


Administration of *Caesalpinia bonduc* Seed Extracts Ameliorates Testosterone-Induced Benign Prostatic Hyperplasia (BPH) in Male Wistar Rats

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Introduction: Benign prostatic hyperplasia (BPH) is a major chronic disease affecting men, and the therapeutic agents currently used to manage it have significant side effects. As a result, an alternative medicine with improved therapeutic properties with no side effects is desperately needed. The current investigation aims to study whether the *Caesalpinia bonduc* seed extracts (ethanolic-A, hydroalcoholic-B, and aqueous-C) have inhibitory potential on testosterone propionate (TP)-induced BPH in Wistar rats.

Methods: Wistar rats (male) were randomly allocated to one of five groups: control, BPH (TP-3 mg/kg, subcutaneously daily), low dose (TP + *C. bonduc* seed extracts – 200 mg/kg body weight), high dose (TP + *C. bonduc* seed extracts – 400 mg/kg body weight), and standard drug (TP + finasteride – 10 mg/kg body weight). At the end of drug treatment, the rats were sacrificed and their serum and prostates were taken for biochemical and histological studies.

Results: *C. bonduc* seed extracts treatment significantly decreased prostate weight and prostatic index in rats with TP-induced BPH. The seed extracts exhibited a potent inhibitory effect on dihydrotestosterone (DHT) in serum and prostate. In addition, the PSA level in the serum showed a noteworthy decrease in comparison with the BPH group. Histopathological examination also indicated that extracts improved the tissue morphology of the prostate significantly. Out of three extracts tested, ethanolic and hydroalcoholic extract recorded significant effect. Finally, liquid chromatography quadrupole time-of-flight mass spectrometry (LC/MS-QTOF) analysis showed that the major compounds present in the extracts were tocopherols, fucosterol, linoleic acid, β -amyrin, β -sitosterol, campesterol, cassane furanoditerpene, norcassane furanoditerpene and other diterpenes.

Conclusion: Thus, *C. bonduc* seed extracts could be a potential source for the formulation of new drug for managing BPH. To the best of our knowledge, this is the first scientific animal investigation into the use of *C. bonduc* seed extract for the management of BPH.

Keywords: *Caesalpinia bonduc* seed extracts, benign prostatic hyperplasia, testosterone, Wistar rats, prostate gland

Introduction

Benign prostatic hyperplasia (BPH) is the topmost prominent disease affecting the prostate gland in older men and approximately one-half of all men aged above 50 years will have this disease.^{1–3} The BPH condition is characterized by an abnormally enlarged prostate gland, which induces urethral constriction, resulting in increased frequency, urgency, and hesitancy of urination, as well as disrupted urine flow, which eventually affects the quality of life of the patients.³ Thus far, the pathophysiology of BPH is not fully elucidated, although several attempts have been made in the previous years. However, previous research studies have established that a discrepancy between androgens and estrogens plays a critical role in the development and progression of BPH.^{4,5}

Even though the pathway associated with prostatic enlargement is not fully understood, androgens are regarded as one of the most significant factors. Although androgens do not directly cause BPH, testicular androgens play an important role in the development of prostatic growth in men.⁶ Dihydrotestosterone (DHT) is likely to be the most vital factor associated with the enlargement of prostate. It was reported that the DHT concentrations in the serum are higher in BPH

patients than in unaffected men of similar age.⁷ In the prostate, 5 α -reductase converts testosterone into DHT.⁸ Because of its higher affinity for the androgen receptor, DHT is a more potent androgen than testosterone. DHT is an active metabolic product of testosterone reduction by 5 α -reductase. It is essential for prostate growth because it binds to the nuclear androgen receptor, causing the synthesis of growth factors acting on prostatic epithelia and stroma, resulting in prostate enlargement.⁹ Experiments have also revealed that estrogen levels rise with age, potentially increasing the expression of DHT, the progenitor of BPH.¹⁰ The current use of 5 α -reductase inhibitors in the treatment of BPH is based on the incrimination of DHT in the pathogenesis of BPH.

The management options available for BPH include watchful waiting, drug treatment and finally surgery. Conventional drugs that manage BPH include 5 α -reductase inhibitors (finasteride and dutasteride) and α 1-adrenergic antagonists (alfuzosin, doxazosin, tamsulosin and terazosin). The α 1-adrenergic antagonists are the early drugs for treating BPH, and they improve lower urinary tract symptoms (LUTS) by relaxing the smooth muscle in the prostate and the neck of the bladder.¹¹ However, the 5 α -reductase inhibitors inhibit the enlargement of BPH by reducing the production of DHT.¹² Even though the drugs mentioned above have ample effectiveness in treating BPH, their severe side effects should not be neglected. To put this into perspective, some of the severe adverse effects of 5 α -reductase inhibitors include impotence, gynecomastia, impairment of muscle growth, and reduced libido. On the other hand, α 1-adrenergic antagonists cause orthostatic hypotension, fatigue, dizziness and abnormal ejaculation. Additionally, the required long-term drug treatment to manage the condition and any surgical treatments are expensive and risky for aged men, which ignores their use as routine treatment.¹³ This warrants a search for new effective and safe drugs for the BPH patients. Medicinal plants are currently being researched for the treatment of BPH patients, and shallow research shows that many BPH patients use complementary and alternative medicines along with their prescriptions to manage their condition.^{14,15}

Regardless of the several endeavors in medication therapy, there is still a need to dig out a new effectual treatment for managing this disease that can deal with its significant potency and fewer side effects in the long-term use.¹⁶ Hence, the present investigation aimed to study the effects of the seed extracts of a renowned medicinal plant called *Caesalpinia bonduc*. *C. bonduc* (L.) Roxb., one of the pantropical leguminous scandent shrubs that has been explored by numerous researchers for multiple medicinal uses worldwide.^{17,18} This plant belongs to the family of Caesalpiniaceae which is generally known as Fever Nut or Nicker Nut.^{19,20} Since ancient times, all parts of this plant have had significant therapeutic value and have been used in traditional medicine.²¹ Additionally, this plant is considered as an essential remedy for several major ailments because of its significant therapeutical effects.²¹

In folklore medicine, the seeds of *C. bonduc* have been used as antiinflammatory agents. The seed oil and the kernel extracts have been screened for antiinflammatory and antipyretic activity. The steroids and terpenes present in the seed are responsible for the antiinflammatory property.²² Anticancer and cytotoxic activities of various parts of *C. bonduc*^{23,24} and its isolated phytoconstituents^{25,26} have been reported earlier. Furthermore, in a study, the anti-androgenic potential of *C. bonduc* seed extract was highly evidenced in male Wistar rats.²⁷ The seed is known to contain fatty oil, starch, sucrose, phytosterols, stearic, palmitic, oleic, linoceric, linolenic and a mixture of unsaturated acid of low molecular weights¹⁷ and this clearly indicates the presence of various bioactive metabolites. By considering all these facts, an effort was made to evaluate the amelioration of *C. bonduc* seed extract on testosterone propionate (TP) induced BPH in experimental animals.

