

The impact of combination therapy of *Croton membranaceus* and finasteride on benign prostatic hyperplasia (BPH) histo-morphology

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ABSTRACT

Introduction: Benign Prostatic hyperplasia (BPH) is one of the age-related conditions that reduce the quality of life in aging men. Treatment modalities range from surgical, use of allopathic drugs, to phytotherapy. Allopathic drugs such as Finasteride and Tamsulosin are often considered to have adverse side effects such as reduced libido and erectile dysfunction. In some countries finasteride is often recommended for BPH although some prefer the use of *Croton membranaceus* (CM). However, some indigenes combine the two. The study therefore sought to determine impact of this combination therapy of CM and on BPH histomorphologically.

Methods: Thirty-five (35) male Sprague Dawley rats (200–250 g) made up of the following groups were established with 5 rats in each group: Group 1, served as the control group; group 2 (model group); group 3 (0.5 mg/kg.b.wt Finasteride- positive control); group 4 [30 mg/kg b.wt. CM (low dose)]; group 5 [300 mg/kg b.wt. CM (high dose)]; group 6 (30 mg/kg b.wt. CM plus 0.5 mg/kg. b.wt. Finasteride); group 7 (300 mg/kg b.wt. CM plus 0.5 mg/kg b.wt. Finasteride). Groups 2–7 were castrated, allowed to rest for 7 days and BPH-induced by administering 5 mg/kg.b.wt testosterone propionate subcutaneously daily for 28 days. CM and Finasteride were administered for 28 days by oral gavage. The prostate and its accessory organs were weighed and examined histologically.

Results: In the untreated group (model group) fibromuscular stroma showed the typical characteristic of high epithelial folds with apparent typical hyperplasia. The Finasteride group showed increased rate of apoptosis and progressive decrease in epithelial cell size. At the lower dose of CM, not enough apoptotic areas were observed. However, multiple layers of acinar epithelia were noticed. At the higher dose of CM, few apoptotic cells were seen with acini fluid reduction. The combination of Low dose CM and Finasteride led to fluid level reduction in the acini accompanied by fat droplets and reactive cells infiltration. At a higher dose of CM, papillary fronds were not protruded into the acini. However, acini fluid appeared coagulated. The combination treatment did not have any adverse hepatorenal effect, nor did it change PSA significantly.

Conclusion: No overt advantage was seen in this combination therapy, although higher doses of CM in tandem with finasteride may not be advisable. However, there was good tolerability.

Abbreviations: 5AIR, 5-alpha reductase inhibitors; AIR, Alpha-1-adrenoceptor blockers; BPH, Benign prostatic hyperplasia; CMI, Clinically meaningful improvement; DHT, Dihydrotestosterone; ED, Erectile dysfunction; IPSS, International Prostate Symptoms Score; LUTS, Lower urinary tract symptoms; PSA, Prostate Specific Antigen; QoL, Quality of life; UFR, Urine Flow Rate; IIEF, International Index of Erectile Function.

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Introduction

Benign prostatic hyperplasia is a condition that often occurs among men aged 40 years and above. Globally 60 % of men may suffer from BPH by the age of 60 years and this increases to 80 % by the age of 80 years. The mainstream treatment is the use of synthetic drugs such as 5- α -reductase inhibitors and α -blockers. When these options fail, surgical interventions may be needed.

Allopathic medicine

The use of allopathic medicine has its own challenges. Common side effects of major concern to men include persistent sexual dysfunction [low libido, erectile dysfunction (ED), and problems with orgasm] (Mysore, 2012). The non-selective numerous side effects of short acting α -1-blockers such as Phenoxybenzamine, Prazosin, and Alphuzosin, prompted the development of others such as Doxazosin, Terazosine and Tamsulosin, that reduced the incidence of cardiovascular diseases because they were long acting. The preference of Tamsulosin over the others was because it did not require dose titration. However, side effects included low libido and ejaculatory dysfunction among others (Giuliano, 2006). The treatment of lower urinary tract symptoms (LUTS) is mainly carried out by α -adrenergic blockers. However, the treatment of the obstruction is by using 5- α -reductase inhibitors (5ARI) which work to shrink the prostate volume by reducing the conversion of testosterone into di-hydrotestosterone (DHT) via inhibition of the enzyme 5- α -reductase (5AR). Dutasteride, for example is an inhibitor of 5AR type I and II with 45 times more effectiveness for the treatment of type I and 2.5 times stronger effect for type II, when compared to those of Finasteride®, which is a type II inhibitor (Frye et al., 1998; Evans and Goa, 2003). The side effect of Finasteride® often includes breast enlargement, ejaculatory disorders, decreased libido and erectile dysfunctions (McConnell et al., 1998). These undesired sex related dysfunctions in aging men have culminated in the desire to seek natural alternatives.

Combination therapy in allopathic medicine

The Doxazosin and Finasteride (MTOPS), and the Tamsulosin and Dutasteride (ComBAT) studies support combination therapy for patients with moderate to severe LUTS or those with the possibility of disease progression. In the former, there was a 64 % International Prostate Symptom Score (IPSS) reduction compared to 45 % and 30 % in Doxazosin and Finasteride, respectively (McConnell et al., 2003). In another study, IPSS risk reduction and reduction in urinary retention, were also observed (Roehrborn et al., 2010).

The total and transitional zone volumes have been shown to be reduced upon the introduction of 5ARI. Clinically, there were improvements of LUTS and urine flow rate (UFR) as well. On the other hand, α -1 adrenoceptor blockers influence the smooth muscle tone of the prostate, the urethra and the bladder. When 327 BPH patients were administered combination drugs (0.5 mg Dutasteride plus 0.4 mg Tamsulosin) for 6 months, symptoms were relieved rapidly (Barkin et al., 2003).

Molecular studies into the combination therapy of α -Adrenergic Receptor Antagonists and 5ARI demonstrated that the efficacy of post treatment change in total prostate volume was associated with SRD5A1 rs166050 gene. However, IPSS significant change was associated with SRD5A2 rs523349 and rs612224 (Xin et al., 2013). In a systematic review, examining the impact of combination therapy on the risk of libido alteration and erectile dysfunction (ED) using 6131 patients, the combination therapy increased the risk of ED compared to the percentage of alpha reductase inhibitors (ARI) alone use, but had no risk of libido alteration (Favilla et al., 2016).

Phyto-medicine

The use of phyto-medicines for the treatment of BPH has increased over the years. In the US, about 35 % of men resort to this treatment option and this increases to about 90 % in Austria. *Solanum lycopersicum* (from tomatoes, watermelon, peaches and red berries) is purported to contain lycopene which inhibits 5AR (Allkanjari and Vitalone, 2015). *Linum usitatissimum* (oil from common flax) inhibits prostate growth through growth factor modification as well as 5AR inhibition (Tarique et al., 2022). *Pygeum africanum* (bark of an evergreen tree of the Rosacea family), behaves like an α -1-blocker. It may inactivate androgen receptors through nuclear transcription inhibition (Ishani et al., 2000) as well as cellular growth factors (Lawson, 1997), while *Serenoa repens* (fruit of dwarf palm tree) and *Croton membranaceus* are said to be 5ARI (Weisser et al., 1996; Afriyie, 2020).

