

Chemotherapeutic Evaluation of Ethanol Extract of *Chromolaena odorata* on Biochemical Aberrations Associated with Experimentally-induced Benign Prostatic Hyperplasia in Male Rats

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Abstract: The sudden rise in benign prostatic hyperplasia (BPH) cases, severe side effects, and the high cost of conventional methods have necessitated the intensive search for alternative BPH management strategies. This study investigated the restorative effects of ethanol leaf extract of *Chromolaena odorata* (EECO) on testosterone-induced BPH in male albino rats. Thirty male albino rats with a weight range of 150-210 g were randomly distributed into six groups of five rats each. Group 1 was normal rats and not induced. Groups 2-6 were induced via daily subcutaneous injection of testosterone propionate (3 mg/kg) for 28 days. After induction, group 2 received vehicle (carboxyl methylcellulose), group 3 received finasteride (1 mg/kg), while groups 4-6 received 100, 200, and 400 mg/kg of EECO, respectively, for 21 days orally. Prostate and biochemical parameters were determined using standard methods. Treatments with EECO decreased the concentrations of prostate-sensitive antigen, dihydrotestosterone, testosterone, malondialdehyde, cholesterol, low-density cholesterol, and liver enzyme activities compared with BPH-control. Furthermore, there was increased superoxide dismutase, and catalase activities in extract treated groups compared with BPH-control. The findings from this study showed that EECO inhibited testosterone-induced BPH anomalies, making it promising phytotherapy for the management of BPH in males.

Keywords: *Chromolaena odorata*; oxidative stress; benign prostatic hyperplasia; prostate-specific antigen; antioxidant activity.

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1. Introduction

Benign prostatic hyperplasia (BPH), a non-cancerous proliferation of epithelial and stromal cells of the prostate gland, is one of the most common age-related diseases in aging men [1, 2]. It affects about half of the men over 80 years old [3]. This population is expected to increase due to recent lifestyle changes. BPH triggers benign prostatic enlargement (BPE), which could lead to voiding difficulty as a result of benign prostatic obstruction (BPO) [4]. A mild case of BPH obstructs urine flow and leads to a bladder infection. However, as the disease

progresses, there is a slight deposition of stone in the bladder, which causes irreparable bladder damage, kidney failure, sepsis, hepatic failure, and ultimately death [5].

Hyperplasia of micronodular has been identified as the first event that initiates BPH development. This results in enlargement of the macroscopic nodular of the prostate, which could result in obstruction of the bladder outlet and lower urinary tract infection [6,7]. However, multifactorial events like inflammation, oxidative stress, metabolic syndrome in men, and other factors that increase cell proliferation and suppress cell apoptosis pathways are involved in the etiology and prognosis of BPH [8]. Classically, sex hormones and age have been identified as the two key independent risk factors [9]. Notably, an increase in estrogen/androgen ratio due to the conversion of testosterone to estradiol by CYP450 enzymes, aromatase [2], and metabolic alterations in other serum hormones have been strongly linked to the etiology of BPH [9].

Accumulating shreds of evidence have reported different management strategies for BPH, such as medical therapy and surgery [10-13]. Medical therapy involves using alpha-blockers, 5 alpha-reductase inhibitors, B₃-agonist, phosphodiesterase type 5 inhibitors, and anti-muscarinic [10]. However, alpha-blockers and 5 alpha-reductase inhibitors are the most commonly prescribed therapy [4]. The use of alpha-blockers is the first-line treatment option aimed at relaxing prostate smooth muscles, thereby increasing urine flow and preventing urine obstruction [7] with no effect on prostate size reduction. The molecular mechanism of 5 α -reductase inhibitors is the repression of interconversion of testosterone to dihydrotestosterone (DHT), which circumvents prostate enlargement [14]. The surgical approach mostly employed is prostate tissue compression, debulking of the adenoma, and removing the adenoma [11].

Though these synthetic agents, when used as combination therapy, are effective in managing BPH, their prolonged usage leads to side effects such as hypotension, nasal congestion, headache, and sexual dysfunction [13, 14]. The high cost of surgery and other surgery-related problems like urinary tract infection, bleeding, irreversible sexual side effects, urethral stricture disease, and urinary incontinence [11, 13] have limited surgical approaches. This necessitated the recent intensive search for the therapeutic potential of phytonutrients, especially with the recent increase in evidence on the role of polyphenols as 5 α -reductase inhibitors [7]. Recently, many herbs have been employed in managing BPH due to their fewer side effects and low cost [15].

Chromolaena odorata, also known as Siam weed, bitter bush, or Christmas bush, is a member of the Asteraceae family [16]. It is an evergreen plant predominantly seen in Europe, Asia, America, and West Africa [17]. *C. odorata* is used in folk medicine to remedy infertility, malaria, wound healing, diabetes, rashes, and skin infection [16,17]. Pharmacological activities identified in the plant include but are not limited to antioxidant, anti-inflammatory, anti-diabetic, hemostatic, hepatoprotective, anticancer, and immunomodulatory activities [16,17]. Several phenolic compounds have been isolated from *C. odorata*, such as p-coumaric, vanillic acids, p-hydroxybenzoic acid, and flavonoids such as flavones and chalcones and flavonols [17]. Based on the already documented anti-inflammatory and antioxidant properties of *C. odorata*, and the significant roles inflammation and oxidative stress play in BPH prognosis. This research work was embarked on to investigate the chemotherapeutic effects of ethanol leaf extract of *C. odorata* on hormonal induced-BPH in the rat model, which is currently used as an effective model to test BPH drugs due to its similarity to the human prostate gland [18].

2. Materials and Methods

2.1. Chemicals/reagents.

Analytical grade chemicals and reagents used for this research were purchased from Sigma Aldrich (USA), British Drug House (BDH, England), Biochrom Co. (UK), Accord Health Care Ltd (UK), and Randox (UK).

2.2. Plant material collection and extraction procedures.

Fresh leaves of *C. odorata* were collected and identified by a taxonomist (Mr. Alfred Ozioko) from the Bioresource Development and Conservation Program, Nsukka, Nigeria. The extraction procedure followed the method of Ekeyi *et al.* [19]. The shade-dried leaves were pulverized into powder (1000 g) and extracted with absolute ethanol (1.5 L) for 48 h through cold maceration. The mixture was filtered through Whatman No. 1 filter paper with a pore size of 11m. The filtrate was concentrated using a rotary evaporator at 40°C to get a chocolate-like semi-solid extract (EECO).

2.3. Phytochemical analysis of ethanol leaf extract of *C. odorata*

Quantitative phytochemical screening for alkaloids, flavonoids, glycosides, saponins, tannins, and steroids was determined by the Harbone [20] and Trease and Evans [21] methods.