In our study, therefore, we investigated the inhibitory effects of the seed extracts of *C. bonduc* on the TP-induced BPH rat model by measuring changes in prostate weight and the inhibition of DHT, and prostate-specific antigen (PSA) as well as prostate histopathology. The results from the present clearly indicated that seed extract of *C. bonduc* might be a novel candidate medication for treating BPH shortly.

Materials and Methods

Study Plant – *C. bonduc*

In this study, we have used dried seeds of *C. bonduc* to prepare the extracts. The *C. bonduc* seeds were collected from the Ernakulam region of Kerala State, India, and identified by Dr. Dan Mathew, Senior scientist at Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode, Trivandrum, Kerala, India. The voucher specimens were also deposited at the same institute.

Preparation of Seed Extract

The *C. bonduc* seeds were thoroughly washed in distilled water and dried to a constant weight at 50°C in a hot air oven for 1 h. After that, the seeds were crushed using a herb crusher or choppers to smash them into a colloidal mass. The ethanolic, hydroalcoholic and aqueous extracts of the seed materials were prepared separately by macerating 200 gm of powdered seed sample in 1 L of ethanol, water: alcohol (1:1) and water for 72 h, after which the suspended materials were carefully filtered. The extraction procedure was repeated twice, and all filtrates were combined and separately concentrated with a rotary evaporator at 40°C in vacuum. The residual solvent was evaporated to dryness with the help of a water bath. The extracts thus obtained were stored in the refrigerator for future studies.

Ethical Approval

The experimental protocol of the present study was reviewed thoroughly and approved with reference no. CKL/TOX/IAEC/2017-3/89 by the Institutional Animal Ethical Committee of CARE KERALAM Ltd., KINFRA Park P.O, Koratty, Thrissur, Kerala 680309 (Reg. No.1620/PO/RcBi/S/12/CPCSEA). The rats were kept in accordance with the guidelines published by CPCSEA (The Committee for the Purpose of Control and Supervision of Experiments on Animals), Government of India.

Study Animals

The candidates chosen for this study were seven-week-old healthy male Wistar rats (175–250 g). All rats were housed in clean plastic cages with a 12-hour light/dark cycle (temperature 22°C, relative humidity 55%). The rats were allowed to access water and food freely throughout the experiment tenure. Rats were also allowed to acclimatize before the commencement of the study. All the animal experiments were strictly conducted conforming to the protocols approved by the Institutional Animal Care Committee of CARE KERALAM Ltd., Koratty, Thrissur, Kerala, India.

Induction of BPH in the Experimental Rats

BPH was induced in rats through daily subcutaneous injections (s.c.) of TP (3 mg/kg, dissolved in corn oil) for 28 days.

Study Design

The rats were randomized into five groups; Group 1 received distilled water (control), Group 2 received TP injection along with distilled water and acted as a BPH induced control, Group 3 (low dose) was divided into three subgroups and each subgroup separately received 200 mg/kg body weight of ethanolic, hydroxyethanolic and aqueous seed extract of *C. bonduc* along with TP injection, Group 4 (high dose) also divided into three subgroups and each subgroup separately received 400 mg/kg body weight of ethanolic, hydroxyethanolic and aqueous seed extract of *C. bonduc* along with TP injection. Finally, Group 5 (standard drug) received TP injection along with finasteride treatment (10 mg/kg body weight, orally). The animal grouping and treatment process are summarized in Table 1.

Table 1 Grouping of Rats and the Treatment Details

	Groups	Treatment Details for 28 Days
Group-I (Control)		Normal rats received distilled water (1 mL/100g body weight, orally).
Group-II (BPH control)		Rats received TP (3mg/kg, subcutaneously) + Distilled water (1mL/100g body weight, orally).
Group-III Low dose	Subgroup 1	Rats received TP (3mg/kg, subcutaneously) + <i>C. bonduc</i> ethanolic extract (200mg/kg body weight, orally).
	Subgroup 2	Rats received TP (3mg/kg, subcutaneously) + <i>C. bonduc</i> hydroalcoholic extract (200mg/kg body weight, orally).
	Subgroup 3	Rats received TP (3mg/kg, subcutaneously) + <i>C. bonduc</i> aqueous extract (200mg/kg body weight, orally).
Group-IV High Dose	Subgroup 1	Rats received TP (3 mg/kg, subcutaneously) + <i>C. bonduc</i> ethanolic extract (400mg/kg body weight, orally).
	Subgroup 2	Rats received TP (3 mg/kg, subcutaneously) + <i>C. bonduc</i> hydroalcoholic (400mg/kg body weight, orally).
	Subgroup 3	Rats received TP (3 mg/kg, subcutaneously) + <i>C. bonduc</i> aqueous extract (400mg/kg body weight, orally).
Group-V Standard drug		Rats received TP (3mg/kg, subcutaneously) + Finasteride (10mg/kg body weight, orally).

Abbreviations: BPH, Benign prostatic hyperplasia; TP, Testosterone propionate.

The weights of the rats were carefully recorded before the initiation of the experiments and consequently every week until the termination of the experiment. All the test materials (*C. bonduc* seed extracts) were administered to the rats in the morning for 28 days. Following the final administration of test material and overnight fasting, the rats were anesthetized using pentobarbital (50 mg/kg, intraperitoneal injection). After that, blood samples were taken for examination and prostate tissues were dissected and weighed right away. The sections prostate lobe was fixed with paraformaldehyde (4%) for histopathological analysis, and the leftover prostate sections were stored at -80°C for further examination.

Ratio of Body and Prostate Weights

At the terminal sacrifice of the study, the body weight (BW) and prostate weight (PW) of each animal were recorded. Following this, group mean body weights were calculated. For each individual study group animal, the prostate weight (P) to body weight (BW) ratio was calculated by dividing the prostate weight by the animal's body weight.

Percentage of Recovery

We have calculated the percentage recovery in P/BW ratio by test group versus control group based on mean prostatic weight and P/BW ratios. The dilation caused by TP alone was assumed to be 100%, and the values of all other test groups were compared to this reference. The formula used for the calculation is as follows:

Percent recovery by the test sample = $[A - B]$

Where A is the percentage increase in prostatic weight caused by TP, and B is the percentage increase in prostatic weight caused by test materials.