Combination therapy in phytomedicine

A combination of *P. africanum* (25 mg) and *Urtica dioica* (stinging nettle) extract (300 mg) was evaluated over six months using the International Prostate Symptom Score (IPSS) and urine flow dynamics. However, there was no significant difference between the test and the placebo groups. Nevertheless, the drug was well tolerated (Melo et al., 2002). A combination of *S. repens* and *U. dioica* (nettle root) was matched against Finasteride in a study of 431 cases with an early stage of BPH. The final outcome indicated that the efficacy was equivalent and unrelated to prostate volume. However, the herbal drug had better tolerability (Sökland, 2000). In a rare combination of *S. repens*, Lycopene and Selenium, 90 BPH positive patients received the above combination of plant-based materials. The participants were divided into two groups; test and placebo groups. After 90 days of drug or placebo administration, biopsies were taken from either group. Compared to those of the placebo group, histo-pathological changes showed apoptosis in the prostate. This was suggestive of effective treatment in the test group but not in the placebo group (Morgia et al., 2017). Apart from bi-herbal products, multi-herbals have been used in clinical settings. In a Phase II RCT which was also placebo-controlled and double-blind, Prostate EZE Max made up of *Cucurbita pepo*, *Epilobium parviflorum*, lycopene, *P. africanum* and *S. repens* was evaluated. In that study, 57 men aged 40–70 years with BPH but otherwise healthy, were volunteers. Results revealed that IPSS only reduced by 8 % in the placebo group compared to 36 % in the case group ($p < 0.05$) over the 3-month study period (Coulson et al., 2013).

Medicinal plant/allopathic drug combination

A traditional plant product used in treating BPH and LUTs is *Palmyihwang-hwan*. This is a poly herbal product and a combination of six plants (*Rehmannia glutinosa*, *Liboschitz ex Steudel*, *Cornus officinalis*, *Dioscorea batatas*, *Alisma orientale*, *Poria cocos* and *Paenonia suffruticosa*). This was used together with Tamsulosin to determine its pharmacokinetics using rats as an experimental model. The results showed that the pharmacokinetics of Tamsulosin was not affected by the co-administration of the multiherbal product (Seong et al., 2023). In another study, involving two therapeutic approaches of using Traditional Chinese medicines; Herbal Saxifrage Tablet (HST) and Western medicine (WM), Terazosin Hydrochloride Hytrin (THH) were examined. The logistic regression and Chow test of 30 cases studied, demonstrated that the four (4) urethra-related symptoms and the thirty-five (35) non-urethra-related symptoms improved Urine Flow Rate, QoL and PV with the Traditional Chinese Medicine compared to those of WM (Li et al., 2010).

A number of studies have examined *S. repens* Arecaceae in combination with Tamsulosin for the management of BPH. One study confirmed that the (*S. repens*, Arecaceae) extract and Tamsulosin, did not confer any therapeutic advantage among the 60 cases of LUTS

secondary to BPH using key indicators such as IPSS, PSA and PV (Hizli and Uygur, 2007).

The treatment of LUTS using Silodosin in combination with *S. repens* in 186 men for more than 12 months showed clinically meaningful improvement (CMI) as well as better IPSS than those who took silodosin alone (Boeri et al., 2017). In another study on *S. repens* and tamsulosin for the treatment of BPH patients, the independent use of each remedy was effective. However, the combination of the two had no extra advantage using IPSS and UFR as determinants of LUTS (Hizli and Uygur, 2007).

In Ghana, *C. membranaceus* is the plant commonly used for the management of BPH (Asare et al., 2015). Communications with some indigenes in Ghana suggest that *C. membranaceus* is used in combination with Finasteride unprescribed. The aim of this study therefore, was to determine the histopathological alternations of *C. membranaceus* in combination with Finasteride on BPH.

Methods and materials

One (1) kilogram of *C. membranaceus* root extract previously identified by the Centre for Research into Plant Medicine with voucher number CSRP 2110 was used. The roots were air-dried under subdue light for 3 weeks and grounded into fine powder. Aqueous extraction was done according to the method of Afriyie et al. (2013). The extract obtained was freeze-dried and the yield was 2.1 %.

Animal experimentation

The study was approved by the University of Ghana Institutional Animal Care and Use Committee with ethics number UG-IAPUC 007/21–22. A total of 35 male Sprague-Dawley rats weighing 200–250 g were randomly divided into 7 groups. Rats were acclimatized in the Department of Animal Experimentation at the Noguchi Memorial Institute for Medical Research. Thereafter, rats were randomly divided into 7 groups of which one group was set aside as the control group (Group 1). All other groups were castrated and injected with 5 mg/kg body weight testosterone propionate subcutaneously for 28 days to induce BPH according to the protocol of Wu et al. (2003). Testosterone injections were administered 7 days after castration. The following BPH-induced groups were set up:

Group 2 was designated the model group and did not receive any other treatment apart from the daily testosterone administration (that was administered to all the induced groups).

Group 3 was treated with 0.5 mg/kg b.wt. Finasteride. This group served as the positive control group.

Groups 4 received 30 mg/kg b.wt. of *C. membranaceus*.

Group 5 received 300 mg/kg b.wt. *C. membranaceus*.

Group 6 was administered a combination of *C. membranaceus* (30 mg/kg b.wt. and 0.5 mg/kg b.wt. Finasteride).

Group 7 was administered 300 mg /kg b.wt. *C. membranaceus* and 0.5 mg/kg b.wt. Finasteride.

All groups continued their drug administration for 28 days.

Housing conditions

Rats were housed under 12 h light/12 h dark cycle. Humidity was controlled at 60–70 % and room temperature of 21–23°C was maintained throughout. Animals were handled humanely, fed the standard rat chow and had access to food and water ad libitum.

Termination of experiment

After 28 days, animals were sacrificed. Rats being sacrificed were anaesthetized with 0.1 ml/100 g of body weight of Anaknet and Chinazin (4:1). An amount of 5 ml blood was drawn by cardiac puncture; 2 ml of the drawn blood was discharged into EDTA tubes and the remaining 3

ml into gel separator tubes. Samples in the gel separator tubes were centrifuged at 4000 rpm for 10 min. The serum was aliquoted and stored at –20°C until use. EDTA samples were transported on ice and were analysed immediately.

Organ harvesting was carried out. The prostate and its accessory organs, liver, kidneys, heart and lungs were collected. All harvested organs were weighed and fixed in 10 % buffered neutral formalin for histological examination.

Biochemical and haematological analyses

Biochemical analyses were carried out mainly for the liver function, using the Buckman Coulter Chemistry analysis AU480 (New York, USA). Full blood count (FBC) was analysed using ACT5 Buckman (Newyork, USA).

PSA was determined using Elabscience ELISA kit (Wuhan, China). The test was performed according to the manufacturer's instructions. In brief: samples and standards were added to a pre-coated rat PSA antibody-immobilized ELISA microwell plate. The antigenic material in the sample and standard automatically got bound to the immobilized antibodies. The bound antibody-antigen complex was further bound to biotinated-substrate-labelled antibody, thereby creating a sandwich. This was incubated and thereafter, unbound substances were washed off the microplate. Avidin-horseradish peroxidase was added to each microwell plate and incubated followed by the addition of Anti-avidin-HRP conjugate to produce a chromogen. Finally, the reaction was stopped with sulphuric acid. The optical density was determined spectrophotometrically using LabSystems plate reader (Midland, Canada) at a wavelength of 450 nm.