2.4. Animals.

Apparently, healthy thirty (30) male adult Wistar Albino rats (8 ± 1 week, with a weight range of 150-210 g) was used to test anti-BPH effects of the extract due to its similarity to the human prostate gland [18] and eighteen (18) Swiss mice (7 ± 1 week, with a weight range of 29-32 g) which are the most frequently selected rodent species for acute toxicity testing [22] was used for acute toxicity study. The animals were housed in a color-coded stainless cage under standard laboratory conditions of temperature ($25 \pm 1^\circ\text{C}$) and light (12 h light/dark cycle). They had free access to water and rodent food and were acclimatized for at least 14 days before using the experiment. All the animals used were of the same sex, within a small age range, sourced from the same source, placed under the same environmental conditions, and fed to eliminate confounding factors that might influence results. The international and national approved guidelines on the care and use of laboratory animals were strictly complied with as stated by the National Institute of Health Guide for Care and Use of Laboratory Animals (Pub No. 85-23, 1985). Ethical approval with reference number UNN/FBS/EC/1048 was obtained from the Ethics and Biosafety Committee of the Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria.

2.5. Acute toxicity study.

This was investigated with Lorke's method [23] using healthy eighteen (18) male mice. The mice were randomized into six groups of three mice each. In the first phase of the experiment, three groups of mice were administered a single dose of 10, 100, and 1000 mg/kg body weight of EECO gavigated orally (3 mice in group 1 received 10 mg/kg b. w, 3 mice in group 2 received 100 mg/kg b. w, while the remaining group was administered 1000 mg/kg b. w of EECO). The animals were closely monitored for lethality and signs of toxicity, including

increased motor activity, sedation, lachrymation, salivation, muscle spasm, and weakness for 24 h. The second phase was conducted by administering an increased oral dose of 1600, 2900, and 5000 mg/kg b. w. of the extract to mice in groups 4, 5, and 6, respectively. These groups were further observed for another 24 h. LD₅₀ was calculated as follows:

$$LD_{50} = \sqrt{D_{0} \times D_{100}}$$

D₀ and D₁₀₀ represent the maximum dose that gave no mortality and the minimum dose that produced mortality, respectively.

2.6. Experimental design.

A total of thirty healthy male albino rats randomly distributed into six groups (n = 5) used for this study were grouped as follows:

Table 1. Experimental design.

Groups	Details of groups
A	Normal control rats, not induced and given only vehicle (carboxymethylcellulose, CMC) for 21 days.
B	BPH induced + vehicle (BPH- control) for 21 days.
C	BPH induced + treated with 1 mg/kg finasteride (Standard control) for 21 days.
D	BPH induced + treated with 100 mg/kg b. w. of EECO for 21 days.
E	BPH induced + treated with 200 mg/kg b. w of EECO for 21 days.
F	BPH induced + treated with 400 mg/kg b. w of EECO for 21 days

2.7. Induction and treatment of BPH.

Benign prostatic hyperplasia was induced in thirty (30) male albino rats divided into five groups (n = 5) by subcutaneous injection of 3 mg/kg of testosterone (Sigma-Aldrich, St Louis, MO, USA) daily for 28 days using a slight modification of the method described by Sarbishegi *et al.* [14]. The test substances: Vehicle, standard drug, finasteride (CAS No: 98319-26-7 procured from Accord Healthcare Ltd, UK), and varying concentrations of the extract (100, 200, and 400 mg/kg chosen based on their optimal activity in pilot studies) dissolved in CMC were prepared by the lead investigator who concealed their identity before handing it over to the person that administered it (single-blind). A single treatment was given orally daily for 21 days as stated in the experimental design above, after which the animals fasted overnight. On the 22nd day, blood samples were collected through the retro-orbital plexus under mild anesthesia. The blood was centrifuged at 604 x g for 15 mins, and the serum obtained was individually coded with numbers and sent to a biochemical analyst to determine biochemical evaluation. The prostates were removed immediately and weighed.

2.8. Anesthesia and euthanasia.

At the end of the experiment, the death of the rodents (mice and rats) was induced humanely according to the guidelines of the American Veterinary Medical Association (AVMA) using an intraperitoneal injection of a barbiturate agent, sodium pentobarbital (200 mg/kg). The death of the mice and rats was confirmed by checking their heartbeat, pupillary response to light, and respiratory pattern before the carcass was disposed of.

2.9. Biochemical studies.

2.9.1. Determination of serum prostate status.

The Enzyme immunoassay technique was used for the quantitative determination of testosterone, prostate-specific antigen (PSA), and dihydrotestosterone (DHT) concentrations as follows: Testosterone was measured with the method of Turkes *et al.* [24] using an ELISA kit (Monobind Inc., Lake Forest, CA GWB-462C5C, USA). The PSA determination was done based on solid-phase two-site immunoassay as described by Stowell *et al.* [25] using an ELISA kit (Monobind Inc., Lake Forest, CA 92630, USA) while the DHT assay was done with an ELISA kit (ALPCO, 26-G Keewaydin Drive, Salem, NH 03079, USA).

2.9.2. Determination of the extent of lipid peroxidation.

This was evaluated according to the method of Wallin *et al.* [26], which involves measuring the absorbance of a pink color complex formed when lipid peroxidation by-product, malondialdehyde, reacts with a thiobarbituric acid reactive substance at 535 nm.

2.9.3. Determination of antioxidant enzyme activities.

The Superoxide dismutase (SOD) activity was assayed with the Fridovich [27] method. In this assay, superoxide radicals generated from xanthine and xanthine oxidase form a red dye when reacted with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride. The extent of inhibition of this reaction measured at a wavelength of 505 nm gives an index of SOD activity. Catalase activity was assayed using Aebi [28] to quantify the extent of hydrogen peroxide breakdown by measuring the reaction mixture's absorbance at 230 nm using a spectrophotometer.

2.9.4. Determination of liver enzyme activities.

Liver enzymes were assayed as follows: Serum aspartate aminotransferase (AST) was assayed with the Reitman and Frankel [29] method using the Randox test kit (Randox lab. Ltd., UK, Cat. No. AS 101, County Antrim, UK). ALT was determined with the method of Reitman and Frankel [29] with a Randox kit (Randox lab. Ltd., UK, Cat. No. AL 100, County Antrim, UK).

2.9.5. Determination of lipid profile.

The following methods were used to determine the lipid profile using Randox commercial kits: Cholesterol concentration was measured as described by Allain *et al.* [30] using Randox kits (Randox lab. Ltd, UK, Cat. No CH 200, Antrim, UK). The Triacylglycerol level was measured as described by Albers *et al.* [31] using Randox kits (Randox lab. Ltd, UK, Cat. No TR 210, Antrim, UK). High-density lipoprotein in the serum was obtained after precipitating LDL-C and VLDL as described in Albers *et al.* [31] with Randox kits (Randox lab. Ltd., UK, Cat. No. CH 203, Antrim, UK), while low-density lipoprotein concentration was determined with the polyvinyl sulfate method as described by Assmann *et al.* [32].

2.10. Histological examination.

The prostates of the rats were prepared for histology using the procedure described by Drury and Wallington [33]. Thereafter, the slides were examined with a Motic compound microscope, and photomicrographs were taken.