Percentage of Inhibition of Increase in Prostate Weight

The percentage inhibition (%) of the increase in prostate weight caused by the treatment of *C. bonduc* seed extracts was calculated using the formula below:²⁸

$$100 - \left\{ \left[\frac{PW \text{ of treatedgroup} - PW \text{ of negativecontrol}}{PW \text{ of modelgroup} - PW \text{ of negativecontrol}} \right] \times 100 \right\}$$

Determination of DHT Levels in Serum and Prostate

The rats underwent fasting overnight on the 28th day following the final administration of *C. bonduc* seed extracts and sacrificed the next day. Blood samples were carefully collected in test tubes, kept for 1 h and then rotated for 15 min at 3000 rpm to obtain serum, which was used for biochemical investigation. After that, approximately 100 mg of prostate tissue was rinsed with $1\times$ phosphate buffered saline (PBS), homogenized in 1 mL of $1\times$ PBS and stored overnight at -20°C . Following this, the homogenates were centrifuged for 5 minutes at 5000 rpm in 4°C . The supernatant was removed and assayed immediately. The levels of DHT in serum and the prostate were determined using an enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer's instructions (Cusabio Biotech Co., Ltd. China). The absorbance was measured using a microplate ELISA reader at 450 nm. Values are expressed as pg/mL tissue homogenate for the prostate and pg/mL for serum.

Determination of the Levels of Prostate-Specific Antigen (PSA) in the Serum

The serum PSA levels were measured using an ELISA kit following the manufacturer's instructions (Cusabio Biotech Co., Ltd., China). The absorbance of the color intensity was measured at 450 nm. After that, the concentration of PSA in the samples was expressed as ng/mL in the serum.

Histopathological Studies Using Hematoxylin and Eosin (H&E) Staining

The histological examination of the prostate tissue was performed according to the method of Bianchi-Frias.²⁹ The prostate tissue was fixed in 10% neutral buffered formalin. The fixed prostate tissues were dehydrated with subsequent 50%, 70%, 90%, and 100% ethanol and then cleared in xylene. After incubation in paraffin at 60°C in an incubator, they were embedded and blocked-in paraffin at the same temperature. Fine serial sections (5 μm thick) obtained by cutting the

embedded tissue using a microtome then mounted on gelatinous water coated slides and dried for 24 h at room temperature. The sections on the slides were deparaffinized using xylene, rehydrated in a descending series of alcohol and water, before stained with hematoxylin and eosin dyes. After that, the slides were dried and mounted on a light microscope for microscopic examination at 200x magnification for histopathological analysis.

Characterization of Active Compounds Through LC/MS-QTOF Analysis

The characterization of bioactive compounds was carried out by LC-QTOF (Agilent Technologies, Santa Clara, CA, USA, Model no: 6545 QTOF).

Statistical Analysis

The data were expressed as mean \pm standard deviation. The averages were analyzed using a one-way analysis. The post-test analysis was performed using Dunnett's multiple comparison tests to determine the comparison between the treatment and control groups. Values were considered significantly different when P value < 0.05 . The analyses were done using the GraphPad Prism software version 5.00 (GraphPad Prism Software, Inc., San Diego, California, 2007).

Results

Effect of *C. bonduc* Seed Extract on the Prostate Weight

Relative prostate weight is normally used to evaluate the growth of BPH. Rats in the TP-induced BPH group recorded significantly greater absolute and relative prostate weights than those of rats in the normal control group. Prostate weights in the ethanolic (A) and hydroalcoholic (B) extracts treated groups, at 400 mg/kg, were decreased appreciably ($P < 0.05$) and exhibited significant decrease in relative prostate weights or PW index compared to the BPH group ($P < 0.01$ and $P < 0.05$ respectively) (Figure 1). In addition, *C. bonduc* seed extracts inhibited the testosterone-induced increase in prostate weight by 41.74% [ethanolic (A) - 41.94%, hydroalcoholic (B) - 52.47% and aqueous (C) - 30.79%] in the 200 mg/kg dose group and by 53.18% [ethanolic - 80.22%, hydroalcoholic - 67.46% and aqueous - 11.87%] in the 400 mg/kg dose group (Figure 2). The *C. bonduc* seed extracts also recorded a significant percentage recovery of prostate. At 400 mg/kg dose, ethanolic extract recorded 46.27% recovery of the prostate, whereas hydroalcoholic and aqueous extract recorded 38.91% and 6.84% recovery of the prostate when compared to the untreated group (Figure 3). Surprisingly, the finasteride treated groups recorded only 18.92% recovery of the prostate. In the present study, the finasteride treated group did not show any significant treatment-related changes in the prostatic parameters. We could not find any statistically significant differences in body weight changes between the groups throughout the study.

Effect of *C. bonduc* Seed Extract on the Levels of DHT in Serum and Prostate Tissue

Prostate enlargement is dependent on the prostatic androgen DHT. In the prostate gland 5α -reductase metabolizes testosterone to DHT which in turn binds to receptors in the cell, resulting in BPH. The BPH induced control group recorded a significant increase in serum DHT level (1599.33 ± 305.934 pg/mL, $P < 0.001$) compared with the normal control group (153.667 ± 52.413 pg/mL) (Figure 4). However, the high dose of *C. bonduc* seed extracts (A, B & C) treated groups recorded a remarkably reduced serum DHT level (561.50 ± 23.50 , 563.00 ± 24.00 and 563.50 ± 1.50 pg/mL, $P < 0.05$) compared with the BPH group (Figure 4). The extract treatment showed significant reduction in prostate DHT levels (A- 510.00 ± 2.00 , B - 496.500 ± 4.50 pg/mL, $P < 0.01$ and C- 320.00 ± 1.00 pg/mL, $P < 0.001$) in the 200 mg/kg group and (A- 339.00 ± 2.00 , B- 202.50 ± 2.50 and C- 240.00 ± 2.00 pg/mL) in the 400 mg/kg group ($P < 0.001$) compared to the BPH group (Figure 5). There was no treatment related significant reductions in serum and prostate DHT levels in the finasteride treated groups in the present study.

Prostate-Specific Antigen (PSA) Levels in Serum

There was a rise in PSA level to 4.017 ± 0.075 ng/mL in BPH induced rats, which indicated a significant increase ($P < 0.001$) in the value when compared to that of the normal rats (1.517 ± 0.142 ng/mL). The PSA level in the ethanolic and hydroalcoholic extract treatment groups (200 and 400 mg/kg) showed a marked decrease of 39.41% (2.43 ng/mL) and 49.17% (1.98 ng/mL), respectively (Figure 6).