Histological analysis

Necropsy was then carried out looking out for lesions in the visceral organs including prostate and accessory glands. Organs were harvested aseptically and washed with PBS, blot-dried and fixed in 10 % buffered neutral formalin (harvested tissues received three changes of fixatives in 72 h). They were routinely processed in graded alcohol, acetone, xylene and paraffin before embedding in paraffin wax. Thereafter, sections were cut at 5 µm thickness, collected over glass slides coated with Mayer's egg albumin and stained with Harris's hematoxylin and eosin for routine histological screening of tissues by light microscopy (Luna, 1968).

Statistical analysis

Data was entered into SPSS version 24 and descriptive statistics of mean $\pm$ SEM of the haematological and biochemical analyses were determined. A one-way ANOVA was performed to determine the differences across groups. This was followed by Bonferroni *Post hoc* analysis and a *p*-value ≤ 0.05 were considered significant.

Results

The H&E-stained tissue sections obtained from the control group revealed normal architecture of the prostate. It showed characteristic architecture (Fig. 1), with secretory epithelium, cubic/columnar epithelial cells and with secretory vesicles. In addition, numerous basal cells were identified in the epithelium. Lumen of prostatic acini presented, were filled with secretion from epithelial cells, demonstrating the high activity of epithelial secretory cells. The epithelium lining the acinar showed the normal cuboidal cell with the nucleus. The model group (Fig. 2) shows hyperplastic glands of varying sizes with significant papillary foldings. Double layered epithelial cells of the inner columnar and outer cuboidal, lined the surfaces of the glands showing typically high epithelial folds. Representative sections of prostate from Finasteride group showed a progressive decrease in epithelial cell size

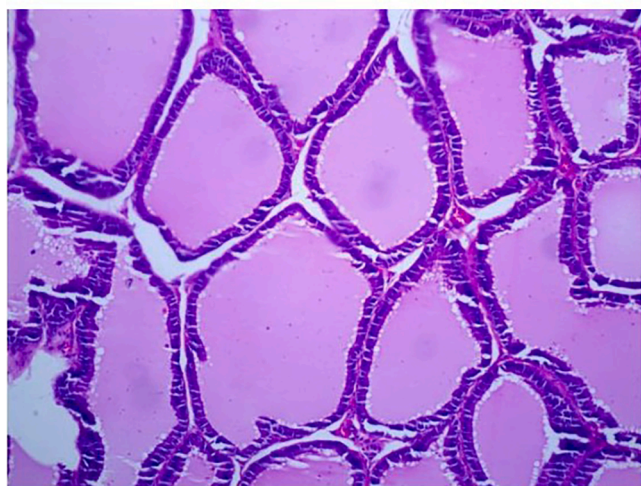


Fig. 1. Section through the prostate from a rat received normal saline to show normal structure. Note: The acini are lined with epithelium and surrounded by loose, fibrous connective tissue containing smooth-muscle fibers and blood vessels. The epithelial cells of the acinar lining are cylindrical, prismatic and or cuboidal with a basal nucleus. H&E x 100.

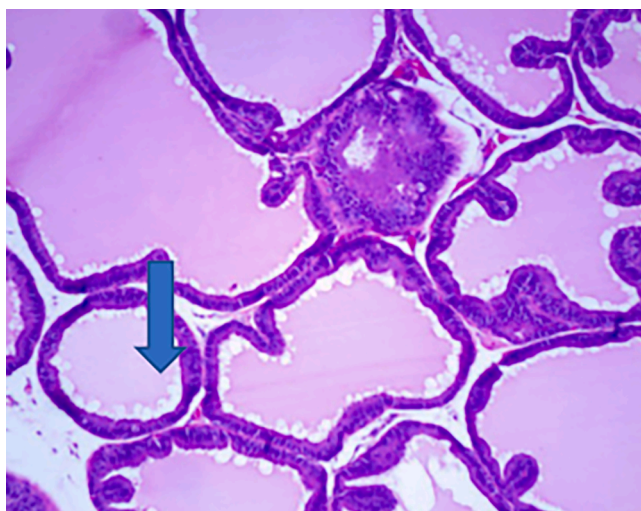


Fig. 2. Section through a rat prostate. It was injected with testosterone to induce hyperplasia of the prostate after being castrated. Microscopically, hyperplastic glands of variable sizes with papillary folding were found in the acini. Glands surfaces were lined with double layered epithelium inner columnar and outer cuboidal. The fibro-muscular stroma showed the typical characteristic of high epithelial folds in the distal region of the gland. Some areas showed apparent epithelial hyperplasia. The empty vacuoles were demonstrated (arrowed). H&E x 100.

(arrowed) coupled with apoptosis (Fig. 3). Furthermore, atrophy of the prostate and apoptosis were also observed. The undulating nature of the epithelium of the acini were lost (Fig. 4). The application of 30 mg/kg b. wt. *C. membranaceus* showed some areas of apoptosis in the presence of hyperplasia. At 300 mg/kg b.wt. *C. membranaceus*, reduced acinar fluid was observed and, in some cases, total absence of the acinar fluid was seen (arrowed). The effects of Finasteride which was used with *C. membranaceus* at 30 mg/ kg b.wt showed apoptosis on the acinar epithelium (Fig. 5). There was fluid reduction in the acinar; however, fat droplets were also observed, and in some cases, the acinar fluid was found to be dried up (Fig. 6). With an increase in the administration of *C. membranaceus* to 300 mg/kg b.wt. plus 0.5 mg/kg b.wt. Finasteride, papillary fronds appeared but not protruded into the acini. The acinar

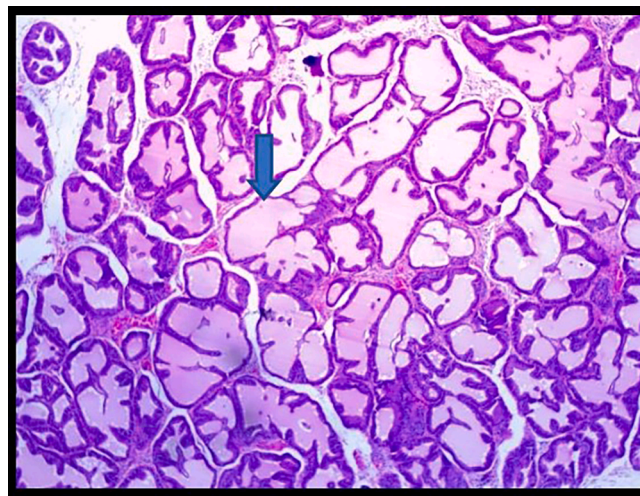


Fig. 3. Representative section through the prostate from a rat that was induced and treated with Finasteride (positive control) H&E x 100. There was a progressive decrease in epithelial cell size (arrow) along with an increased rate of apoptosis on the acinar epithelial cell surface. It also showed prostate involution through a blend of atrophy and cell death. There was marked lobular and acinar atrophy with small, shrunken, round and or comma shaped acini. The basal cells appeared more prominent and hyperplasia was frequently observed. Depletion and vacuolation of the secretory cells were a common feature. Nuclei of the acinar surface were found to be smaller and hyperchromatic. The usual undulating appearance of the epithelium was lost. There was a decrease in the glandular element which was accompanied by hypercellularity and prominent nuclei. Scattered foci of lymphocytic infiltrations were observed in the stromal tissues.