2.11. Statistical Analysis.

Data generated in the research work was analyzed with Statistical product and service solutions (SPSS) for Windows version 18.0 (SPSS Inc., Chicago, IL, USA). Differences between means were separated and analyzed using a one-way analysis of variance (ANOVA) alongside *post hoc* multiple comparisons. The least significant threshold employed was $p < 0.05$ was used at a 95% confidence interval. All analyses were performed in triplicates and reported as mean \pm SD.

3. Results

3.1. Phytochemical screening of the EECO.

The quantity of phytochemicals in the extracts in decreasing order is as follows: Saponins (1.74 ± 0.03 mg/g), alkaloids (1.63 ± 0.02 mg/g), flavonoids (0.93 ± 0.03 mg/g), tannins (0.72 ± 0.01 mg/g), steroids (0.40 ± 0.01 mg/g), and glycosides (0.21 ± 0.02 mg/g) (Table 2).

Table 2. Quantitative phytochemical constituent of EECO.

Phytochemicals	Amount (mg/g)
Saponins	1.74 ± 0.03
Alkaloids	1.63 ± 0.02
Flavonoids	0.93 ± 0.03
Tannins	0.72 ± 0.01
Steroids	0.40 ± 0.01
Glycosides	0.21 ± 0.02

Each value represents Mean \pm SD, n = 3

3.2. Acute toxicity study (LD50).

Mice administered 10-5000 mg/kg b. w. of the leaf extract did not record any behavioral change, signs of toxicity, or death. This shows that the plant is safe for consumption up to a dose of 5000 mg/kg b. w. since no death was recorded. This led to the choice of 100, 200, and 400 mg/kg b. w., which had the highest activities in the pilot studies as doses of the extract to be administered to the rats (Table 3).

Table 3. Acute toxicity study (LD50) of EECO.

Doses in mg/kg body weight	Number of deaths recorded
Phase 1	0/3
10	0/3
100	0/3
1000	0/3
Phase 2	
1600	0/3
2900	0/3
5000	0/3

3.3. Effects of EECO on the prostate weight of BPH-induced rats.

Induction of BPH caused a significant ($p < 0.05$) increase in prostate weight in the BPH-control group compared with baseline. Interestingly, a significant reduction ($p < 0.05$) in prostate weight occurred in rats treated with the standard drug (finasteride) and higher doses of EECO (200 and 400 mg/kg b. w) (Figure 1).

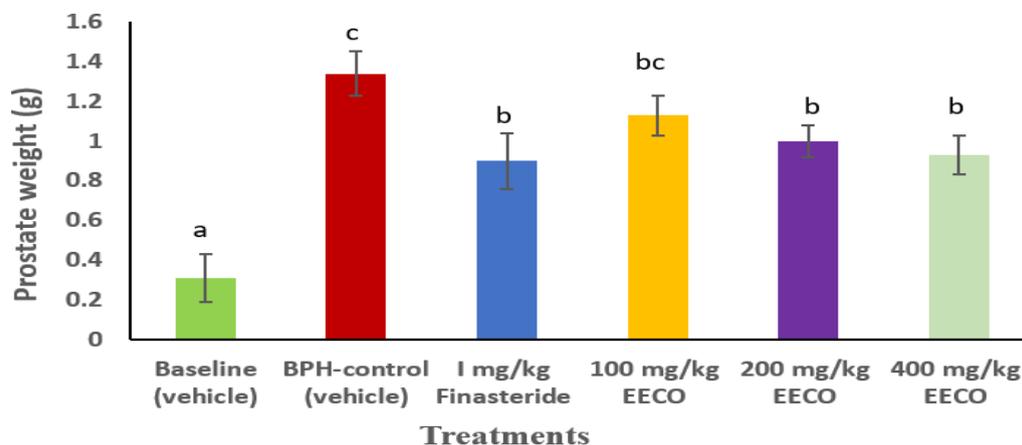


Figure 1. Effect of EECO on the prostate weight of BPH-induced rats. The values are represented as mean \pm SD ($n = 5$). Mean values with different alphabets as superscripts when compared across the groups are significantly different at $p < 0.05$.

3.4. Effects of EECO on prostate status of BPH-induced rats.

In this study, the administration of testosterone caused a significant ($p < 0.05$) increase in the concentrations of PSA and testosterone in the BPH-induced groups compared with the baseline, confirming the induction of benign prostatic hyperplasia. However, treatment with the standard drug and 400 kg/mg bodyweight of the extract resulted in a significant ($p < 0.05$) reduction in the concentrations of PSA and testosterone when compared with BPH-control. In the same vein, groups administered varying doses of the extract, and the standard drug, finasteride, had a significantly ($p < 0.05$) lower concentration of DHT than the BPH-control, which unveils the potency of the extract in reducing prostatic activity in the BPH-induced animals (Table 4).

Table 4. Effects of EECO on prostate markers of BPH-induced male rats.

Treatments	Prostatic specific antigen (PSA) (ng/ml)		Testosterone (ng/ml)	DHT (ng/ml)
	PSA before treatment	PSA after treatment		
Baseline (vehicle)	2.18 \pm 0.51 ^a	2.15 \pm 0.94 ^a	3.40 \pm 1.71 ^a	2.78 \pm 0.70 ^{a,b}
BPH-control (vehicle)	7.58 \pm 1.20 ^b	6.72 \pm 2.13 ^c	8.96 \pm 0.77 ^d	3.26 \pm 0.69 ^b
Finasteride (1 mg/kg)	7.91 \pm 1.07 ^b	2.90 \pm 1.25 ^{a,b}	4.14 \pm 0.88 ^{a,b}	2.24 \pm 0.20 ^a
EECO 100 mg/kg	7.70 \pm 0.54 ^b	4.30 \pm 1.64 ^b	7.68 \pm 1.12 ^{c,d}	2.74 \pm 0.73 ^{a,b}
200 mg/kg	8.10 \pm 2.11 ^b	3.56 \pm 0.39 ^{a,b}	7.02 \pm 2.06 ^{c,d}	2.30 \pm 0.21 ^a
400 mg/kg	7.83 \pm 1.32 ^b	3.22 \pm 0.73 ^{a,b}	6.02 \pm 2.24 ^{b,c}	2.40 \pm 0.46 ^a

Each value represents mean \pm SD, $n = 5$; Mean values in the same column with different alphabets as superscripts, when compared down the groups, are significantly different at $p < 0.05$.

3.5. Effects of EECO on MDA concentration of BPH-induced rats.

Subcutaneous testosterone injection provoked an increase ($p < 0.05$) in MDA concentration in BPH-control compared with baseline. Conversely, there was a dose-dependent decrease in MDA concentration in groups treated with the standard drug and varied doses of the

extract compared with BPH-control. The decrease in MDA concentration of the group treated with standard drug was found to be non-significantly ($p > 0.05$) lower than that of groups treated with 200 and 400 mg/kg of EECO (Figure 2).