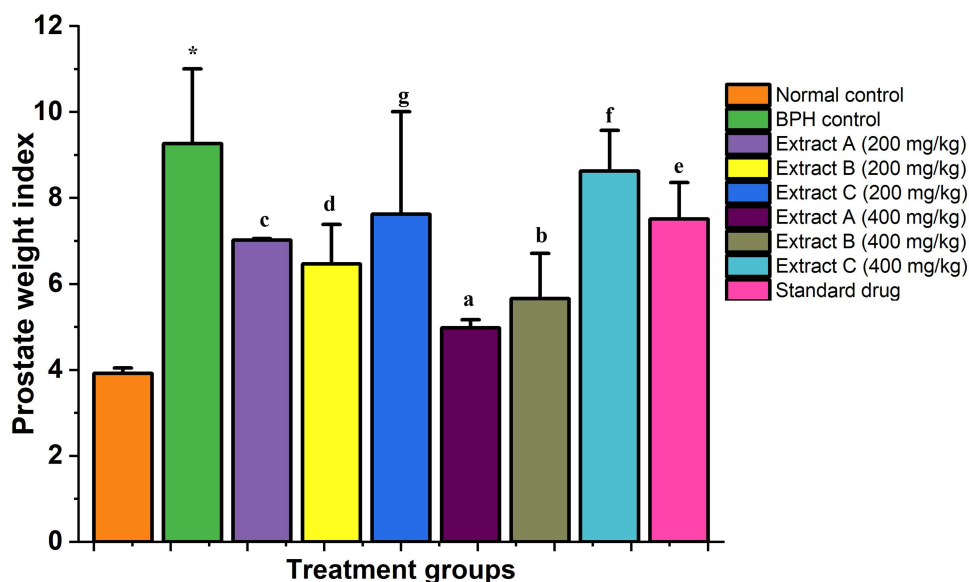


Figure 1 The effect of *C. bonduc* seed extracts on prostate weight index in Wistar rats with TP induced BPH. Values are represented as the mean \pm standard deviation. The * in the BPH control group indicated that the value was significantly different from the normal control group. In the treatment groups, the values with different letters (a–g) significantly differ from the BPH group.

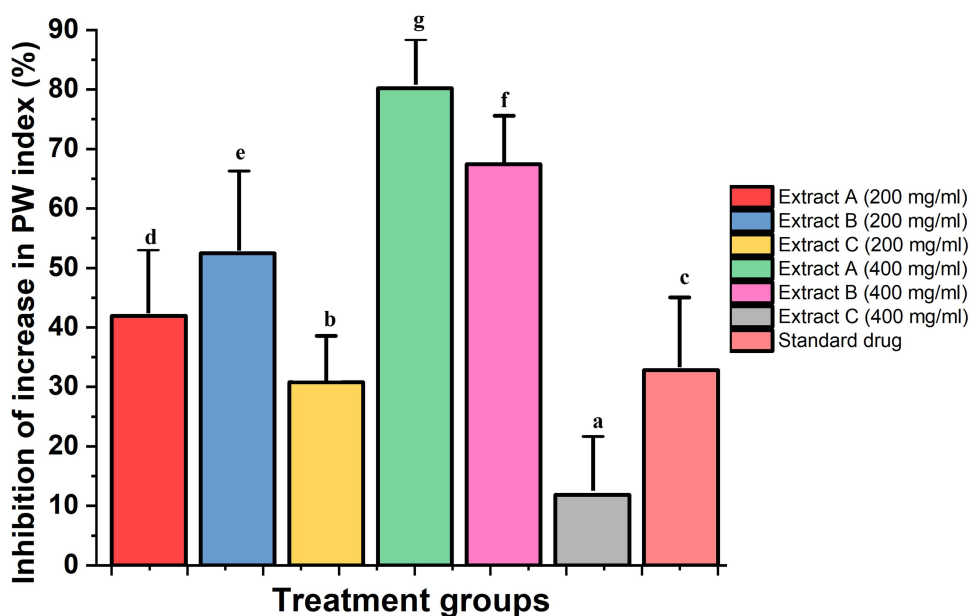


Figure 2 The percentage inhibition of the increase in prostate weight induced by treatments. The values with different letter (a–g) are significantly different ($p < 0.05$) for each group.

Histopathological Evaluations

On gross examination, marked enlargement of the prostates without any visible lesions was observed in the different experimental groups.

The prostate of rats generally consists of three distinct lobes, including dorsal, lateral and ventral lobes. We have analyzed the ventral lobe of prostate tissue for various histopathological changes.^{30,31} Histological sections from the control group showed a normal architecture of the ventral prostate lobe characterized by columnar epithelium with round-shaped nuclei adjacent to the intact basal membrane and regular acini. Furthermore, the lumen contains only a small number of papillary folds. The lumens were filled with prostatic secretions in connective tissue and further the matrix was observed normal. Overall, histopathology revealed a normal prostate gland (Figure 7A).

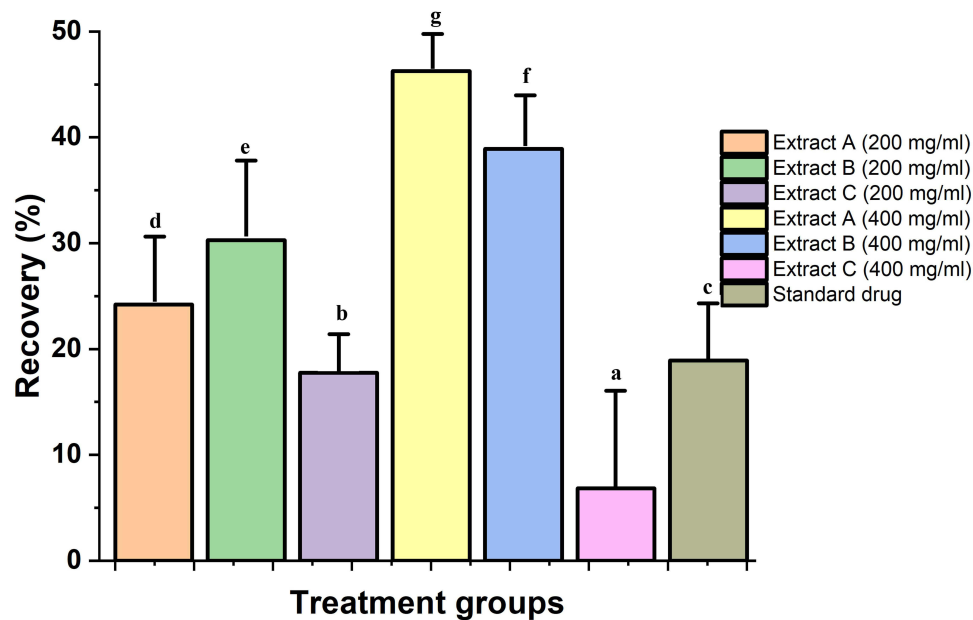


Figure 3 The percentage recovery of animals treated with *C. bonduc* seed extracts at 200 mg/kg and 400 mg/kg. The values with different letter (a–g) are significantly different ($p < 0.05$) for each group.

