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Combined ethanol extract of *Hypselodelphys poggeana* and *Spermacoce radiata* leaves ameliorate benign prostatic hyperplasia in rats via modulation of serum sex hormonal levels

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ABSTRACT

This study evaluated the effects of a combined ethanol extract of and *Hypselodelphys poggeana* and *Spermacoce radiata* leaves (CESHL) on sex hormone levels and prostate weights of benign prostatic hyperplasia (BPH) induced rats. The combined extract was prepared from the plants and was subjected to acute toxicity (LD_{50}) evaluation in rats. The study adopted five groups of rats (n = 6) with group 1 being the normal control without BPH induction, group 2 was the BPH control, group 3 was the finasteride control, and groups 4 and 5 were BPH induced rats treated with 250 and 500 mg/kg CESHL/day for 28 days. Induction of prostate hyperplasia in rats belonging to groups 2-5 was done via daily subcutaneous administration of testosterone propionate (5 mg/kg body weight) for 28 days in accordance with standard protocols. The result obtained in the acute toxicity evaluation indicated an LD_{50} value of above 5000 mg/kg body weight. In the main study, a significant increase ($P<0.05$) in serum testosterone, dihydrotestosterone, prostate weight, prostate index and reduced serum estradiol was observed in the BPH control when compared with the normal control. Treatment of BPH induced rats (groups 4 and 5) with CESHL significantly reduced serum concentrations of testosterone and dihydrotestosterone, and also elevated serum estradiol concentration when compared with the BPH control, comparing favourably with the values of these hormones in the finasteride-treated group. The BPH induced rats treated with CESHL also had significantly reduced prostate weight and prostate index relative to the BPH control and higher percentage inhibition of prostate weight and prostate index when compared with the finasteride control. Our findings show that CESHL may ameliorate benign prostate hyperplasia via mechanisms that lower serum testosterone and dihydrotestosterone levels and increase estradiol levels.

KEYWORDS: Benign prostatic hyperplasia, *Spermacoce radiata*, *Hypselodelphy spoggeana*, Sex hormones, Prostate weight, Prostate index

1. INTRODUCTION

Benign Prostatic Hyperplasia (BPH) is a multi-factorial medical condition characterized by the putative proliferation of the stromal and epithelial cells of the prostate, which leads to the swelling of the prostate and eventual obstruction of urine exit in ageing men [1]. Various theories have been purported in a bid to explain the aetiology of BPH with none of them offering a comprehensive explanation on the mechanism of BPH development and progression [2, 3, 4]. Genetic theories according to De Souza *et al.*, on the aetiology of BPH faults the occurrence of numerous polymorphisms responsible for BPH via encoding the androgenic receptors which are important for the activation of other genes and the production of the androgen hormone directly involved in the progression of BPH [1]. Whereas, other researchers Nicholson and Ricke, and Vignera *et al*., opined that the underlying genetic mechanism of BPH is inconsistent, as other studies have shown that many men with BPH do not possess any mutations on their CAG genes [2, 4]. Changes in the levels of androgenic and estrogenic hormones with ageing in men are responsible for the initiation and progression of

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BPH [5]. A high level of testosterone is converted to dihydrotestosterone (DHT) by the 5α-reductase enzyme and an increased serum level of DHT in association with estradiol triggers the growth and proliferation of the stromal and epithelial cells present in the prostate [6]. New findings have also linked BPH initiation and progression to the increasing levels of proinflammatory cytokines and oxidative stress [3]. The use of 5α-reductase inhibitors including Finasteride and Dutasteride to treat BPH have always yielded the desired results but are associated with some undesirable side effects, which have necessitated the need to search for a more potent drug with less toxicity and have a low cost [4].