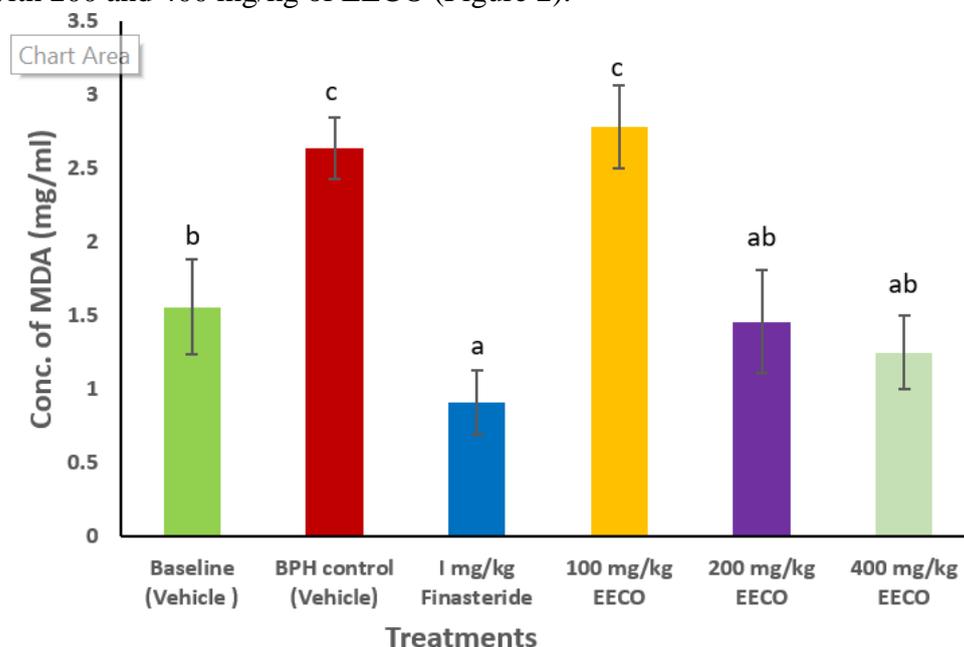


Figure 2. MDA concentration of BPH- induced rats. The values are represented as mean \pm SD (n = 5). Mean values with different alphabets as superscripts when compared across the groups are significantly different at $p < 0.05$.

3.6. Effects of EECO on antioxidant enzyme activities of BPH-induced rats.

Subcutaneous testosterone injection provoked a significant ($p < 0.05$) decrease in SOD and CAT activities in the rats induced with BPH and not treated when compared with the baseline. Conversely, there was a dose-dependent increase in SOD and CAT in groups administered 200 and 400 mg/kg of EECO compared with BPH-control. (Table 5).

Table 5. Effect of EECO on antioxidant enzymes of BPH-induced rats.

Treatments	SOD (U/mg)	CAT (U/mg)
Baseline (vehicle)	11.12 \pm 0.17 ^b	2.50 \pm 0.23 ^{a,b}
BPH control (vehicle)	10.76 \pm 0.21 ^a	2.05 \pm 0.18 ^a
Finasteride (1 mg/kg)	10.92 \pm 0.31 ^{a,b}	3.35 \pm 0.54 ^{a,b,c}
EECO 100 mg/kg	10.92 \pm 0.13 ^{a,b}	3.21 \pm 0.25 ^{b,c}
200 mg/kg	11.06 \pm 0.15 ^b	4.00 \pm 0.64 ^{c,d}
400 mg/kg	11.10 \pm 0.15 ^b	4.20 \pm 1.33 ^d

Each value represents mean \pm SD, n = 5; Mean values in the same column with different alphabets as superscripts, when compared down the groups, are significantly different at $p < 0.05$.

3.7. Effects of EECO on serum liver function enzyme activities of BPH-induced rats.

In this study, the results in Figure 3 showed that although the activity of aspartate aminotransferase (AST) did not increase significantly ($p > 0.05$) in BPH-control compared with baseline, treatments with 200 and 400 mg/kg b. w of the extract elicited a significant ($p < 0.05$) decrease in AST activity than that of BPH-control. The ALT activity of rats administered 400 mg/kg of EECO was similar to that of baseline. Similarly, AST and ALT activities of the standard group were comparable with the group treated with high doses of EECO but significantly lower ($p < 0.05$) than that of BPH-control.

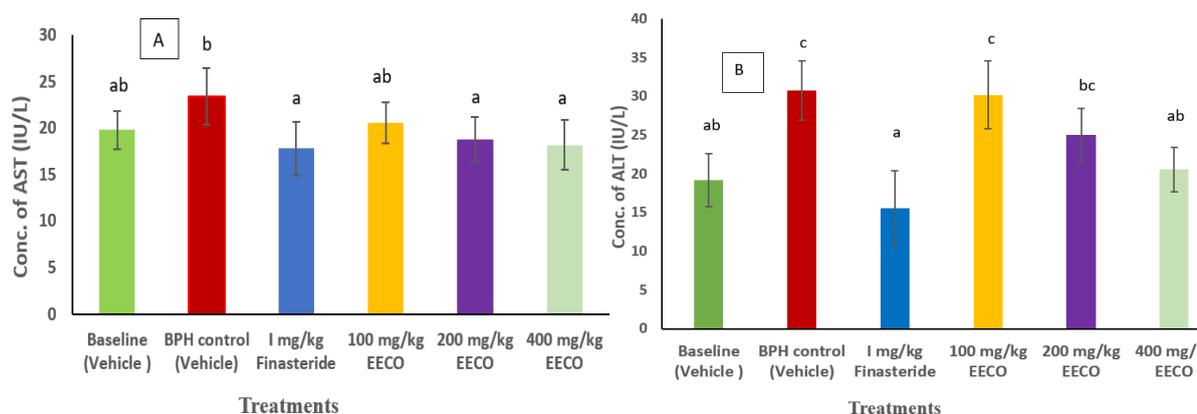


Figure 3. AST (A) and ALT (B) liver function enzymes activities of BPH- induced rats. The values are represented as mean \pm SD (n = 5). Mean values with different alphabets as superscripts when compared across the groups are significantly different at $p < 0.05$.

3.8. Effects of EECO on serum lipid profile of BPH-induced rats.

As shown in Table 6, the concentration of serum cholesterol, triacylglycerol (TAG) as well as low-density lipoprotein cholesterol (LDL-C) were higher ($p < 0.05$) in BPH-control than in the baseline. Compared with BPH-control, 400 mg/kg of EECO provoked a lower ($p < 0.05$) concentration of cholesterol. No significant ($p > 0.05$) differences in both cholesterol and TAG were observed in EECO, and baseline indicates inhibition of lipid peroxidation by the extract. In the same vein, treatment with higher doses of the extract (200 and 400 mg/kg b.w) and standard drug substantially increased ($p < 0.05$) high-density lipoprotein cholesterol (HDL-C), and decreased LDL-C levels in a manner comparable to baseline, also indicating inhibition of lipid anomalies in these groups (Table 6).

Table 6. Effects of EECO on serum lipid profile of BPH-induced rats.