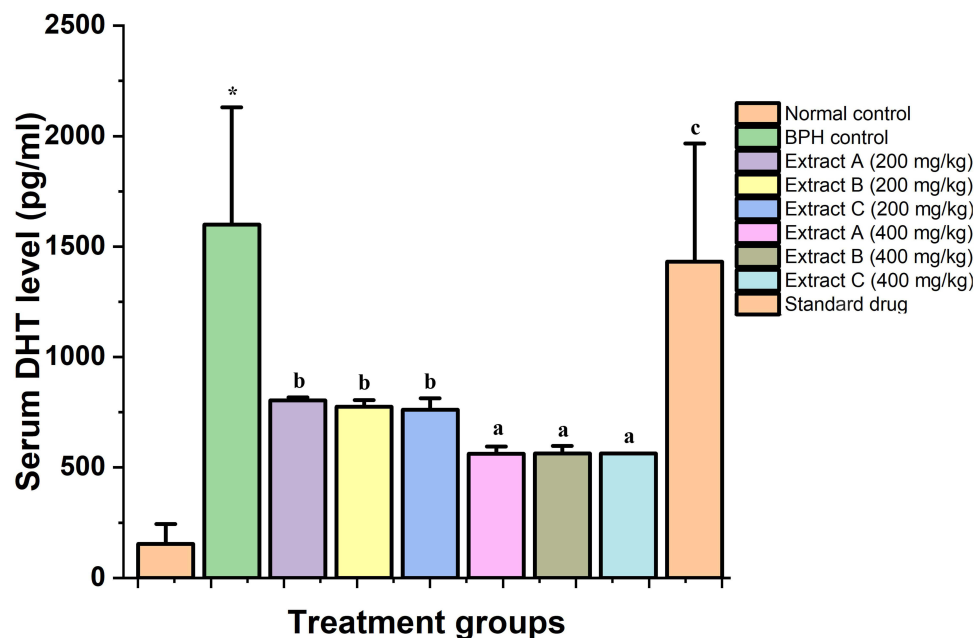


Figure 4 The effect of *C. bonduc* seed extracts on the levels of DHT in serum. Values are mean \pm SD. Values are represented as the mean \pm standard deviation. The *In the BPH control group indicated that the value was significantly different from the normal control group. In the treatment groups, the values with different letters (a–c) significantly differ from the BPH group.

In contrast, the BPH-induced group showed marked epithelial hyperplasia with numerous intraluminal folds, resulting in the lumen narrowing. These animals exhibited greatly increased prostatic epithelial thickness compared to those from the normal control group. In addition to this, the walls of tubules were observed thickened when compared to the control group (Figure 7B). Nearly every tubule developed large involutions which project into the lumen, resulting in the reduction of lumen volume compared with the control group. Overall histopathology for this group revealed BPH condition (Figure 7B).

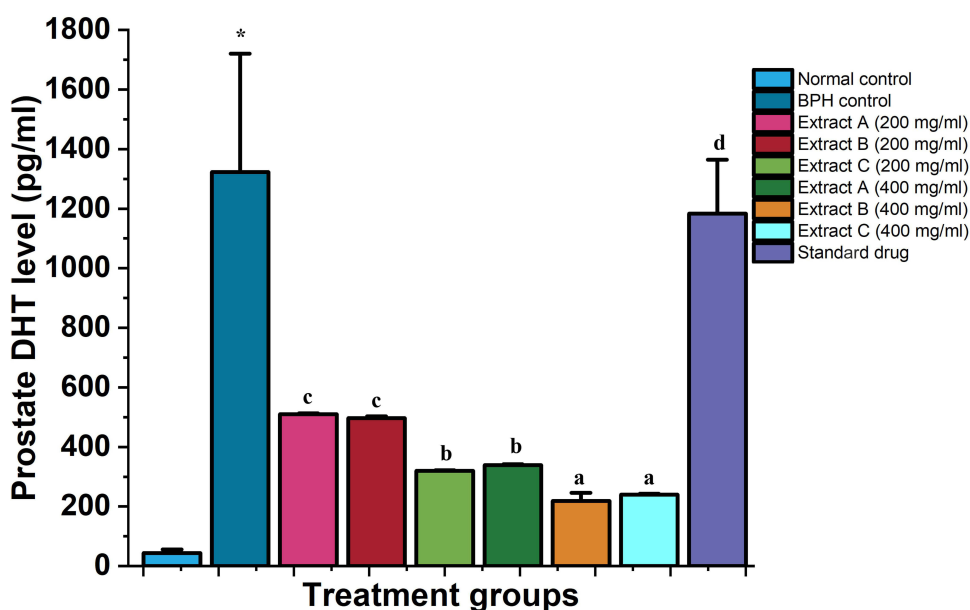


Figure 5 The effects of *C. bonduc* seed extracts on DHT levels in prostate tissue. Values are represented as the mean \pm standard deviation. The * in the BPH control group indicated that the value was significantly different from the normal control group. In the treatment groups, the values with different letters (a–d) significantly differ from the BPH group.

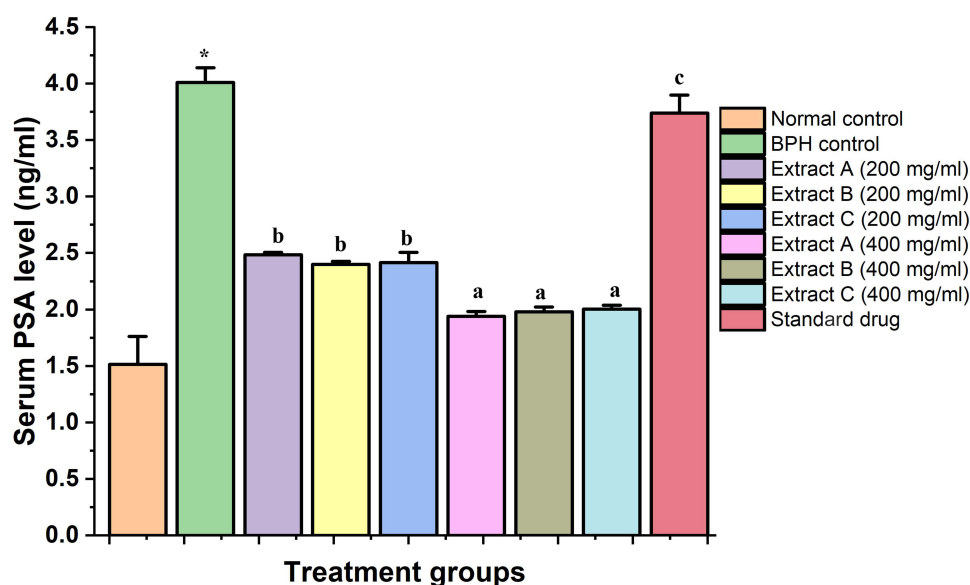


Figure 6 Effects of *C. bonduc* seed extracts on serum PSA level. Values are represented as the mean \pm standard deviation. The * in the BPH control group indicated that the value was significantly different from the normal control group. In the treatment groups, the values with different letters (a–c) significantly differ from the BPH group.