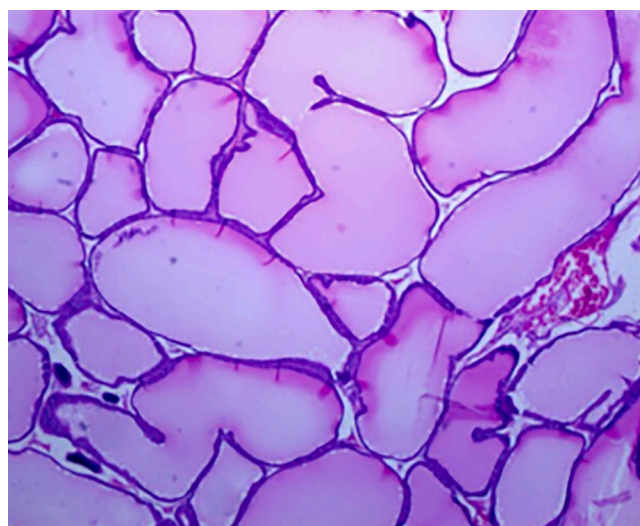


Fig. 4. Section through a rat prostate that was BPH-induced and later treated with *Croton membranaceus* (30 mg/kg). Note: not enough apoptotic areas were found. However, multiple layers (hyperplasia) of the acinar epithelia were noticed. H&E x 100.

fluid apparently seemed to be coagulated (Fig. 7).

Seminal vesicle

Histologically, the seminal vesicles of the control group show smooth muscle cells in the tunica muscularis which was found to be strongly stained (Fig. 8). In the model group, the seminal vesicle showed clamps of mononuclear cells (Fig. 9). Representative sections from the prostate obtained from rats treated with Finasteride, showed a reduction in the

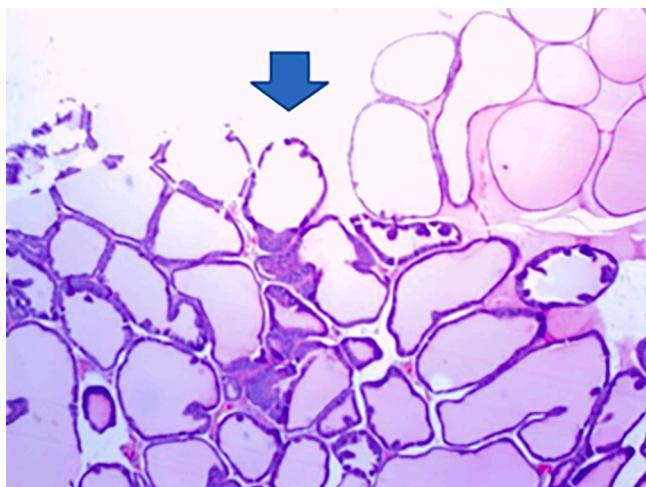


Fig. 5. Section through a rat prostate that was BHP-induced and later treated with *Croton membranaceus* (300 mg/kg). Note: Acinar fluid was found to be reduced and some of them were empty (arrow). There were a few apoptotic cells on the epithelial surface of the acini. Interstitial stroma showed infiltrations of mononuclear cell. H&E x 100.

fronds on the luminal surface (Fig. 10). On the other hand, the papillary projections in the acinar were found to have increased with no significant alterations in the stroma or acinar (30 mg/kg b.wt. *C. membranaceus* treatment (Fig. 11). Treatment with 300 mg/kg b.wt. *C. membranaceus* showed a decrease in fibrous tissue in the seminal vesicle (Fig. 12). The stroma showed infiltrations of inflammatory cells. The combination of Finasteride and *C. membranaceus* incorporated at 30 mg/kg b.wt. showed atrophy of muscles in the stroma which narrowed the inter acinar spaces (arrowed) (Fig. 13). At a higher dose of *C. membranaceus* 300 mg/kg b.wt. plus Finasteride, the acini showed a reduction in size, and the fluid appeared to be condensed, coagulated and dried up, compared to those of control (Fig. 14).

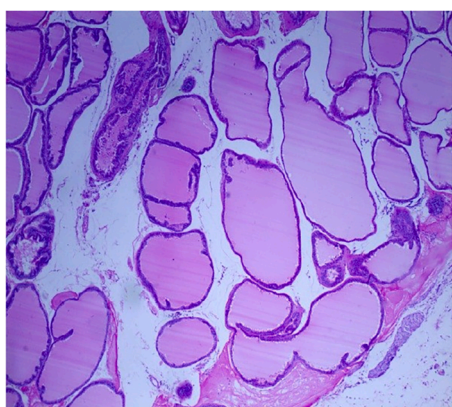
The values of various biochemical parameters are presented in Tables 1 and 2. Results show that the combination therapy could not produce any significant changes on haematological values compared to those of control rats. However, significant differences with AST and total protein in the liver function tests were recorded compared to the control. The serum potassium and creatinine levels were found to be significantly different in Group 4 and Group 7 ($p = 0.026$) for potassium, and Group 2 and Group 4 ($p = 0.016$) for creatinine (Table 2). Testosterone was found to be elevated in all the test groups (Table 3). Organ weights for liver and

kidney, seminal vesicles and prostate are shown in Table 3. Significant differences were seen between Group 1 and Group 3 ($p = 0.031$) for the kidneys. Differences were not seen however, for the prostate and its accessory organs. Organ weights showed some reduction in all treatment groups when compared to the model group apart from the Finasteride treated group. However, all groups had higher weights for the prostate and its accessory organ than the control group, although not statistically significant.

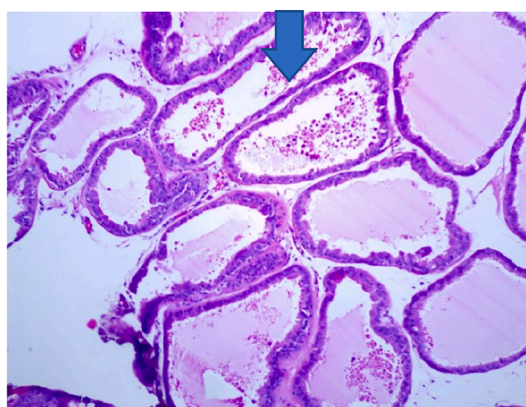
Discussion

The present report contains a description of the histomorphometric impact of combination therapy of *C. membranaceus* and Finasteride on benign prostatic hyperplasia (BPH). The aim of this investigation was to resolve an issue of whether this combination therapy could be extrapolated to human to alleviate BPH. Nasr El-Din & Abdel Fattah (2019) showed from their four-week experiments the potential curative and preventive effects of garlic on testosterone-induced orchiectomized BPH rats. The authors reported that histologically and morphometrically, the BPH group showed epithelial hyperplasia, stromal expansion and reduced acinar lumens that were significantly improved in both curative and preventive groups of rats used. Other workers (Peehl and Sellers 1997) were of this opinion that the prostate connective tissues which have spaces containing smooth muscles, fibre, blood vessels and cells, increase activity in this area and contributes to the development of BPH (Auffenberg et al., 2009).