Treatments	CHOL (mmol/L)	TAG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Baseline (vehicle)	4.74 \pm 1.76 ^a	1.82 \pm 0.20 ^{a,b}	5.04 \pm 0.39 ^{a,b,c}	1.48 \pm 0.41 ^a
BPH-control (vehicle)	7.82 \pm 1.36 ^b	2.29 \pm 0.42 ^c	3.88 \pm 1.18 ^a	2.32 \pm 0.52 ^b
Finasteride (1 mg/kg)	4.57 \pm 0.65 ^a	1.66 \pm 0.31 ^a	5.60 \pm 0.48 ^{b,c}	1.04 \pm 0.26 ^a
EECO 100 mg/kg	7.62 \pm 1.67 ^b	2.22 \pm 0.29 ^{b,c}	4.63 \pm 0.34 ^{a,b}	2.22 \pm 0.34 ^b
200 mg/kg	7.27 \pm 1.09 ^b	2.15 \pm 0.31 ^{b,c}	5.15 \pm 1.18 ^{b,c}	1.50 \pm 0.39 ^a
400 mg/kg	5.32 \pm 1.23 ^a	1.92 \pm 0.24 ^{a,b,c}	5.98 \pm 1.03 ^c	1.32 \pm 0.71 ^a

Each value represents mean \pm SD, n = 5. Mean values in the same column with different alphabets as superscripts compared to the groups are significantly different at $p < 0.05$.

3.9. Effects of EECO on prostate tissue histology.

The prostate glands in Group A had typical histomorphology, with normal acini (A) of different sizes, lined by cuboidal to low columnar epithelial cells with basally situated heterochromatic nuclei, thin fibromuscular stroma (S), and a few epithelial infoldings. Group B's prostate exhibited considerable benign prostatic hyperplasia aberrations compared to group A. Sloughing of the acinar epithelium (white arrow), loss of euchromatic nuclei, and polarity (red arrow), and thickening of the prostatic acini were all seen in group B. The histomorphology of groups B and D administered 100 mg/kg b. w of EECO did not differ significantly. However, treatment with the standard drug and 400 mg/kg body weight of EECO restored the central acini (A), intraepithelial stratification (arrow), and decreased epithelial height (Figure 4).

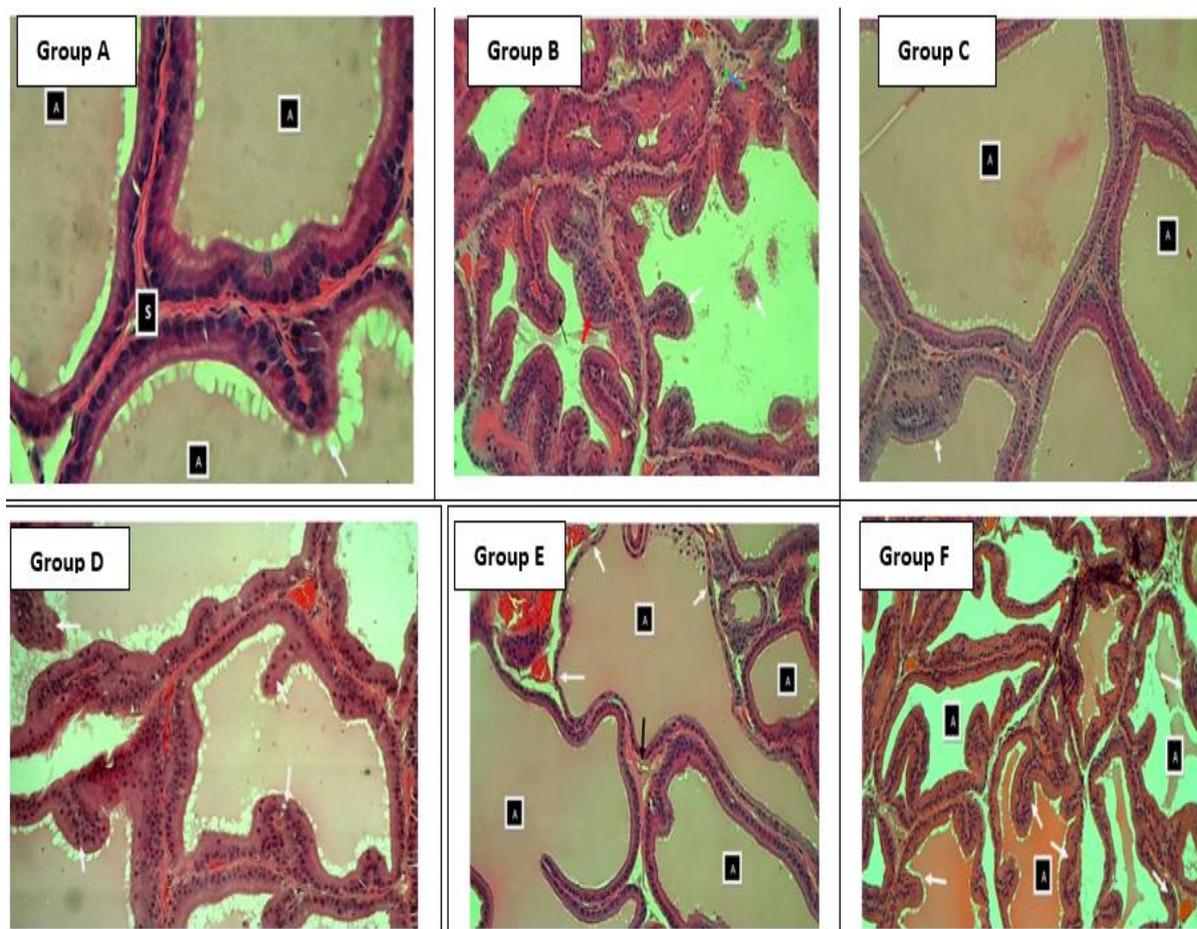


Figure 4. Photomicrographs of the prostate tissues of the rats in groups 1-6. **Key:** Group A: Baseline, group B: BPH-control, group C: treated with 1 mg/kg of standard drug, finasteride, groups D-F were treated with 100, 200, and 400 mg/kg b. w of EECO respectively.

4. Discussion

The results of this study show that *C. odorata* is endowed with abundant phytochemicals with pharmaceutical activities, such as tannins, saponins, flavonoids, alkaloids, steroids, glycosides, and other phytonutrients. The presence of these phytochemicals is the basis for the recent application of *Serenoa repens* (saw palmetto), *Prunus Africana*, and other herbal preparations to manage BPH [12]. These secondary metabolites have recently been harnessed as therapeutic agents in many diseases due to their biocompatibility and less toxic effects [34]. The mechanisms of action of these nutrients are mainly through inhibition of key enzymes involved in these pathways [35]. For instance, Nyamai *et al.* [12] reported the inhibitory effect of *Serenoa repens* stem bark extract on 5- α reductase isoenzymes.