The animals from the group treated with the extracts at 200 mg/kg recorded only a mild reduction in the epithelial thickness compared with those from the BPH group (Figure 7C–E). However, rats treated with *C. bonduc* seed extracts (A, B and C) at 400 mg/kg displayed remarkably reduced hyperplasia symptoms compared with those from the BPH group. Furthermore, this group demonstrated a significant reduction in the thickness of the epithelium wall of their prostatic tubules, implying a significant reduction in histoarchitectural disruption of the prostate (Figure 7F–H). The finasteride treated group also showed only a mild reduction in the epithelial thickness when compared to the BPH group (Figure 7I).

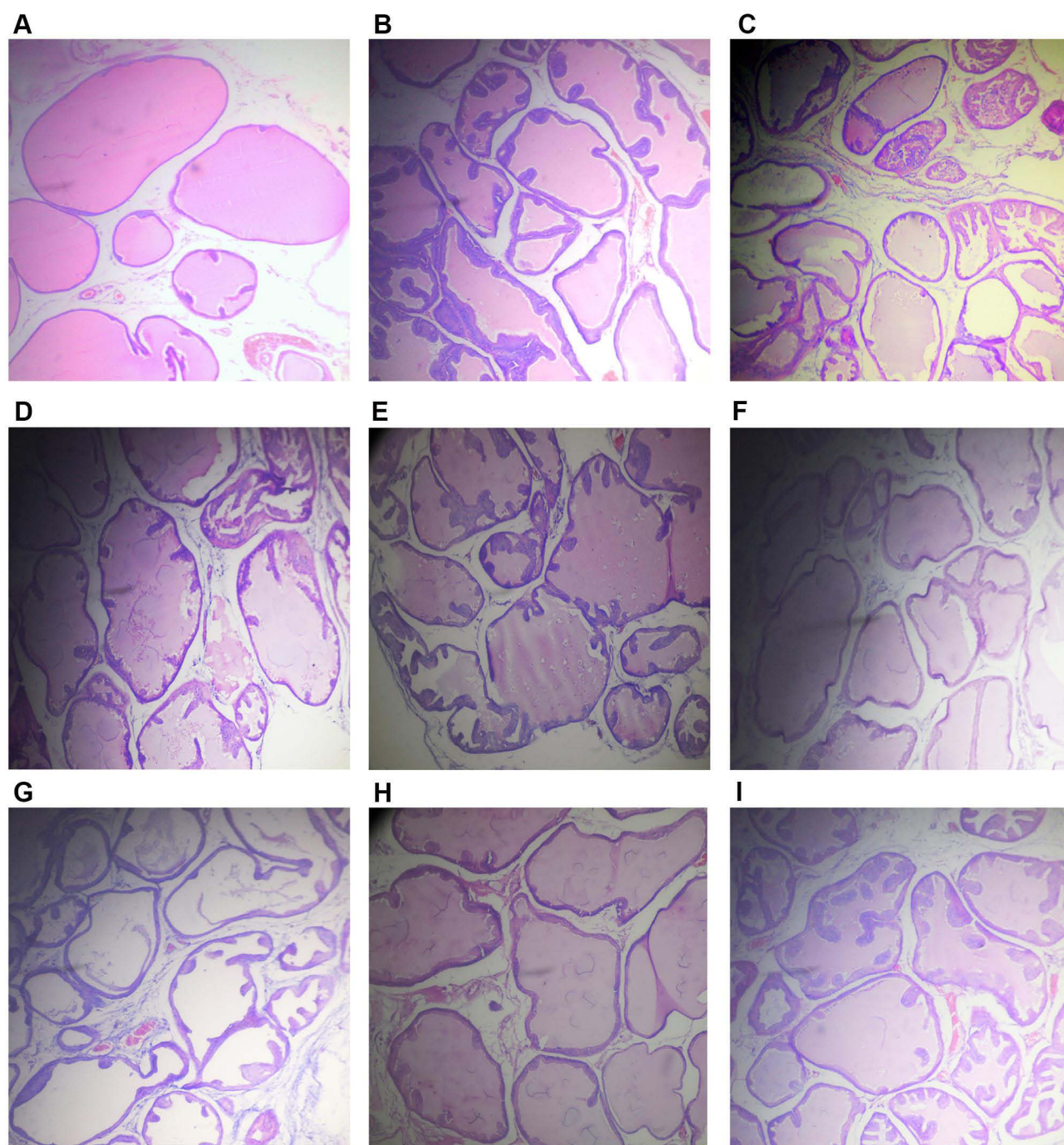


Figure 7 Photomicrograph of prostate ventral lobe (H&E-x40) from (A) Normal control group - Normal histological architecture of the prostate lobe, (B) BPH induced control group - marked increase in thickness of epithelium with prominent villous projections into the lumen, which result in the narrowing of the lumen (C). *C. bonduc* seed ethanolic extract (A) (200 mg/kg) group - mild increase in epithelial thickness with intraluminal projections, (D) *C. bonduc* seed hydro alcoholic extract (B) (200 mg/kg) group - mild increase in epithelial thickness with intraluminal folds, (E) *C. bonduc* seed aqueous extract (C) (200 mg/kg) group - moderate increase in epithelial thickness with intraluminal folds, (F-H). *C. bonduc* seed extracts (A-C) (400 mg/kg) groups - marked reduction in epithelial thickness with sparse intraluminal folds, and (I). Finasteride (10 mg/kg) group - moderate increase in epithelial thickness with intraluminal projections.

Identification of Compounds in the Extracts of *C. bonduc*

The LC/MS-QTOF spectral results and database search tentatively confirmed 16, 17 and 9 major compounds from the ethanolic, hydroxyethanolic and aqueous extracts of *C. bonduc*, respectively (Table 2). The LC/MS-QTOF analysis recorded that the major compounds present in the extracts were cassane furanoditerpene (Caesalpinin), Norcassane furanoditerpene (Norcaesalpinin) and other diterpenes (Caesaldekarin) (Table 2).