Histologically, the model group demonstrated the typical proliferation of nodules of glands with stroma proliferations in this study. This is typical with glands of varying sizes and papillary foldings. This testosterone BPH-induced model has been used in various studies (Wu et al., 2003) with similar effects and in some cases, more prominent hyperplasia was observed than what was seen in this study (Afriyie et al., 2014). The age of orchiectomization and the weight of the rats may have had a significant role which could not have been clarified from the present experiments. Nonetheless, rats used in this study, had lower final live body weight than those used by Afriyie et al. (2014) whose final live body weight was about 400 g. On the other hand, it is highly likely that the testosterone/oestrogen BPH-induction (3–6 weeks without orchiectomization) may provide extrapolation much better to the human patho-physiological conditions. In that model, a gradual development of prostatic inflammation was observed with migration of lymphocytes into the stroma and peri-glandular spaces, leading to voiding dysfunction when treatment was prolonged to a period of 13 weeks (Bernoulli, 2008). Nonetheless, the model group of this study demonstrated glandular proliferation characteristics (Table 4) that simulate the effects that



A



B

Fig. 6. Section through a rat prostate that was BHP-induced and later treated with Finasteride (0.5 mg/kg) and *Croton membranaceus* (30 mg/kg). Note: A. fat droplets appeared on the mucous surface. Fluid level was reduced in acinus, H&E x 40. B. Fat droplets appeared in the acini and there were infiltrations of reactive cells. Acinar fluid was dried up (arrow). H&E x 100.

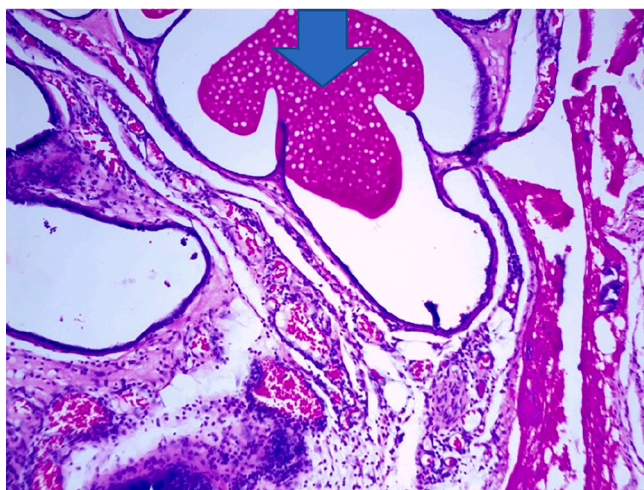


Fig. 7. Section through a rat prostate that was BHP-induced and later treated with finasteride (0.5 mg/kg) and *Croton membranaceus* (300 mg/kg). Note: Papillary fronds were not protruded into the acinii. The fluid in the acini appeared to be coagulated and turned deep purple in colour. The stromal tissues showed infiltrations of inflammatory cells. H&E x 100.

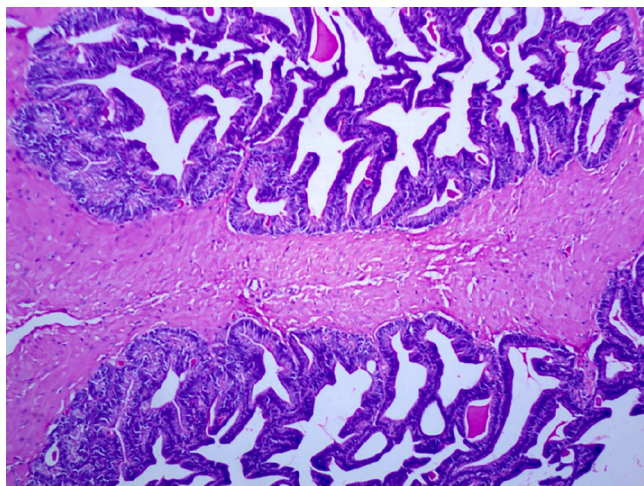


Fig. 8. Seminal vesicle from a control rat. Note: The smooth muscle cells in the tunica muscularis were strongly stained. The nuclear staining intensity of these cells was similar to luminal epithelial cells. Staining intensity in luminal cells was stronger than in basal cells. H&E x 100.

happen in BPH in humans.

The group that was treated with Finasteride® showed marked improvement by way of a progressive decrease in the size of epithelial cells coupled with increased apoptosis. Additionally, secretory cells showed reduced vacuolation and depletion. The undulating nature of the epithelium was found to be lost. Additionally, glandular elements decreased. These are similar to those that have been observed from a similar study where Finasteride was administered to testosterone-induced BPH rats. In that study, there was reduction in stroma cells as well as restoration of the lumen (Afriyie et al., 2014). Similarly, when rats were induced to develop BPH and subsequently treated with Finasteride, another study showed increased density of interglandular smooth muscle fibre and thick intraglandular epithelia (Mbaka et al., 2017).

The evidence gathered from our present experiments that treatment with *C. membranaceus* had comparatively less potential to generate much apoptosis compared to those of Finasteride® treatment group. Additionally, multiple hyperplasticity layers could still be seen with the

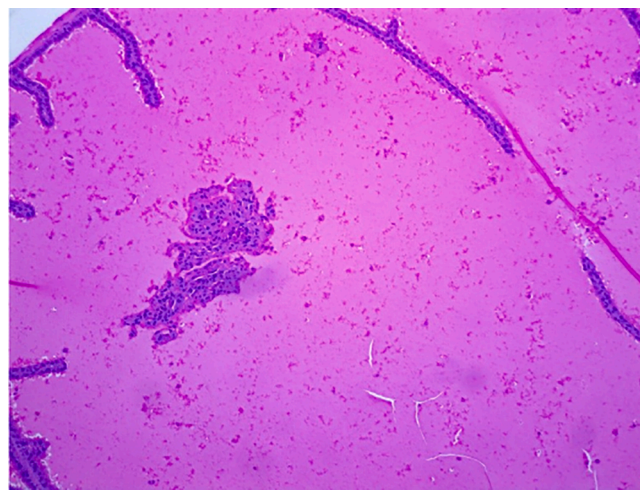


Fig. 9. Section through the seminal vesicle of a rat. It was injected with testosterone to induce hyperplasia of the prostate after being castrated. Note: clump of mononuclear cells in the vesicular fluid. H&E x 100.

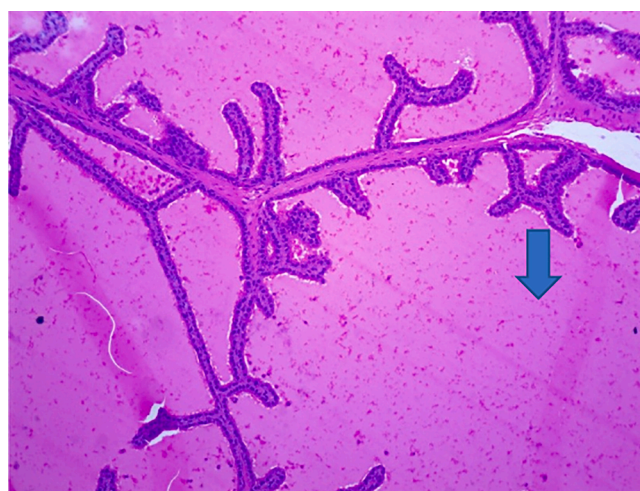


Fig. 10. Representative section through the seminal vesicle of a rat that was induced and treated with finasteride (positive control). Note: Acini were found to be filled with pink fluid. Number of fronds on luminal surface has been reduced. H&E x 100.

administration of 30 mg/kg b.wt. *C. membranaceus*. The presence of multiple hyperplasticity is an indication of an ongoing treatment process. However, at 300 mg/kg b.wt. *C. membranaceus*, there was reduction in acini fluid with some literally appearing empty (atrophic acini) which could be associated with a decrease in testosterone levels and a more profound effect. Mononuclear cell infiltration as a response of the body's immune system to inflammation, was also observed. Thus, a higher dose appeared to have elicited a better treatment response. A similar study demonstrated packed prostatic acini, regular shaped and reduced fibro-muscular stroma, implying loss of collagen and elastin. Thus, a reduced structural support coupled with loss of the muscular contraction and relaxation of the prostate had happened. Similarly in a previous study, both low and high dose *C. membranaceus* (30 and 300 mg/kg b.wt) resulted in widening of the lumen and thinning of the stroma (Afriyie et al., 2014).