Hypselodelphy spoggeana (K. Schum.) Milne-Redh is a member of the *Marantaceae* family found in tropical areas in Africa especially in the southeastern part of Nigeria and in other parts of the world. It is a partly woody-herb that grows to about 2-6m in height and bears white or violet flowers. There is limited available information on its ethnopharmacological properties despite its use for general healing, depressant and as an aphrodisiac agent to enhance genital stimulation [7]. *Spermacoce radiata* (DC.) Hiern belongs to the family of Rubiaceae [8]. Spermacoce has been useful in traditional medicine due to its pharmacological properties such as anti-inflammation, antibacterial, anti-ulcer, anti-cancer, antitumour and anti-malaria properties, which were attributed to high phytochemical contents including terpenoids, flavonoids, alkaloids and phenols [9, 10]. The leaves of different species of *Spermacoce* have demonstrated potent therapeutic activities against gonorrhoea, gastrointestinal disorders, gallstones, headache and haemorrhoids [11]. *Hypselodelphy spoggeana* and *Spermacoce radiate* leaf extracts are effective in the treatment of benign prostatic hyperplasia and different urinary tract infections by local traditional medicine practitioners in the southeastern part of Nigeria with both those that used them independently or in combination claiming total recovery from BPH (12). This study evaluated the effects of a combined ethanol extract of *Hypselodelphy spoggeana* and*Spermacoce radiata* leaves (CESHL) on the sex hormonal levels and prostate weight of benign prostatic hyperplasia (BPH) induced rats.

2. RESULTS

2.1. Percentage yield of the extraction

The extraction of 700 g of the combined plant samples gave a percentage yield of 8.26 % equivalent to 57.82 g of the CESHL.

2.2 Acute toxicity effects of CESHL on rats

The acute toxicity effects of the CESHL on the rats indicated that it is safe for consumption as none of the treated rats showed any signs or symptoms of toxicity. The rats were physically stable, no death was recorded, and the LD_{50} of the CESHL was found to be greater than 5000 mg/kg.

2.3. Effects of CESHL on the serum testosterone levels of BPH induced rats

The result in Figure 1 indicated significant ($P<0.05$) increases in the serum testosterone concentrations of the BPH control and BPH induced rats treated with 250 and 500 mg/kg CESHL/day when compared with the normal control respectively. However, the serum testosterone concentration of the finasteride control rats showed no significant ($P>0.05$) decrease relative to the normal control. Besides, the serum testosterone levels of the finasteride control and BPH induced rats treated with 250 and 500 mg/kg CESHL respectively, decreased significantly ($P<0.05$) in comparison with the BPH control. Moreover, the serum testosterone concentration of BPH induced rats treated with 500 mg/kg CESHL/day showed a significant ($P<0.05$) reduction relative to the finasteride control. Contrarily, the BPH induced rats administered 250 mg/kg CESHL/day should have no significant ($P>0.05$) increase in serum testosterone concentration when compared with the finasteride control.

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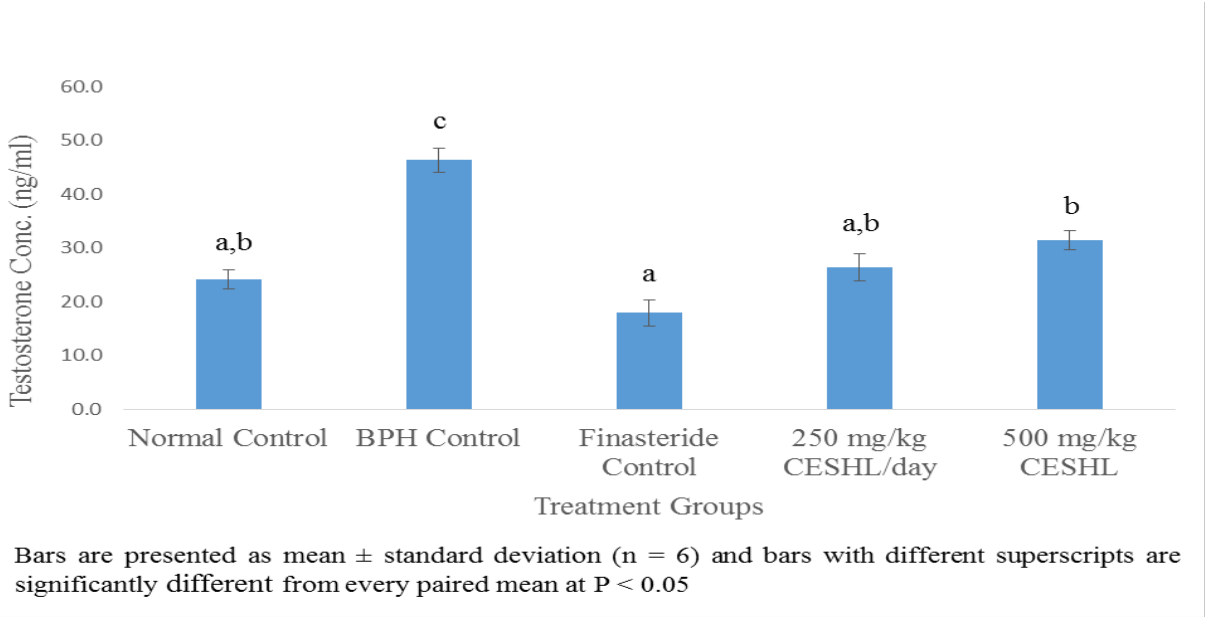