Table 2 The Compounds from the *C. bonduc* Seed Extracts

S.No	Ethanollic Extract		Hydroxyethanollic Extract		Aqueous Extract	
	Name	RT	Name	RT	Name	RT
1	α -Tocopherol	17.752	γ -Tocopherol	18.257	γ -Tocopherol	18.248
2	Fucosterol	19.148	δ -tocopherol	15.749	Fucosterol	19.134
3	Linoleic acid	15.692	Fucosterol	19.154	Caesalpinine A	15.144
4	Caesalpinine A	15.044	Linoleic acid	15.682	Caesalpinin N	14.234
5	Caesalpinin C	15.509	Caesalpinine A	14.934	Caesalmin E	15.416
6	Caesalpinin D	14.030	Caesalpinin C	15.499	β -Amyrin	18.413
7	Caesalpinin N	14.197	Caesalpinin N	14.203	β -Sitosterol	16.718
8	Caesalmin E	15.526	Caesalmin E	10.731	Campesterol	18.933
9	Caesalmin B	13.549	Caesalmin B	14.187	Caesaldekarin H	15.223
10	Norcaesalpinin D	13.814	Norcaesalpinin D	14.419		
11	β -Amyrin	18.400	Norcaesalpinin E	14.885		
12	β -Sitosterol	16.838	β -Amyrin	17.427		
13	Campesterol	18.948	β -Sitosterol	16.729		
14	Caesaldekarin H	15.227	Campesterol	18.822		
15	Caesaldekarin J	12.386	Caesaldekarin H	15.234		
16	Caesaldekarin K	15.509	Caesaldekarin J	15.333		
17			Caesaldekarin K	16.048		

Abbreviations: RT, Retention time; α , alpha; β , beta; γ , gamma; δ , delta.

Discussion

Irrespective of the progression in the diagnosis and management of BPH, it remains the most dominant urologic health problem troubling elderly men worldwide.¹¹ The current study was aimed to evaluate the inhibitory or antiproliferative effects of *C. bonduc* seed extracts on the development of BPH using a TP-induced model in rats. The rats with TP-induced BPH displayed elevated absolute and relative prostate weights, increased DHT and PSA levels and prostatic epithelial hyperplasia compared to normal control rats. We found that *C. bonduc* seed extracts reduced the testosterone-induced increment in prostate weight and then improved the histology of prostate tissue. Further, the extracts decreased the expression of DHT and PSA.

A handful of research studies have proven that an increase in the prostate is a vital indicator of the development of BPH,^{32–34} and our study reconfirmed this since BPH rats recorded significant enlargement in the size of prostate gland. It is well understood that when the prostate enlarges, the urethral canal constricts, resulting in partial or complete urinary canal obstruction. According to Veeresh et al,³⁵ increased relative prostate weight is used as one of the significant marker indicating the development of BPH and in earlier studies, where experimental rats with BPH have shown an increased relative prostate weight. BPH is characterized by epithelial and stromal hyperplasia of the prostate, which results in an increase in prostate weight. When the prostate dilates, it results in the constriction of urethral canal, causing partial or complete obstruction. Due to these reasons, many studies have tested the inhibitory effects of many compounds, especially phytochemicals, on the development of BPH by measuring prostate weights.³⁶ In the present study also, oral administration of ethanollic (A) and hydroxyalcoholic (B) extracts of *C. bonduc* seed, at 400 mg/kg, resulted in substantial reductions in absolute and relative prostate weights. Hence, the ability of *C. bonduc* seed extracts to reduce the prostate weight of BPH-induced rats clearly pointed the ameliorative potential of this plant.

Testosterone and DHT are involved in the pathogenesis of BPH and play an important role in the development of male reproductive organs.^{37,38} The serum concentrations of testosterone and DHT may vary with age.³⁹ The levels of DHT in the serum of BPH patients are significantly higher than those of unaffected men of comparable age.⁴⁰ DHT is primarily synthesized from circulating testosterone in the prostate, hair follicles, and testes via the enzymatic action of 5 α -reductase. Interestingly, DHT binds to androgen receptors more strongly than testosterone and adrenal androgens. This is due to the greater affinity of DHT towards androgen receptors when compared to that of testosterone and adrenal

androgens.³⁷ As a result, a number of studies have been conducted to investigate how 5 α -reductase regulates DHT levels. Finasteride is a known 5 α -reductase inhibitor commonly used to manage BPH conditions. It reduces testosterone and DHT levels in serum and the prostate gland, contracting prostate size and BPH-related symptoms like LUTS.^{41,42} Scientists, however, are vigorously investigating to find an alternative medicine for finasteride to treat and manage BPH because long-term use of this drug has serious adverse effects.⁴³ In our studies, the testosterone-induced increase in the production of serum DHT was significantly inhibited by oral administration of *C. bonduc* seed extracts in a dose-dependent manner. This suggests that the inhibitory potential of *C. bonduc* seed extracts in our TP-induced rat model was due to the downregulation of DHT production in serum and prostate. These results suggest that *C. bonduc* seed extracts may be a viable alternative to finasteride to manage BPH conditions in the future.

Enlargement of the prostate gland is viewed as a histological diagnosis characterized by the proliferation of the prostate's cellular elements, including stromal and epithelial components.⁴⁴ In our study, histopathological data clearly suggested the overall histological improvement in the *C. bonduc* seed extracts group compared to the BPH control. The histomorphological changes in prostate tissue sections of the *C. bonduc* seed extract treated with the features earlier reported.⁴⁵ The observed hyperplasia mechanism may be associated with DHT accumulation in the prostate, which binds to nuclear hormone receptors and stimulates cellular growth. The majority of hyperplasia is caused by glandular proliferation, but the stroma is also thickened. Histopathology of the prostate in rats with BPH revealed epithelial hyperplasia with an increase in epithelial thickness when compared to normal control animals in this study. In contrast, the *C. bonduc* seed extracts treated rats recorded mild prostatic epithelial hyperplasia with decreased epithelial thickness compared with BPH-induced animals. These results totally agreed with the data of prostate weight. Thus, the *C. bonduc* seed extracts were highly effective in attenuating prostatic hyperplasia. The histopathological observations further support the results of serum analyses. Hence, the BPH-induced rats with *C. bonduc* seed extracts normalize aberrations associated with BPH.