An increase in PSA and prostate androgens (testosterone and DHT) are common biomarkers of BPH and testicular function [4, 36-39]. Interestingly, a remarkable normalization of these BPH biomarkers was observed in groups treated with *C. odorata* extract compared with the BPH -control. An increase in PSA is due to age-related prostate enlargement and decreased prostatic epithelium retention [40]. Concurrently, the synthesis of PSA is also dependent on the concentration of DHT in the prostatic tissue [37]. BPH patients have disrupted DHT and estrogen-mediated homeostasis which controls prostate proliferation and apoptosis [40]. The reduction of these prostate biomarkers suggests the anti-BPH effect of the plant and its potency as a novel agent in BPH treatment. Though the mechanisms of action of this extract were not determined, it could be hypothesized that the phytoconstituents present in the plant

had a modulatory effect on the BPH induction pathways, possibly by inhibiting 5 α -reductase or mopping up testosterone and thus reducing the level that will be converted to dihydrotestosterone as shown in the result. The attenuation of BPH by our extract agrees with what was reported by Ikeyi *et al.* [41] that crude methanol extract of *Zapoteca portoricensis* root and its fraction decreased DHT and PSA levels in BPH-induced rats. Our result is also in tandem with the observation made by Sun *et al.* [42] in BPH-induced rats treated with *Metapanax delavayi* leaf extract.

Oxidative stress is the key player in the pathogenesis of several diseases, including BPH [43], and plant antioxidants have a marked impact on averting BPH progression [38]. To ascertain the oxidative status of the BPH-induced albino rats, we measured the rats' MDA level and antioxidant enzyme activities. Our results portrayed a reduction in MDA level and amplification of the activities of antioxidant enzymes in groups treated with the extract, depicting a rebate of oxidative stress in these groups. A wealth of evidence has shown that an imbalance in the oxidative status of the prostate towards overproduction of high oxidants and radicals induces peroxidation of lipids, proteins, and DNA [44], thereby releasing a high level of MDA in the serum of patients [14]. Kaya *et al.* [43] opined that DNA damage in BPH patients initiated by a higher level of MDA activates cytotoxic and mutagenic pathways leading to prostate stimulation and genetic changes that induce BPH development [2,14]. Restoration of oxidative status by the phytoconstituents present in the extracts might have inhibited anomalies associated with BPH. Plant polyphenols repair the prostate antioxidant defense mechanisms, which ultimately reduce peroxidation, levels of pro-inflammatory markers and up-regulate apoptotic factors [7]. The antioxidant potency of this plant has been reported by several authors [17, 45-46]. Specifically, flavonoids found in the extract are known to play many roles in preventing the peroxidation of biomembranes [47]. Lending credence to the plants' antioxidant capacity is the recent flavanone, odoratenin (1), with very high antioxidant activity, isolated from the leaves by Putri and Fatmawati, [46]. Our findings support the reports of other researchers who also recorded an elevated level of MDA and a decrease in SOD and CAT activities in BPH patients [14, 43, 48].

Haemolysis and alteration in membrane permeability due to testosterone administration enhances leakage of hepatic enzymes into the serum [39]. Hence, the activities of AST and ALT are used as indicators of liver function [5]. Alteration in liver architecture (liver injury or bile duct blockage) is attributed to cell rupture, an influx of cellular components into the liver together with an increase in hepatocyte permeability [41]. The observed inhibition of the testosterone-induced increase in these liver markers compared with the control in this study could partly be attributed to the observed reduction in the serum testosterone concentration in rats treated with this plant extract in our study. Besides, it could be possible that the antioxidant potency of the plant also has immense benefits in protecting the integrity of the hepatic membrane, thereby preventing leakage of these liver markers into the serum. The observed aberrations in these liver markers in this study align with the report of Meludu *et al.* [5], who also identified a higher level of liver enzymes in BPH patients.

Age-induced metabolic abnormalities, including obesity, smoking, metabolic syndrome, and dyslipidemia, are implicated in both the onset and prognosis of BPH [49]. Hence, the need to investigate the effect of BPH-induction on the lipid indices of rats. Invariably, our extract's reduction in CHOL and other lipid markers suggests a decline in BPH progression. Dyslipidaemia in BPH patients is usually provoked by oxidative stress [41]. It is also worth noting that although cholesterol is a precursor for the synthesis of steroid hormones, elevated

cholesterol is a predisposing factor for BPH onset [50]. Our study's observed restoration of lipid indices could be due to the high quantity of saponins in the extract exerting hypolipidemic activity by forming a complex with cholesterol [51]. A positive correlation between BPH and dyslipidemia has been observed by other authors [4,9,41].

The limitations associated with this study, among others, are the slight genetic difference between rats' prostate and that of humans, which may interfere with the total generalization of the results to humans. Limitations associated with our study also include non-verbal communications between the researcher and the animals and peculiarities associated with individual rats. Such limitations were taken care of by the statistical tool (ANOVA) employed to analyze the results.

5. Conclusions

The results of this study showed that EECO has anti-BPH activity as portrayed by its attenuative effects on prostate biomarkers. Though the exact mechanisms of its action were not verified, the observed antioxidant activity might have prevented oxidative stress-induced aberrations, which are one of the key players in BPH progression. The rich phytonutrients in the extract could be responsible for its anti-BPH potency. Thus, EECO could be a promising phytotherapy in managing BPH in males. However, there is a need to characterize and identify the exact phytonutrient (s) responsible for this activity, which could serve as a lead compound (s) for anti-BPH drug design.

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Conflicts of Interest

None to declare.

References

1. Song, K.H.; Seo, C-S.; Yang, W-K.; Gu, H-O.; Kim, K-J.; Kim, S-H. Extracts of *Phyllostachys pubescens* Leaves Represses Human Steroid 5-Alpha Reductase Type 2 Promoter Activity in BHP-1 Cells and Ameliorates Testosterone-Induced Benign Prostatic Hyperplasia in Rat Model. *Nutrients* **2021**, *13*, 884–896, <https://doi.org/10.3390/nu13030884>.
2. Asare, G.A.; Andam, S.E.; Asare-Anane, H.; Ammanquah, S.; Anang-Quartey, Y.; Afriyie, D.K.; Musah, I. Lipid associated antioxidants: Arylesterase and paraoxonase-1 in benign prostatic hyperplasia treatment-naive patients. *Prostate Int* **2018**, *6*, 36–40, <https://doi.org/10.1016/j.pnrl.2017.04.002>.
3. Madersbacher, S.; Sampson, N.; Culig, Z. Pathophysiology of benign prostatic hyperplasia and benign prostatic enlargement: A mini-review. *Gerontology* **2019**, *65*, 458–464, <https://doi.org/10.1159/000496289>.
4. Fogaing, C.; Alsulihem, A.; Campeau, L.; Corcos, J. Is Early Surgical Treatment for Benign Prostatic Hyperplasia Preferable to Prolonged Medical Therapy: Pros and Cons. *Medicina* **2021**, *57*, 368–378, <https://doi.org/10.3390/medicina57040368>.