Fig. 1: Serum testosterone concentrations of BPH induced rats treated with CESHL

2.4. Effects of CESHL on the serum dihydrotestosterone levels of BPH induced rats

The dihydrotestosterone (DHT) concentrations in Figure 2 showed significantly (P<0.05) increases in the BPH control, finasteride control and BPH induced rats treated with 250 mg/kg CESHL/day in comparison with the normal control respectively. Asides, there was no significant (P>0.05) increase in the serum DHT concentration of BPH induced rats that received 500 mg/kg CESHL/day relative to the normal control. In addition, the serum DHT concentrations of the finasteride control and BPH induced rats treated with 250 and 500 mg/kg CESHL/day significantly (P<0.05) increased respectively when compared with the normal control. Furthermore, there was no significant (P>0.05) reduction in the serum DHT of BPH induced rats treated with 250 mg/kg CESHL/day but a significant (P<0.05) reduction in the serum DHT concentration of BPH induced rats treated with 500 mg/kg CESHL/day when compared with the finasteride control.

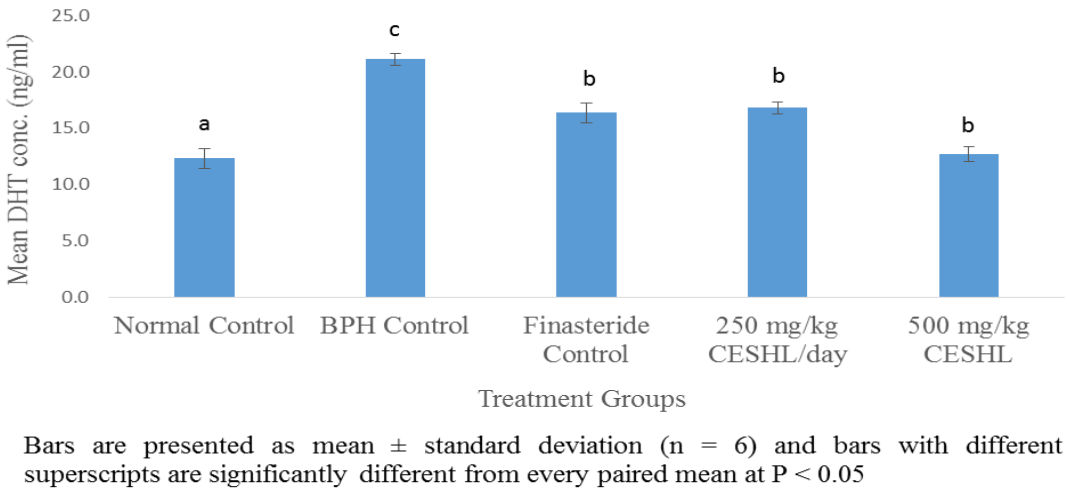

Figure 2: Dihydrotestosterone (DHT) concentrations of BPH induced rats treated with CESHL