PSA is a glycoprotein that is produced by the secretory epithelium of the prostate gland and it has been reported to be mildly increased in BPH.⁴⁶ PSA level will significantly increase in both benign and malignant lesions of the prostate but is usually marked in case of prostatic cancer.^{47,48} Thus, the enhanced serum PSA levels of the disease control group suggest the development of BPH for this group of rats and a decrease in PSA is linked to a reduction in prostatic hyperplasia due to inhibition of prostatic 5 α -reductase, the enzyme that converts DHT.⁴⁹ Therefore, a reduction in PSA level after the administration of any test materials displays the effectiveness of the treatment of BPH.⁵⁰ In the present study, PSA levels were increased in BPH-induced rats but reduced markedly in all three *C. bonduc* seed extract treated rats (200 and 400 mg/kg) compared with those in the BPH/vehicle control group. Adding to this, the decreased serum PSA levels of the BPH rats following administration of *C. bonduc* seed extracts (both doses) further underscores the potential of this plant in the management of BPH.

Because of its numerous pharmacological relevant properties, *C. bonduc* has been used traditionally by several people for managing various ailments. Numerous research studies have been conducted to demonstrate its anti-diabetic, anti-cancer, anti-inflammatory, and anti-pyretic properties with recent studies concentrating on its antiestrogenic and anti-androgenic properties.⁵¹ Gupta et al⁵² reported that *C. bonduc* seeds demonstrated a significant anti-cancer property when studied using the Ehrlich ascites carcinoma model in Swiss albino mice. In this study, ethanol seed extract treated mice showed a substantial decrease in tumor size and a higher life span than the non-treated control tumor-induced mice. It is also reported that androgen stimulates the growth of prostate cells, testosterone and related hormones that are shown to play a vital role in the development of BPH.⁵³ Meerwal and Jain²⁷ established the anti-androgenic potential of *C. bonduc* ethanolic seed extract (200 mg and 400 mg/kg body weight) in male Wistar rats, resulting in a significant decrease in sperm count, viability, and motility, as well as lower testosterone levels.

C. bonduc has been found to exert a soothing anti-inflammatory action, making it particularly beneficial for improving an enlarged prostate.⁵⁴ Furthermore, this plant is used for the treatment of prostatic adenoma. A 90-day oral toxicity study of ethanolic root extract of *C. bonduc* conducted in Wistar rats significantly decreased absolute prostate weight,⁵⁵ and this result clearly indicated that the phytochemicals present in the extract might have anti-BPH properties. Thus, this plant was selected for investigating the anti-BPH properties. The screening of phytochemicals from the *C. bonduc* seeds recorded the presence of diverse bioactive metabolites like flavonoids, alkaloids, sterols, saponins, phenols, glycosides/cardiac glycosides, tannins, and resins.⁵⁶ Tocopherol derivatives purified and identified

from seed kernels include α -tocopherol, γ -tocopherol, and δ -tocopherol. The major sterols reported were β -sitosterol, campesterol, and stigmasterol. In addition to this, seed kernel oil was rich with linoleic acid.⁵⁷

In the present study, *C. bonduc* seed extracts inhibited BPH progression, which was indicated by significant reductions in absolute and relative prostate weights. These results were further supported by the declines in the serum and prostate DHT levels and PSA levels in the serum. Histological changes indicated that *C. bonduc* seed extract treatment ameliorated prostatic epithelial hyperplasia. The anti-BPH property of the extracts from *C. bonduc* detected in our investigation may be due to the occurrence of various bioactive compounds, especially phenolics and flavonoids. It was reported that a variety of flavonoids derived from various plants possessed α 1-adrenergic receptor antagonists and reduced the contraction of the prostate smooth muscles of experimental animals.⁵⁸ In this study, besides the efficacy of flavonoids presented in seed extracts, the phytosterols in the extracts from these plants may have acted as α 1-blockers and alleviated the prostate contractions produced by adrenaline.⁵⁸ A phytochemical investigation conducted by us found the presence of sterols in the seed extracts. β -sitosterol is also known as plant sterol, which is richly found in the seed extract of *C. bonduc*. The LC/MS-QTOF analysis reveals the presence of high amounts of three phytosterols, campesterol, stigmasterol and β -sitosterol in the seed extracts. The structure of β -sitosterol is very similar to that of cholesterol. Moreover, β -sitosterol might help to reduce cholesterol levels by limiting the amount of cholesterol entering the body. In addition to this, it can also help to reduce swelling in the prostate and other tissues and thus improve the symptoms associated with the enlargement of the prostate.¹⁴ Besides, sitosterol recorded significant anticancer activity.¹⁴ An earlier study confirmed the potential role of β -sitosterol in managing BPH in human and animal models. Pagano et al reported that β -sitosterol could improve BHP men's urological symptoms and urine flow rate.⁵⁹

γ -tocopherol is one of the major compounds identified in the seed extract, which is reported to have a protective effect against prostate cancer.⁶⁰ Another important compound identified in the extract was linoleic acid. Linoleic acid effectively inhibits 5 α -reductase present in the prostatic epithelium and stroma homogenates.⁶¹ Adaramoye et al reported that the hexane fraction of *Annona muricata* seed ameliorates testosterone-induced benign prostatic hyperplasia in rats, and the phytochemical analysis of the hexane extract reveals the presence of β -amyirin in a significant quantity, which may be one of the major phytochemicals contributing to the activity.⁶² Similarly, in our study, β -amyirin was one of the major phytochemicals present in all the extracts. According to our best knowledge, the anti-BPH properties of caesalpinine, caesalpinins, caesalmin, norcaesalpinins and caesaldekarins were not reported. But our structural activity relationship studies clearly revealed that these compounds also exert significant anti-BPH activities. As said earlier, the seed extracts of *C. bonduc* recorded significant anti-BPH effects, especially reducing the serum and prostate DHT levels and serum PSA levels. Finally, more research at the precise cellular and molecular levels is still needed to elucidate the underlying mechanisms of seed extract in the treatment of BPH.

Conclusions

To the best of our knowledge, this is the first animal study on BPH management with *C. bonduc* seed extracts. Considering the potent side effects associated with the conventional drugs currently being used in the management of BPH, the need for a new therapeutic drug for meliorating BPH is obvious. The results of our study clearly demonstrated that the *C. bonduc* seed extracts would be a novel candidate drug for managing BPH in the future. However, more advanced research is needed to identify bioactive components and the associated molecular mechanisms for managing BPH.

Data Sharing Statement

All the data supporting the current study were included in the article. The raw data used to support the findings of this study are available from the corresponding author upon request.

Statements of Ethics

Our research did not include any human subjects. All animal experiments were approved by the Animal Ethics Committee of CARE KERALAM Ltd., KINFRA Park PO, Koratty, Thrissur, Kerala-680309 and carried out in harmony with guidelines CPCSEA, Government of India.

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Disclosure

The authors report no conflicts of interest in this work.

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