The combination at low dose *C. membranaceus* (30 mg/kg b.wt) produced fat droplets in the acini and mucous surface. These fat droplets could sometimes be a normal feature but could also contribute to the enlargement. Additionally, reactive cell infiltration occurred as a response to some prostate insults which could be attributed to chronic

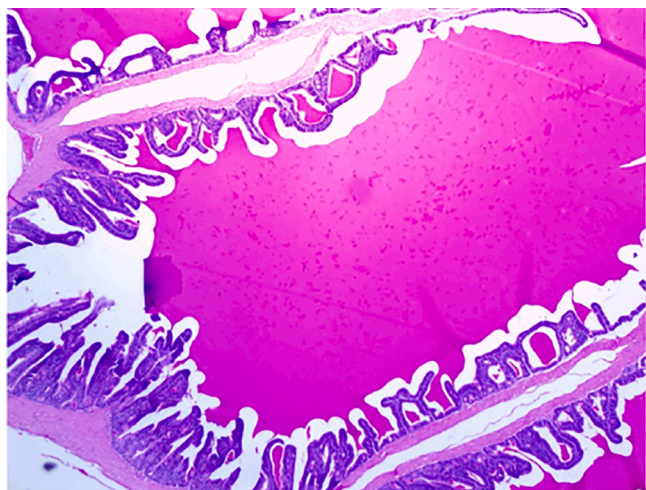


Fig. 11. Section through the seminal vesicle of a rat that was BPH-induced and later treated with *Croton membranaceus* (30 mg/kg). Note: Papillary projections in the acini were found to be increased. There was no significant alteration and or infiltrations in the stromas or acini. H&E x 100.

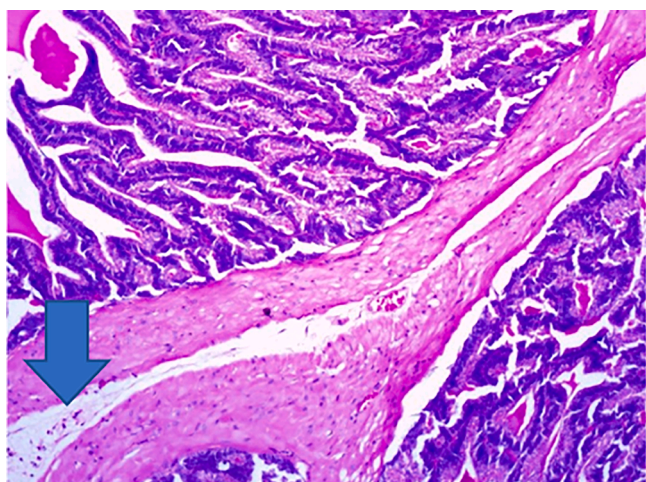


Fig. 12. Section through the seminal vesicle of a rat that was BPH-induced and later treated with *Croton membranaceus* (300 mg/kg). Note: Seminal fluid appeared to be reduced and condensed. The obvious decrease of fibrous tissue was found in the seminal vesicles, and inflammatory cell infiltration was also found in the stroma. Inflammatory cell infiltrated in the stroma of the seminal vesicle (arrow). H&E x 100.

inflammation. At the higher dose (300 mg/kg. b.wt) of *C. membranaceus* infiltrations of inflammatory cells and protrusion of papillary fronds did not exist. Reduction in the thickened epithelial cells that protruded into the lumen of the prostatic tubules, subsequently leads to restoration of the luminal space. In this group, acini with coagulated fluid was seen. Not too many studies have examined such combination effects histomorphologically. However, *Prunus africanus* herbal extracts reversed and ameliorated histological and histo-morphometric features. The treatment group showed a reduction in epithelial thickness similar to that of the Finasteride® and the control group (William and Russa, 2021).

The positive control of the seminal vesicle had a reduced number of fronds on the luminal surface. This could be due to hormonal imbalance and changes in testosterone levels. After having been treated with 30 mg/kg b.wt. *C. membranaceus*, no significant infiltration of stroma or acini were seen in rats although papillary projections were still prominent. This could be an indication of the positive effect of the treatment.

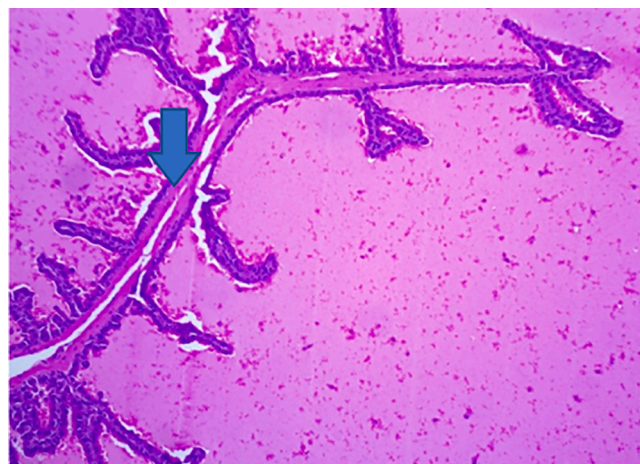


Fig. 13. Section through the seminal vesicle of a rat that was BPH-induced and later treated with finasteride (0.5 mg/kg) and *Croton membranaceus* (30 mg/kg). Note: smooth muscles in the stroma were found atrophied. It had narrowed inter acinar space (arrow). H&E x 100.

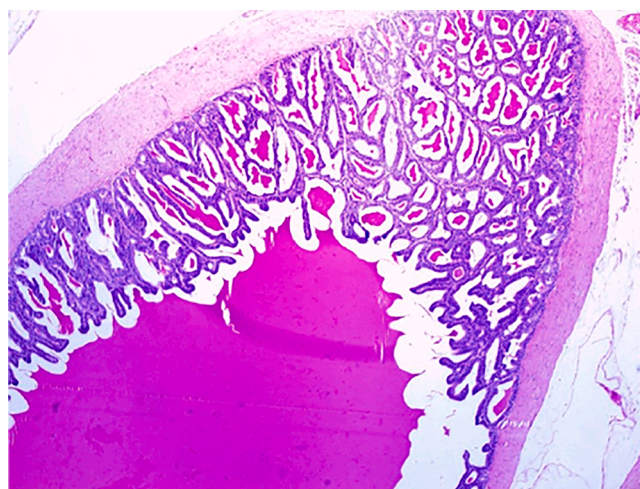


Fig. 14. Section through the seminal vesicle from a rat that was BPH-induced and later treated with finasteride (0.5 mg/kg) and *Croton membranaceus* (300 mg/kg). Note: Acini were found reduced in size. Acinar fluid appeared dried and condensed. The condensed materials took a strong pink colour. H&E x 100.