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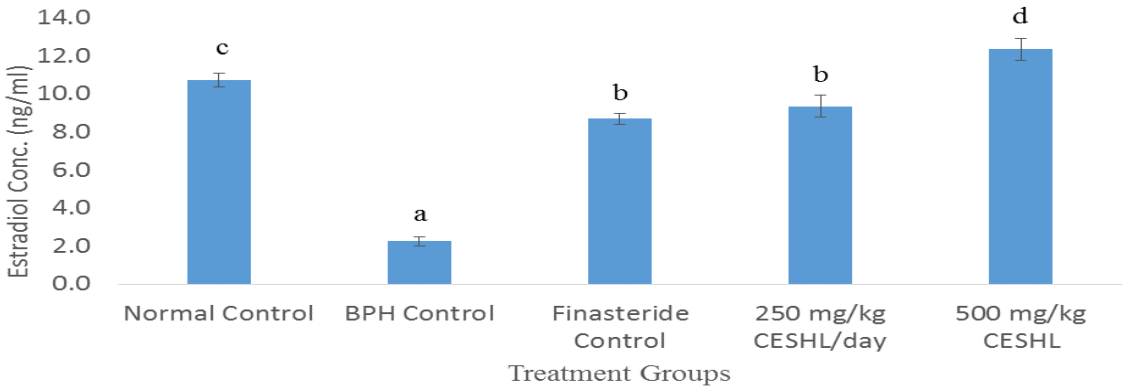
2.5. Effects of CESHL on the serum estradiol levels of BPH induced rats

In Figure 3, there were significant (P<0.05) reductions in the estradiol (E_2) concentrations in the BPH control, finasteride control and BPH induced rats treated with 250 mg/kg CESHL/day when compared with the normal control. Whereas, there was a significant (P<0.05) increase in the serum E_2 concentration of the BPH induced rats treated with 500 mg/kg CESHL/day relative to the normal control. Also, there were significant (P<0.05) increases in the serum E_2 concentrations of the finasteride control and BPH induced rats treated with 500 mg/kg CESHL/day in comparison with the BPH control respectively. Moreover, there was no significant (P>0.05) increase in the E_2 concentrations of BPH induced rats treated with 250 mg/kg CESHL/day but a significant (P<0.05) increase in the E_2 concentration of BPH induced rats treated with 500 mg/kg CESHL/day relative to the finasteride control.



Bars are presented as mean ± standard deviation (n = 6) and bars with different superscripts are significantly different from every paired mean at P < 0.05

Figure 3: Estradiol concentrations of BPH induced rats treated with CESHL

2.6. Effects of CESHL on the body weight of BPH induced rats

The baseline body weight (BBW) in Table 1 showed that the BPH and finasteride control had significantly (P<0.05) lower BBW when compared with the normal control, and BPH induced rats treated with 250 and 500 mg/kg CESHL/day respectively. Also, the BBW of BPH induced rats treated with 250 mg/kg CESHL/day was not significantly (P>0.05) higher in comparison with the normal while the BBW of the BPH induced rats treated with 500 mg/kg CESHL/day had significantly (P<0.05) high BBW when compared with any of the groups.

The results in Table 1 indicated that the BPH control had no significantly (P<0.05) lower body weight after 28 days (BWA) and the BPH induced rats treated with 250 had no significantly (P>0.05) high BWA when compared with the normal control. The finasteride control had significantly (P<0.05) lower BWA when compared with the normal control, BPH induced rats treated with 250 and 500 mg/kg CESHL/day but not significantly (P>0.05) BWA when compared with the BPH control. The BWA of BPH induced rats treated with 500 mg/kg CESHL/day was significantly (P<0.05) high in comparison with the BWA observed in every other group.

The body weight gain (BWG) in Table 1 showed no significant (P>0.05) increase in the body weight gain in the BPH control when compared with the normal control. However, there was no significant (P>0.05) reduction in the body weight gain in the finasteride control and BPH induced rats treated with 250 and 500 mg/kg CESHL/day relative to the normal control and BPH control respectively. Although the BPH induced rats, treated 500 mg/kg CESHL/day showed decreased BWG, this was not significantly different from other groups.