5. Meludu, S.C.; Ezenwelu, V.I.; Manafa, P.O.; Onah, C.E.; Ekuma-Okereke, O. Biochemical characteristics of liver enzymes, prolactin, zinc, and selenium in benign prostatic hyperplasia and cancer of the prostate patients attending urology clinic at Nnamdi Azikiwe University Teaching Hospital, Nnewi. *IJIR* **2017**, *3*, 223-232.
6. Yu, Z.J.; Yan, H.L.; Xu, F.H.; Chao, H.C.; Deng, L.H.; Xu, X.D.; Huang, J.B.; Zeng, T. Efficacy and Side Effects of Drugs Commonly Used for the Treatment of Lower Urinary Tract Symptoms Associated With Benign Prostatic Hyperplasia. *Front pharmacol* **2020**, *11*, 658, <https://doi.org/10.3389/fphar.2020.00658>.
7. Eleazu, C.; Eleazu, K.; Kalu, W. Management of benign prostatic hyperplasia: Could dietary polyphenols be an alternative to existing therapies? *Front Pharmacol* **2017**, *8*, 234, <https://doi.org/10.3389/fphar.2017.00234>.
8. Shoieb, A.E.; Sherif, M.; Amani, E.K.; Ashraf, B.A. Chrysin attenuates testosterone-induced benign prostate hyperplasia in rats. *Food Chem Toxicol* **2018**, *111*, 650–659, <https://doi.org/10.1016/j.fct.2017.12.017>.
9. Hashim, N.A.; Al-Ali, Z.A.J.R.; Syhood, A.A. Association between metabolic syndrome (MetS) and benign prostatic hyperplasia (BPH) in Amara city, Iraq. *Eurasia J Biosci* **2020**, *14*, 93-98, <https://doi.org/10.1097/MD.0000000000003243>.
10. Morton, A.; Williams, M.; Perera, M.; Teloken, P.E.; Donato, P.; Ranasinghe, S.; Chung, E.; Bolton, D.; Yaxley, J.; Roberts, M.J. Management of benign prostatic hyperplasia in the 21st century: temporal trends in Australian population-based data. *BJU Int* **2020**, *126*, 18–26, <https://doi.org/10.1111/bju.15098>.
11. Chughtai, B.; Forde, J.C.; Thomas, D.D.M.; Laor, L.; Hossack, T.; Woo, H.H.; Te, A.E.; Kaplan, S.A. Benign prostatic hyperplasia. *Nat Rev Dis Primers* **2016**, *2*, 16031, <https://doi.org/10.1038/nrdp.2016.31>.
12. Nyamai, D.W.; Arika, W.M.; Rachuonyo, H.O.; Wambani, J.R.; Ngugi, M.P. Herbal management of benign prostatic hyperplasia. *J Cancer Sci Ther* **2016**, *8*, 130-134, <https://doi.org/10.4172/1948-5956.1000404>.
13. Batai, K.; Phung, M.; Bell, R.; Lwin, A.; Hynes, K.A.; Price, E.; Meiklejohn, K.M.; Bracamonte, E.R.; Funk, J.T. Correlation between body mass index and prostate volume in benign prostatic hyperplasia patients undergoing holmium enucleation of the prostate surgery. *BMC Urol* **2021**, *21*, 88-95, <https://doi.org/10.1186/s12894-020-00753-9>.
14. Sarbishegi, M.; Khani, M.; Salimi, S.; Valizadeh, M.; Aval, F.S. Antiproliferative and antioxidant effects of *Withania coagulans* extract on benign prostatic hyperplasia in rats. *Nephro Urol Mon* **2016**, *8*, e33180, <https://doi.org/10.5812/numonthly.33180>.
15. Daniel, W.G.; Bashar, N.; Simon, P.; Rick, P.; Jens-Uwe, S.; Stuart, A.S. Prostate Benign Prostatic Hyperplasia. 3rd ed.; Omar M. Aboumarzouk. John Wiley & Sons Ltd. Leipzig, Germany, **2019**, 531-561.
16. Olawalea, F.; Olofinisan, K.; Iwaloye, O. Biological activities of *Chromolaena odorata*: A mechanistic review. *S Afr J Bot* **2022**, *144*, 44-57, <https://doi.org/10.1016/j.sajb.2021.09.001>.
17. Kanase, V.; Shaikh, S.A. Pharmacognostic and pharmacological review on *Chromolaena odorata* (siam weed). *Asian J Pharm Clin Res* **2018**, *11*, 34-38, <https://doi.org/10.22159/ajpcr.2018.v11i10.26863>.
18. Li, J.; Tian, Y.; Guo, S.; Gu, H.; Yuan, Q.; Xie, X. Testosterone-induced benign prostatic hyperplasia rat and dog as facile models to assess drugs targeting lower urinary tract symptoms. *PLoS one* **2018**, *13*, e0191469, <https://doi.org/10.1371/journal.pone.0191469>.
19. Ekeyi, Y.; Uchendu, N.O.; Anaduaka, E.G.; Ezeanyika, L.U.S. Ethanol extract of *Cassiasieberiana* leaves ameliorates deviances associated with benign prostatic hyperplasia in rats. *All Life* **2021**, *14*, 473-483, <https://doi.org/10.1080/26895293.2021.1927857>.
20. Harborne, J.B. Phytochemical Methods: A guide to modern technique of plant analysis. Chapman and Hall, London, **1998**, 49-188.
21. Trease, G.E.; Evans, W.C. Textbook of Pharmacology. 13th Ed.; Macmillan Publishers Limited, London, **1989**, 343-383.
22. Gad, S.C. Rodents model for toxicity testing and biomarkers. In Biomarkers in Toxicology, Cambridge, MA: Academic Press, **2014**, 7-69.
23. Lorke, D. Determination of acute toxicity. *Arch Toxicol* **1983**, *53*, 275-287, <https://doi.org/10.1007/bf01234480>.
24. Turkes, A.; Turkes, A.O.; Joyce, B.G.; Fahmy, D.R. A sensitive solid phase enzyme immunoassay for testosterone in plasma and saliva. *Steroids* **1979**, *33*, 347, [https://doi.org/10.1016/0039-128x\(79\)90011-4](https://doi.org/10.1016/0039-128x(79)90011-4).
25. Stowell, L.; Sharman, L.E.; Hamel, K. An enzyme-linked immunosorbent assay (ELISA) for prostate-specific antigen. *Forensic Sci Int* **1991**, *50*, 125-138, [https://doi.org/10.1016/0379-0738\(91\)90141-5](https://doi.org/10.1016/0379-0738(91)90141-5).