At the higher dose, seminal fluid appeared to have reduced and appeared to be condensed. Furthermore, inflammatory cells infiltrated the stroma of the seminal vesicle. Thus, the hyper unprescribed doses of *C. membranaceus* may be a disadvantage to semen production.

In this study, low dose *C. membranaceus* combination with Finasteride® led to smooth muscle in the stroma being atrophied thus narrowing the acini space which later reduced in size at a higher dose and remained dried and condensed. Atrophy of smooth muscle in the stroma of the seminal vesicle can lead to the narrowing of the acini space within the vesicle. This narrowing could potentially obstruct the normal flow of seminal fluid, which may create an impact of the volume or composition of semen. A reduction in acini size may compromise their functional capacity to produce and release of seminal fluid during ejaculation. Therefore, further investigation should include an attempt to look into the long-term effects of the combination therapy in heavier rats; studies on the effects of body weight change and nitrogen balance and as well as on the effects on the local and systemic metabolism are also needed.

Differences in organ weights were seen with the lungs. Significant difference was seen in *C. membranaceus* (300 mg/kg b. wt.) and the control, with the treatment causing an increase in organ size. No

Table 1
Mean haematological parameters of rats on various drug regimen.

Analyte/ Unit	Negative control	Model	CMARE 30 mg/ kg b.wt	CMARE 300 mg/ kg b.wt	Finasteride/ mg/kg b.wt	CMARE 30 mg/kg b.wt	Finasteride/ mg/kg b.wt	CMARE 300 mg/kg b.wt	Finasteride	p- value
WBC (10 ⁹ / L)	4.96 ± 0.9	9.43 ± 0.75 *	9.98 ± 1.86	8.32 ± 2.06	8.32 ± 2.06		6.74 ± 2.14		7.18 ± 2.49	0.41
RBC (/pL)	8.52 ± 0.72	8.82 ± 0.36	6.16 ± 1.43	6.83 ± 1.38	6.83 ± 1.38		8.61 ± 0.42		8.43 ± 0.75	0.55
HBG (g/dL)	14.32±1.09	14.83 ± 0.21	10.22 ± 2.23	10.76 ± 2.01	10.76 ± 2.01		13.86 ± 0.32		13.65 ± 1.23	0.37
HCT (L/L)	0.45 ± 0.04	0.46 ± 0.01	0.31 ± 0.07	0.34 ± 0.06	0.34 ± 0.06		0.44 ± 0.01		0.43 ± 0.04	0.34
MCV(fL)	52.4 ± 0.6	52 ± 1.15	52.17 ± 1.42	50.8 ± 1.66	50.80 ± 1.66		51.20 ± 1.24		51.5 ± 1.19	0.97
MCH (pg)	16.88±0.22	16.85 ± 0.62	13.93 ± 2.8	16.32 ± 0.73	16.32 ± 0.73		16.20 ± 0.49		16.18 ± 0.31	0.54
MCHC (g/ dL)	32.2 ± 0.54	32.53 ± 0.83	27.32 ± 5.47	32.18 ± 0.49	32.18 ± 0.49		31.66 ± 0.32		31.43 ± 0.28	0.64
RDW-SD	12.66±0.43	13.13 ± 0.23	11.75 ± 0.67	10.9 ± 1	10.9 ± 1.0		12.84 ± 1.05		13.03±0.34	0.14
PLT (10 ⁹ /L)	691 ± 236	774 ± 211	314 ± 127	297 ± 142	297 ± 142		689 ± 220		528 ± 237	0.33
LYP (%)	72.04±3.98	71.2 ± 2.51	66.62 ± 4.16	69.6 ± 8.14	69.54 ± 4.03		67.18 ± 5.32		69.0 ± 5.02	0.98
LYA (10 ⁹ / L)	1.11 ± 0.21	2.06 ± 0.21	2.5 ± 0.50	2.764 ± 1.54	2.764 ± 1.54		1.50 ± 0.33		1.38 ± 0.21	0.63
NEA (10 ⁹ / L)	3.61 ± 0.71	6.72 ± 0.68	6.69 ± 1.42	5.226 ± 0.75	5.226 ± 0.75		4.91 ± 1.74		5.30 ± 2.13	0.50
MOA (10 ⁹ / L)	0.25 ± 0.1	0.33 ± 0.04	0.41 ± 0.09	0.19 ± 0.06	0.19 ± 0.06		0.24 ± 0.12		0.34 ± 0.14	0.58
EOA (10 ⁹ / L)	0.04 ± 0.02	0.26 ± 0.12	0.29 ± 0.18	0.10 ± 0.04	0.10 ± 0.04		0.05 ± 0.02		0.09 ± 0.05	0.36
BAO (10 ⁹ / L)	0.04 ± 0.01	0.05 ± 0.01	0.07 ± 0.02	0.04 ± 0.01	0.04 ± 0.01		0.04 ± 0.02		0.05 ± 0.02	0.68

Data presented as mean ± SEM.

* $p < 0.05$ compared to the control group.

Table 2
Mean biochemical parameters of rats on various drug regimen.

Analyte/ Unit	Control	Model	CMARE 30 mg/kg b.wt	CMARE 300 mg/kg b.wt	Finasteride/ CMARE 30 mg/ kg b.wt	Finasteride/CMARE300 mg/ kg b.wt	Finasteride	p- value
RENAL FUNCTION TEST								
Urea (mmol/L)	6.16 ± 0.56	7.69 ± 0.50	6.46 ± 0.50	6.33 ± 0.27	7.43 ± 0.28	6.45 ± 0.14	7.16 ± 0.17	0.08
Creatinine (μmol/L)	29.00 ± 2.00	37.50 ± 4.35	22.40±3.23 [†]	25.00±1.78 [†]	26.50 ± 2.22	30.00 ± 2.16	30.60 ± 1.78	0.02
HCO ₃ ⁻ (mmol/L)	21.00 ± 0.63	25.50 ± 3.28	22.60 ± 1.21	22.75 ± 1.55	19.75 ± 0.85	19.00 ± 0.71	21.80 ± 1.07	0.12
Cl (mmol/L)	96.60 ± 1.63	112.00 ± 9.06	98.20 ± 2.84	103.80 ± 3	102.75 ± 5.22	103.25 ± 1.11	101.80 ± 0.86	0.21
K (mmol/L)	6.59 ± 0.72	7.61 ± 1.31	4.97 ± 0.38 [‡]	6.17 ± 0.70	8.18 ± 1.17	9.88 ± 0.54	7.71 ± 1.18	0.03
NA (mmol/L)	136 ± 2	160 ± 12	138±4	145±2	141 ± 7	142 ± 2	143±1	0.12
LIVER FUNCTION TEST								
ALT (IU/L)	67.2 ± 7.8	87.7 ± 15.1	63.2 ± 8.3	51.7 ± 4.3	102.5 ± 33.9	54.5 ± 14.5	70.8 ± 9.3	0.27
AST (IU/L)	248 ± 31	311 ± 34	316±21	268±30	368±45 [‡]	209 ± 22	310±27	0.04
ALP (IU/L)	255 ± 6	290 ± 28	278±44	256 ± 33	248 ± 27	258 ± 36	231±28	0.90
TBIL (μmol/L)	1.80 ± 0.37	1.75 ± 0.63	1.00 ± 0.63	2.00 ± 0.40	1.25 ± 0.25	1.00 ± 0.41	2.00 ± 0.45	0.52
DBIL (μmol/L)	0.72 ± 0.13	0.35 ± 0.22	0.26 ± 0.09	0.55 ± 0.17	0.30 ± 0.14	0.43 ± 0.16	0.40 ± 0.13	0.31
TP (g/L)	62.00 ± 2.39	75.00 ± 6.1	58.20 ± 2.30 [†]	61.50 ± 0.90	63.25 ± 0.00	68.00 ± 2.48	66.60 ± 1.36	0.03
ALB (g/L)	29.40 ± 1.29	34.50 ± 3.71	26.40 ± 1.29	27.75 ± 1.03	27.25 ± 2.78	29.00 ± 1.96	29.00 ± 0.71	0.15

Data presented as mean ± SEM.