Table 1: Changes in body weight of BPH induced rats treated with CESHL

Treatment groups	BBW (g)	BWA (g)	BWG (g)
Normal Control	133.21±4.64^{b}	158.92±8.79^{b}	25.72±5.17^{a}
BPH Control	117.53±9.56^{a}	146.75±7.22^{a,b}	29.22±3.97^{a}
Finasteride Control	113.22±5.36^{a}	137.78±7.37^{a}	24.58±8.65^{a}
BPH + 250 mg/kg CESHL/day	136.75±6.82^{b}	160.17±9.62^{b}	23.42±6.56^{a}
BPH + 500 mg/kg CESHL	152.83±5.66^{c}	175.23±6.22^{c}	22.41±2.37^{a}

Values are presented as mean ± standard deviation (n = 6) and values with different superscripts are significantly (P < 0.05) different from any paired mean. BBW = baseline body weight; BWA = body weight after 28 days of treatment; BWG = body weight gain.

2.7. Effects of CESHL on the prostate weight (PW)

There were significant (P<0.05) elevated prostate weight (PW) in the BPH control, finasteride control and BPH induced rats treated with 250 and 500 mg/kg CESHL/day respectively when compared with the normal control (Table 2). The finasteride control, BPH induced rats treated with 250 and 500 mg/kg CESHL/day showed significant (P<0.05) reductions in PW in comparison with the BPH control respectively. Besides, The BPH induced rats treated with 250 mg/kg CESHL/day indicated no significant (P>0.05) reduction in PW when compared with the finasteride control. Contrarily, the BPH induced rats treated with 500 mg/kg CESHL/day had significantly (P<0.05) reduced PW relative to the finasteride control.

Table 2: Prostate weight and relative prostate weight of BPH induced rats treated with CESHL

Treatment	Prostate weight (g)	Prostate index (%)
Normal Control	0.26±0.05^{a}	0.16±0.04^{a}
BPH Control	0.76±0.10^{d}	0.52±0.06^{d}
Finasteride Control	0.52±0.03^{c}	0.38±0.09^{c}
BPH + 250 mg/kg CESHL/day	0.43±0.06^{b,c}	0.27±0.03^{b}
BPH + 500 mg/kg CESHL/day	0.36±0.03^{b}	0.20±0.01^{a}

Values are presented as mean ± standard deviation (n = 6) and values with different superscripts are significantly (P < 0.05) different from any paired mean.

2.8. Effects of CESHL on the prostate index (PI)

The prostate index (PI) in Table 1 indicated a significant (P<0.05) increase in the PI of the finasteride control and BPH induced rats treated with 250 mg/kg CESHL/day when compared with the normal control respectively. The BPH induced rats treated 500 mg/kg CESHL/day showed no significant (P>0.05) increase in PI relative to the normal control. Asides, there were significant (P<0.05) reductions in the PI of the finasteride control, BPH induced rats treated with 250 and 500 mg/kg CESHL/day respectively in comparison with the BPH control. In addition, the PI of the BPH induced rats treated with 250 and 500 mg/kg CESHL/day decreased significantly (P<0.05) when compared with the finasteride control.

2.9. Effects of CESHL on the percentage inhibition of the prostate weight

The percentage inhibition of the prostate weight in Table 3 showed that the BPH induced rats treated with 250 and 500 mg/kg CESHL/day indicated increased inhibition of PW when compared with the finasteride control. The value of percentage PW inhibition showed that the BPH induced rats treated with 500 mg/kg CESHL/day had the highest PW inhibition, followed by the BPH induced rats treated with 250 CESHL/day and then the finasteride control with the least percentage PW inhibition.

Table 3: Percentage inhibition of prostate weight (PW) and prostate index (PI) in BPH induced rats treated with CESHL

Treatment Groups	Percentage inhibition of PW (%)	Percentage inhibition of PI (%)
Normal Control	-	-
BPH Control	-	-
Finasteride Control	72.96±0.83	85.77±0.56
BPH + 250 mg/kg CESHL/day	85.38±1.02	91.75±0.34
BPH + 500 mg/kg CESHL/day	92.80±0.66	97.78±0.75

Values are presented as mean ± standard deviation (n = 6)

2.10. Effects of CESHL on the percentage inhibition of the prostate index (PI)

The percentage inhibition of prostate index (PI) in Table 3 indicated a dose-dependent increase in the prostate index inhibition in the BPH induced rats treated with 250 and 500 mg/kg CESHL/day when compared with the normal control. The BPH induced rats treated with 500 mg/kg CESHL/day indicated the highest PI inhibition, followed by the BPH induced rats treated with 250 mg/kg CESHL/day and the finasteride control in descending order.