26. Wallin, B.; Rosengren, B.; Shertzer, H.G.; Camejo, G. Lipoprotein oxidation and measurement of TBARS formation in a single microtiter plate: Its use for evaluation of antioxidants. *Anal Biochem* **1993**, *208*, 10-15, <https://doi.org/10.1006/abio.1993.1002>.
27. Fridovich, I. Superoxide dismutase: An adaptation to a pragmatic gas. *J Biol Chem* **1989**, *264*, 7762-7764.
28. Aebi, H.E. Catalase. In: *Methods of enzymatic analysis*. 3rd ed.; Weinheim, Deerfield Beach, Florida, **1983**, 273-285.
29. Reitman, S.; Frankel, S.A. Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am J Clin Pathol* **1957**, *28*, 56-63, <https://doi.org/10.1093/ajcp/28.1.56>.
30. Allain, C.C.; Poon, L.S.; Chan, C.S.; Richmond, W.; Fu, P.C. Enzymatic determination of total serum cholesterol. *Clin Chem* **1974**, *20*, 470-475, <https://doi.org/10.1093/clinchem/20.4.470>.
31. Alber, J.J.; Warmick, G.R.; Cheng, M.C. Determining of high density lipoprotein (HDL)cholesterol. *Lipids* **1978**, *13*, 926-932, <https://doi.org/10.1007/BF02533852>.
32. Assmann, G.; Jabs, H.U.; Kohnert, U.; Nolte, W.; Schriewer, H. LDL-cholesterol determination in blood serum following precipitation of LDL with polyvinylsulfate. *Clin Chim Acta* **1984**, *140*, 77-83, [https://doi.org/10.1016/0009-8981\(84\)90153-0](https://doi.org/10.1016/0009-8981(84)90153-0).
33. Drury RAB, Wallington EA. *Careton's histological technique*, 4th ed. London: Oxford University Press. **1967**, 120-123.
34. Ekin, S.; Bayramoglu, M.; Goktasoglu, A.; Ozgokcen, F.; Kiziltas, H. Antioxidant activity of aqueous and ethanol extracts of *Crataegusmeyeri* pojarck leaves and contents of vitamin, trace element. *J Chil Chem Soc* **2017**, *62*, 3661-3667.
35. Crocetto, F.; Boccellino, M.; Barone, B.; Zazzo, E.D.; Sciarra, A.; Galasso, G.; Settembre, G.; Quagliuolo, L.; Imbimbo, C.; Bo, S.; Angelillo, I.F.; Domenico, M.D. The Crosstalk between prostate cancer and microbiota inflammation: Nutraceutical products are useful to balance This interplay? *Nutrients* **2020**, *12*, 2648-2668, <https://doi.org/10.3390/nu12092648>.
36. Mesbahzadeh, B.; Hassanzadeh-Taheri, M.; Aliparast, M.; Baniasadi, P.; Hosseini, M. The protective effect of crocin on cisplatin-induced testicular impairment in rats. *BMC Urol* **2021**, *21*, 117, <https://doi.org/10.1186/s12894-021-00889-2>.
37. Swerdloff, R.S.; Dudley, R.E.; Page, S.T.; Wang, C.; Salameh, W.A. Dihydrotestosterone: Biochemistry, Physiology, and Clinical Implications of Elevated Blood Levels. *Endocr Rev* **2017**, *38*, 220-254, <https://doi.org/10.1210/er.2016-1067>.
38. Mbaka, G.; Anunobi, C.; Ogunsina, S.; Osiagwu, D. Histomorphological changes induced in benign prostatic hyperplasia with exogenous testosterone and estradiol in adult male rats treated with aqueous ethanol extract of *Secamone afzelii*. *Egypt J Basic Appl Sci* **2017**, *4*, 15-21, <https://doi.org/10.1016/j.ejbas.2016.11.003>.
39. Soumanou, F.K.Y.; Avakoudjo, J.D.G.; Hodonou, D.F.; Ouake, K. Benign prostatic hyperplasia in 69 years old man with highest serum PSA level >3500 ng/mL. *Med Sur Urol* **2019**, *8*, 219.
40. McNally, C.J.; Ruddock, M.W.; Moore, T.; McKenna, D.J. Biomarkers that differentiate benign prostatic hyperplasia from prostate cancer: A literature review. *Cancer Manag Res* **2020**, *12*, 5225-5241, <https://doi.org/10.2147/CMAR.S250829>.
41. Ikeyi, A.P.; Okagu, I.U.; Ezeanyika, L.U.S.; Alumanah, E.O. *Zapoteca portoricensis* root crude methanol extract and its fractions normalize aberrations associated with benign prostatic hyperplasia in rats. *Front Life Sci* **2020**, *13*, 360-372, <https://doi.org/10.1080/26895293.2020.1788653>.
42. Sun, C.; Peng, Y.; Wu, Y.; Zhang, Y.; Li, X. The effect of *Metapanax delavayi* leaf extract on testosterone-induced benign prostatic hyperplasia in rats. *J Funct Foods* **2020**, *66*, 103797, <https://doi.org/10.1016/j.jff.2020.103797>.
43. Kaya, E.; Ozgok, Y.; Zor, M.; Eken, A.; Bedir, S.; Erdem, O.; Ebiloglu, T.; Ergin, G. Oxidative stress parameters in patients with prostate cancer, benign prostatic hyperplasia and asymptomatic inflammatory prostatitis: A prospective controlled study. *Adv Clin Exp Med* **2017**, *26*, 1095-1099, <https://doi.org/10.17219/acem/66837>.
44. Shukla, S.; Srivastava, J.K.; Shankar, E.; Kanwal, R.; Nawab, A.; Sharma, H.; Bhaskaran, N.; Ponsky, L.E.; Fu, P.; MacLennan, G.T.; Gupta, S. Oxidative Stress and Antioxidant Status in High-Risk Prostate Cancer Subjects. *Diagnostics* **2020**, *10*, 126-136, <https://doi.org/10.3390/diagnostics10030126>.
45. Kikiowo, B.; Ogunleye, J.A.; Iwaloye, O.; Ijatuyi, T.T. Therapeutic potential of *Chromolaena odorata* phytoconstituents against human pancreatic α -amylase. *J Biomol Struct Dyn* **2020**, <https://doi.org/10.1080/07391102.2020.1833758>.

46. Putri, D.A.; Fatmawati, S.A. New Flavanone as a Potent Antioxidant Isolated from *Chromolaena odorata* L. Leaves. *Evid Based Complement Alternat Med* **2019**, 1453612, <https://doi.org/10.1155/2019/1453612>.
47. Tiwari, S.C.; Husain, N. Biological activities and role of flavonoids in human health—a review. *Indian J Sci Res* **2017**, *12*, 193-196.
48. Zhang, L.; Fan, X-R.; Xie, H.; He, Q-H.; Nie, Y-S.; Zhang, M.; Yan, M. Anti-inflammatory and antioxidant effects of Kelong-Capsule on testosterone-induced benign prostatic hyperplasia in rats. *Evid Based Complementary Altern Med* **2018**, <https://doi.org/10.1155/2018/5290514>.
49. Matsushita, M.; Fujita, K.; Nonomura, N. Influence of Diet and Nutrition on Prostate Cancer. *Int J Mol Sci* **2020**, *21*, 1447-1464, <https://doi.org/10.3390/ijms21041447>.
50. Habu, J.B.; Ibeh, B.O. *In vitro* antioxidant capacity and free radical scavenging evaluation of active metabolite constituents of *Newbouldia laevis* ethanolic leaf extract. *Biol Res* **2015**, *48*, 16-25, <https://doi.org/10.1186/s40659-015-0007-x>.
51. Orczyk, M.; Wojciechowski, K.; Brezesinski, G. The influence of steroidal and triterpenoid saponins on monolayer models of the outer leaflets of human erythrocytes, *E. coli* and *S. cerevisiae* cell membranes. *J Colloid Interface Sci* **2020**, *563*, 207-217.