[†] $p < 0.05$ compared to the model group.

[‡] $p < 0.05$ compared to the Finasteride/ 300 mg/kg CMARE group.

differences were seen in other organs. Notably, is the fact that the prostate and its accessory organs did not show improvement statistically. For some unexplained reason, the model group had a lower weight

compared to all the treatment groups. However, with the PSA, the 30 mg/kg b.wt. *C. membranaceus* administered group had the lowest value compared to all the groups. It can be inferred that the model group did

Table 3
Mean PSA and testosterone levels of rats on various drug regimen.

Analyte/ Unit	Control	Model	CMARE 30 mg/ kg b.wt	CMARE 300 mg/ kg b.wt	Finasteride/CMARE 30 mg/kg b.wt	Finasteride/ CMARE 300 mg/kg b.wt	Finasteride	p- value
PSA/(ng/ml)	0.63 ± 0.03	0.55 ± 0.04	0.44 ± 0.03	0.55 ± 0.06	0.60 ± 0.01	0.54 ± 0.02	0.59 ± 0.08	0.14
Testosterone/ (nmol/l)	6.87 ± 0.82	154.63 ± 7.47 *	169.50 ± 10.50 *	143.10 ± 8.50 *	186.50 ± 38.41 *	143.75 ± 17.13 *	158.88 ± 9.88 *	0.00

Data presented as mean ± SEM.

* $p < 0.05$ compared to the control group.

Table 4
The table represents relative organ weights [Mean ± Sem (g x 10⁻³)].

	G1	G2	G3	G4	G5	G6	G7	p-value
ORGAN								
Heart	3.15 ± 0.13	3.50 ± 0.17	3.82 ± 0.10	3.40 ± 0.15	3.46 ± 0.15	3.64 ± 0.09	3.51 ± 0.04	0.054
Lungs	5.84 ± 0.74*	7.10 ± 0.44	7.12 ± 0.38	6.63 ± 0.39	8.73 ± 0.58*	6.88 ± 0.20	7.74 ± 0.81	0.026
Liver	26.18 ± 4.13	30.24 ± 1.11	27.38 ± 2.30	25.65 ± 1.06	26.39 ± 1.94	31.66 ± 1.37	34.34 ± 1.06	0.084
Spleen	9.11 ± 3.97	3.58 ± 0.37	3.83 ± 0.48	3.56 ± 0.12	3.83 ± 0.23	4.12 ± 0.34	4.30 ± 0.26	0.216
Brain	5.91 ± 0.71	6.27 ± 0.20	5.33 ± 0.20	6.28 ± 0.31	6.49 ± 0.29	6.48 ± 0.21	6.14 ± 0.18	0.393
Kidney	3.48 ± 0.10	3.86 ± 0.29	4.29 ± 0.55	3.66 ± 0.15	3.31 ± 0.16	3.97 ± 0.24	4.04 ± 0.20	0.159
SV + P	6.96 ± 0.15	10.85 ± 4.19	13.88 ± 0.97	13.19 ± 1.47	13.52 ± 1.98	12.35 ± 0.75	11.19 ± 2.29	0.179

G1 = Control group; G2 = Model group (untreated); G3 = Finasteride treatment (0.5 mg/kg); G4 = *C. membranaceus* treatment group (30 mg/kg b.wt.); G5 = *C. membranaceus* treatment group (300 mg/kg b.wt.); G6 = 0.5 mg/kg. b.wt. Finasteride + 30 mg/kg b.wt. *C. membranaceus*; G7 = 0.5 mg/kg. b.wt. Finasteride + 300 mg/kg b.wt. *C. membranaceus*.

not respond too well as a model group. PSA is not a reliable marker that correlates the prostate weight. Hence the focus of this paper is on the histomorphology.

Most studies worked with Tamsulosin in combination with medicinal plants. For example, curcumin is said to reduce inflammatory symptoms. Hence, BPH patients who undergo TURP are administered curcumin in addition to the Tamsulosin and Finasteride combination. In a study that examined the effect of natural products plus allopathic medicine over six months, PSA did not show any change as seen in this study. Prostate volume increased. However, IPSS, International Index of Erectile Function (IIEF) and QoL improved significantly (Qiao et al., 2021). Other studies had different outcomes with different medicinal plants. For example, *Phellodendron bawei* tablets in combination with the standard treatment (α 1-receptor blockers plus 5 α reductase inhibitors) in a double-blind study for six months, saw an increase in IPSS and prostate volume but not PSA (Gong et al., 2021).

AST was highest in the group that received 300 mg/kg b.wt. *C. membranaceus* compared to the Finasteride group; this was significant. Other studies on the other hand demonstrated a decrease in AST on the application of i.e. combined extract of *Spermacoce radiata* (DC.) In an acute toxicity study of *C. membranaceus* the highest dose at 1500 mg/kg b.wt. did not elicit any statistical difference in AST levels compared to the control group (Asare et al., 2011). AST levels at 300 mg/kg. b.wt. decreased but not significantly in a 90-day study using Sprague Dawley rats (Afriyie et al., 2013). In a human study with 60 mg/d *C. membranaceus* use over 3 months, AST increased slightly although not significant (Asare et al., 2015). The current increase in AST is not suggestive of hepatotoxicity as this is not supported by the ALT levels that are more liver specific. Furthermore, the combination therapy had AST levels comparable to other treatment groups. In a study on the ethanolic extract of *Melandrium firmum* using testosterone BPH-induced Wistar rats, AST and ALT remained relatively unchanged in all treatment groups for 4 weeks (Lee et al., 2012).

Creatinine was lowest in the Finasteride group compared to the control. There was a similar effect in the group that received 30 mg/kg. b.wt. *C. membranaceus*. From other studies with *C. membranaceus*, creatinine did not show any increase (Afriyie et al., 2013; Asare et al., 2011). Therefore, the relative organ weights do not support any hepatorenal dysfunction.

Most studies have not examined the haematological effect of combination therapy on BPH. In this study, no significant differences in haematological parameters were observed. The sharp decline in platelet with *C. membranaceus* has been noted and will require further investigation.

Thus, the evidence provided here is similar to other studies that found little or no adverse effect of combination therapy both in animals and human studies. Tolerability was recorded in most studies and IPSS, Qmax and QoL improvement in some human studies.

In conclusion, the histomorphological evidence provided generally suggests no added beneficial effects of the *C. membranaceus*/Finasteride combination. However, higher doses of this combination may not be advisable in terms of semen production.

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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