3. DISCUSSION

The study evaluated the effects of a combined ethanol extract of *Spermacoce radiata* and *Hypselodelphy spoggeana* leaves (CESHL) on the sex hormonal levels and prostate weight of benign prostatic hyperplasia (BPH) induced rats. BPH is one of the health disorders affecting ageing men in our societies and available drugs like Finasteride and Dutasteride are not fully effective in treating and elicit some adverse health effects to the user. Androgenic and estrogenic hormones are responsible for the development and progression of BPH and their downregulation in ageing men or BPH patients play a vital role in the effective prevention and management of BPH and this has necessitated this study [13].

The results revealed a high serum concentration of testosterone in BPH induced and BPH control models when compared to normal control. This agrees with earlier studies by Kinter *et al.*, which implied that testosterone plays a role in the progression of BPH through its conversion to Dihydrotestosterone [5]. In addition, this study evaluated the effect of various treatment options on testosterone including the use of finasteride, an allopathic drug and the CESHL extract in two different concentrations. However, when compared with BPH control, serum testosterone concentration decreased when Finasteride and 250 mg/kg of CESHL/day were administered, though between finasteride and 250 mg/kg CESHL/day, a less significant concentration of Serum testosterone occurred in the latter. This concentration was further reduced when the doses of CESHL was increased to 500 mg/kg/day implying that it is possibly contributing to ameliorating BPH by suppressing 5-alpha reductase [14]. The results suggest that CESHL controlled serum testosterone concentration better than finasteride.

Dihydrotestosterone (DHT) an active metabolite of testosterone is one of the major factors implicated in the pathogenesis of BPH and agents that reduce its circulating concentration mostly by inhibiting the 5α-reductase enzyme activity that converts testosterone to DHT prevents BPH development and progression [15, 16]. However, there are increasing interests in the role of other factors like growth factors, estrogen and inflammation in BPH since some ageing men with low testosterone and DHT levels have developed BPH, which implies that many factors could predispose men to BPH [4]. The very high DHT level in the BPH control in this study showed that much of the testosterone concentration earlier observed in this group might be responsible for the increased DHT levels unhindered and correlates with the increased prostate weight and prostate index observed in this study. The high DHT level in the BPH control could have induced increased division and proliferation of the prostate epithelial and stromal tissues responsible for BPH development and

progression. Whereas, the significant reductions in the serum DHT concentrations in the finasteride control and BPH induced rats treated with 250 and 500 mg/kg CESHL/day showed both the finasteride and CESHL independently inhibited the conversion of testosterone to DHT by the 5α-reductase enzyme. These results further show that 500 mg/kg CESHL/day had a better effect in reducing DHT concentration and may be more useful in the management and prevention of BPH progression as shown by the decreased prostate weight and prostate index recorded in this study.

The significant decline in the serum estradiol (E_2) level in BPH control with increased prostate weight and prostate index show that low estradiol level promotes BPH development. This is contrary to earlier reports that elevated estrogens like estradiol bind to estrogen receptors in the prostate and induce BPH and increased prostate volume while an increase in serum testosterone does not play any role in BPH progression [2, 16]. The treatment of BPH induced rats with finasteride and CESHL respectively caused a significant increase in the serum estradiol levels contrary to the BPH control, which correspond to the significant reduction in prostate weight, prostate index and high percentage inhibition of prostate weight and prostate index. Our findings show that a high dose of CESHL maintains a high of estradiol and reverses BPH growth and vice versa. The increased E_2 concentration is in alignment with an observation made by Vignera *et al.,* and Ajayi *et al.*, concerning increased estradiol (E_2) concentrations and their role in facilitating the progression of BPH [4, 16]. These results correlate with earlier literature reports that some plant extracts combined showed activity against BPH pathogenesis [17].

The no significant elevation of the final body weight after 28 days observed in the normal control rats, BPH control, finasteride control, and BPH induced rats treated with CESHL were in line with the trend observed in the baseline body weights and showed that BPH has no negative impact on the body weight. The BPH induced rats had no reductions in the quantities of food they consumed throughout the experiment which when coupled with normal energy metabolism in the rats could account for the normal changes in the body weight observed in this study which is in agreement with the findings Hongcai *et al.,* and Uroko *et al*., [13, 18]. In addition, the relatively lower body weight gain in the finasteride control and BPH induced treated with CESHL could be attributed to the lower prostate weight recorded in the rats.

