and other free radicals [66,67].

Inflammation is extremely prevalent in the prostates of aging men [68], and involves an infiltrate of lymphocytes (specially T population) and macrophages into the prostate; suggesting a progression of immune response in BPH. These infiltrating cells are responsible for the production of various inflammatory cytokines, such as interleukins (IL-1β, IL-2, IL-4, IL-6, IL-8), IFNγ and TNF-α, which may contribute to prostate hypertrophy [47,69]. In this study, a significant increase in prostatic levels of TNF-α and IL-8 was observed in the negative control group compared with the normal control group. These results are consistent with data from previous studies showing that levels of IL-8 and TNF-α, pro-inflammatory cytokines considered to be potent growth factors for prostate epithelial and stromal cells, are increased in BPH models [70,34]. Indeed, cytokines secreted by inflammatory cells can promote angiogenesis and the local production of growth factors in tissues [71-73]. Groups of animals treated with finasteride and with aqueous and methanolic extracts of *M. arboreus* showed a significant reduction in the levels of these cytokines compared with the negative control group. These effects could be attributed to the secondary metabolites found in this plant by such authors, such as ursolic acid which is able to reduce the oxidative stress indicator iNOS and the production of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) [67,74], and finally act by preventing the release of inflammatory mediators capable of disrupting the normal growth of the prostate gland. According to several authors, compounds that inhibit the release of inflammatory cytokines can be used as potentially effective therapeutic agents to treat BPH [1,47, 75]. In this study, a significant increase in PSA levels was also observed in the negative group compared to the normal control. Prostate-specific antigen (PSA) is a protein produced by glandular and endothelial cells of the prostate [76]. It is one of a biomarker used to investigate the progression of BPH and prostate cancer. Its serum level is abnormally high in patients with prostate cancer, and BPH, as well as in patients with inflammatory prostate diseases [43]. It therefore represents an important indicator of the status of the prostate gland, and in this case of BPH; there is a good correlation between prostate volume and serum PSA level [77,78]. Administration of *M. arboreus* extracts and finasteride for 28 days resulted in a decrease in prostate PSA levels compared to the negative control group. According to some authors, the reduction of PSA levels by substances or treatments is an indication of their inhibitory activity of 5a-reductase, an enzyme that catalyzes the formation of dihydrotestosterone (DHT) from testosterone, and of the effectiveness in the treatment of prostatic hyperplasia [43,79].

However, additional research using alternative models is essential to provide further evidence supporting the positive effects of *M. arboreus* extracts on the prostate. Similarly, evaluating their influence on testosterone concentrations, DHT (dihydrotestosterone) concentrations, 5α-reductase activity, and androgen receptor activity, is crucial for enhancing our understanding of the molecular mechanism of action of *M. arboreus* extracts and its compounds on inflammation linked to Benign prostatic hyperplasia (BPH). BPH and prostate cancer are two distinct prostate conditions that share similarities in their biological mechanisms, symptoms, and risk factors. This convergence underscores the complexity of prostatic physiology

and suggests an interrelation between these two pathologies. Considering the beneficial effects of the plant on BPH, the idea emerges that it could potentially exert similar positive effects in the context of prostate cancer. This hypothesis emphasizes the importance of conducting in-depth research to explore the therapeutic potential of the plant in the treatment or prevention of this prostatic condition.

Conclusion

All these results show that the aqueous and methanolic extracts of *M. arboreus* can inhibit cell proliferation or glandular expansion stimulated by testosterone, and thus the development or establishment of BPH. This inhibiting effect of *M. arboreus* could be explained either by the inhibition of 5α-reductase, by their antioxidant and anti-inflammatory properties, or by the combination of all these mechanisms. *M. arboreus* could therefore be an effective candidate for drug development for the treatment of BPH.

Author contributions

SD and DN designed the study, supervised data collection and analysis, and proofread the manuscript. FTM and CFA performed the experiments and data collection, and participated in writing the first draft of the manuscript. KSK carried out the in vitro experiments and revised the manuscript. BLN and JGK participated in the collection and extraction of the plant and revised the manuscript. CBN performed the biochemical assays and analyzed the data.

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