Elevated prostate weight (PW) and prostate index (PI) often points to an impending BPH because of its contribution to the symptoms preceding BPH, which includes lower urinary tract symptoms [19]. This study also recorded an increase in prostate weight in BPH control relative to the normal control suggesting BPH progression and proliferation of prostate tissues and cells. Treatment with finasteride and different doses of CESHL significantly reversed prostate weight to near normal compared to normal control, which indicated that CESHL has anti-benign prostatic hyperplasia affects more than finasteride. The high therapeutic effects of CESHL against BPH coupled with its low toxicity, availability, and low cost are strong indications that there should be further researches on CESHL to maximize its therapeutic potentials and e benefits for BPH patients. Thus, therapeutic agents that lower serum testosterone and DHT levels as demonstrated by CESHL could be useful in reversing an enlarged prostate weight and are contrary to the earlier findings by Xia *et al*., that increased testosterone levels caused a proportional decrease in prostate weight [20]. The changes observed in the prostate weight, prostate index of BPH induced rats without any treatment, and rats with BPH treated with CESHL are in agreement with the findings of Nicholson, and Rick, and Sasagawa*et al*., [2, 21]. Their findings showed that an increase in testosterone level promotes proliferative of prostate tissues and BPH whereas reduction in testosterone level decreases prostate weight and reverses BPH.

The high percentage inhibitions of prostate weight and prostate index observed in the BPH induced rats treated with finasteride and CESHL respectively showed the therapeutic effects of the treatments on BPH and further indicated that the rats were recovering from the adverse effects of BPH and suggest that CESHL has better therapeutic effects against BPH. The dose-dependent inhibitory effects of CESHL on prostate weight and prostate index of the BPH treated rats indicated that with the administration of 500 mg/kg CESHL/day the prostate weight of the BPH induced rats achieved almost restoration to normal size of rats without BPH similar to the findings of Hongcai *et al.,* and Uroko *et al*., [13, 22]. We, therefore suggest that a high dose of CESHL is more effective in the treatment of BPH than finasteride as the CESHL may have diverse mechanisms of action other than the inhibition of 5-alpha-reductase activity exhibited by finasteride.

4. CONCLUSION

The findings of this study indicated that a combined ethanol extract of *Spermacoce radiata* and *Hypseldelpghy spoggeana* leaves ameliorate benign prostatic hyperplasia progression through lowering of testosterone, dihydrotestosterone and estradiol concentrations. The low cost of CESHL and its ability to reduce an enlarged prostate weight or volume significantly makes it a better therapeutic agent for the management of BPH rather than finasteride.

5. MATERIALS AND METHODS

5.1. Materials

5.1.1 Chemicals and reagents

The absolute ethanol solvent was d from Guangdong GuanghuaSci-Tech Company Ltd, India. Besides, the testosterone propionate and finasteride were from Laborate Pharmaceuticals Limited, India and BafnaPharmaceuticals Limited, India respectively. The test kits used were from Randox Laboratories, United Kingdom.

5.1.2. Collection and identification of plant materials

The fresh leaves of *Spermacoce radiata* and *Hypselodelphy spoggeana* were collected from the botanical garden of the Department of Forestry, Michael Okpara University of Agriculture, Umudike and identified at the Taxonomy Unit of the same Department and dried under shade at room temperature (25℃) until constant weights were obtained. After drying, the plant samples were pulverized with a mechanized grinder into coarse powder samples for extraction.

5.2. Methods

5.2.1. Experimental animal

In this study, we used fifty-one male Wistar albino rats (140 – 150 g) body weight range. The experimental animals were from the Animal production unit of the Department of Zoology, University of Nigeria, Nsukka, Nigeria. The animals were acclimatized to our animal house for 14 days and handled humanely. The study also complied with the terms and conditions of the ethical approval issued for the study by the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike.

5.2.1. Experimental design

The study had five groups of rats (n = 6) with groups 1 – 3 serving as the normal control, BPH control (BPH induced untreated) and finasteride control (BPH induced with 5 mg/kg finasteride/day) respectively. Groups 4 and 5 were BPH induced rats treated with 250 and 500 mg/kg CESHL/day for 28 days respectively. The normal control was treated with 2 mg/kg olive oil/day, The rats were induced BPH by the subcutaneous administration of testosterone propionate mixed with olive oil (2:1) 5 mg/kg/day for 28 days. The baseline body weight (BBW) and the final body weight after 28 days of the study (BWA) were taken, the rats fasted overnight on the 28th day, euthanized and blood samples were taken from them via cardiac puncture and prostate tissues removed, and their weights measured accordingly on the 29th day.

5.2.2. Preparation of combined ethanol extract of *S. radiata* and *H. poggeana* leaves (CESHL)

The combined ethanol extract of *S. radiata* and *H. poggeana* leaves (CESHL) were obtained by weighing 350 g of each of the pulverized plant leaf samples into a clean-sterile flask (i.e. 700 g of combined plant samples) and dissolving it with 1.8 L of absolute ethanol for 72 h with intermittent shaking according to Uroko *et al*. [18]. After 72 h, the macerated combined plant sample was filtered. In addition, the combined extract was concentrated at 45°Cin a water bath to move ethanol, the weight and e net percentage yield of calculated.

5.2.3. Acute toxicity study of CESHL

The acute toxic effects of CESHL were evaluated according to the method of Lorke [23]. Phase I of the acute toxicity study of CESHL was evaluated with nine rats while and the phase II study was evaluated with another nine rats. In addition, the acute toxicity result was confirmed with three rats. In phase I the nine rats randomly distributed into three groups (n = 3) and groups 1-3 were administered with 10, 100 and 1000 mg/kg CESHL respectively and observed for 24 hours for adverse reactions or death. Similarly, the phase II had groups 1-3 containing 3 rats each which received 1600, 2900 and 5000 mg/kg CESHL respectively, and monitored for 24 hours for signs of CESHL toxicity and when no death was observed after the phase I and II, the LD50 of CESHL was assumed to be greater than 5000mg/kg.

5.2.4. Hormonal assays

The assays for serum testosterone (TT), dihydrotestosterone (DHT) and estradiol (E_2) concentrations in the rats were according to the methods of Tietz as outlined in the respective assay kit for each of the hormones [24].

5.2.5. Evaluation of prostate index (PI), and percentage inhibitions of prostate weight (PW) and prostate index (PI)

The prostate weights obtained after weighing the harvested prostate tissues, body weights of the rats were used to determine PI, and the percentage inhibitions of PW and PI according to the equations illustrated Hongcai *et al.* [13].

$$PI\ (\%) = \frac{PW}{BW}\ x\ 100$$

Where PW = prostate weight, BW = body weight

$$\text{Percentage inhibitions of PW and PI} = 100 - \mathrm{T}(\frac{\{T-C\}}{\{B-C\}}\ x\ 100)$$

Where C, B, T were the values of either PW or PI for the normal control group, BPH control, and CESHL treated BPH induced rats respectively.

5.3. Ethical approval

The Ethical Committee of the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike approved the study with approval number: MOUAU/VPP/EC/18/003

5.4. Statistical analysis

The data collected were analysed statistically with one-way analysis of variance (ANOVA) using a Statistical Products and Service Solutions (SPSS) version 22 and means separated with the Duncan multiple range comparison test at 95% confidence level ($P < 0.05$).

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Author contributions: Concept – R.I.U., S.N.I.; Design – R.I.U., S.N.I., E.L.O.; Supervision – R.I.U.; Resources – R.I.U., S.N.I., E.L.O.; Materials – E.L.0.; Data Collection and/or Processing – R.I.U., S.N.I., F.M.A.; B.Y., V.T.; Analysis and/or Interpretation – R.I.U., S.N.I., E.L.O.; Literature Search –R.I.U., S.N.I., F.M.A.; Writing – R.I.U.; Critical Reviews – S.N.I., E.L.O., F.M.A.

Conflict of interest statement: The authors declared no conflict of interest in the manuscript.

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