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AMELIORATING ACTION OF *CNIDOSCOLUS ACONITIFOLIUS* ON TESTOSTERONE PROPIONATE-INDUCED BENIGN PROSTATE HYPERPLASIA RATS

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ABSTRACT

Unhealthy lifestyle, high cost of living, non-balance feeding habit and poor exercise are among the major contributory factors to high incidence of benign prostate hyperplasia especially among men of 40 years and above. Improvement of biochemical parameters in testosterone propionate-induced benign prostate hyperplasia (BPH) rats by *Cnidoscolus aconitifolius* was studied. The 28days study was done using 30 male albino rats grouped into six with 5 rats each. The extraction and characterization of the leaves were done using standard methods. Enzyme assays and other biochemical parameters were determined using spectrophotometric techniques. The results obtained revealed that the administration of *Cnidoscolus aconitifolius* significantly reduced the Prostate Specific Antigen (PSA), Dihydrotestosterone (DHT), Alkaline phosphatase (ALP) and Inflammatory marker levels when compared with the BPH (negative) control group whose values were elevated. The histopathology assays of the treated groups showed little healing in the prostate as compared to the negative control group. The results suggest that *Cnidoscolus aconitifolius* possesses anti-BPH potentials and may be encouraged for management of BPH.

Keywords: Prostate, Hyperplasia, Dihydrotestosterone, and Inflammatory marker, *Cnidoscolus aconitifolius*

INTRODUCTION

Benign prostate hyperplasia (BPH) is gaining more attention nowadays because of the high incidence of the disease condition among men of 40 years and above (Tusubira *et al.,* 2023; Praven, 2013). Histologically, BPH can be said to be a non-malignant abnormal increase in the size of the prostate tissue that leads to urethra constriction which affects elderly men globally (Delvin *et al.,* 2019). The prostate plays important roles in the protection and nourishment of the sperm during ejaculation and being a small sized gland found in front of the rectum and under the bladder, it also plays important role during the passage of urine along the urethra (Hoffman, 2020; Madersbacher *et al.,* 2019).

It is worthy to note that though, BPH is a non-cancerous prostate disorder resulting in the enlargement of the prostate and causes lower urinary tract symptoms (LUTS) (Shabani *et al*., 2021); it is a chronic and slowly progressive disease. It evolves into an enlarged macroscopic nodular that resulted from simple microscopic nodular hyperplasia (Tusubira *et al.,* 2023). Lower urinary tracts symptoms (LUTS) can be defined by several symptoms including urgency, nocturia, frequency, dysuria, difficulty emptying the bladder, difficulty initiating micturition, and weak or interrupted stream during micturition (Lokeshwar *et al*., 2019). As such, the clinical manifestation of BPH could affect the quality of life owing to urinary hesitancy or straining in initiating urination that deteriorates the urinary function (Ishola *et al*., 2017).

The exact causes of BPH are not yet known, Batista and Mendonca (2022) implicated inappropriate action of steroid 5α-reductase type 2 enzyme (SRD5A2) which catalyzes the conversion of testosterone into dihydrotestosterone, as playing a crucial role in BPH development. In addition, Shabani *et al.* (2021) reported that oxidative stress could contribute to BPH debelopment. According to their report, there is a connection between oxidative stress and an increased risk of cellular proliferation, which leads to hyperplasic growth in prostatic tissues. The following are risk factors for BPH: aging, poor diet, diabetes, heart disease, and family history.

Therapeutically, options for benign prostate hyperplasia (BPH) may include Drug therapy (e.g. alpha-blockers, 5-alpha-reductase inhibitors) or surgery (Deters, 2021). Medications that are used for managing BPH contain 5-alpha reductase inhibitors which inhibits the conversion of testosterone to dihydrotestosterone (such as finasteride) and 1-alpha receptor antagonist which can be a means of treating benign prostatic hyperplasia by relaxing the bladder neck and the smooth muscle around the prostate (such as tamsulosin) and surgical treatments (Al-Trad *et al*., 2019). Nevertheless, their application is restricted because of complications with surgical prostatectomy and drug side effects, like dizziness, headache, sexual concerns (erectile dysfunction, loss of libido), allergic reaction, blood pressure changes, brain-related effects (hallucinations and sleepiness) among others (Ross, 2021). Surgery would have been a better option, but Edmond *et al.* (2023) have reported complications of surgery for benign prostate hyperplasia patients. Regarding these issues, it is of crucial to identify natural and affordable product that can prevent and be used to manage BPH development with no

or considerable side effect (Csikos *et al.*, 2021).

Several plant bioactive compounds can inhibit enzymes associated with the development of human diseases and have been used to treat various common human ailments (Mominur *et al.,* 2021). It is well proved that several of these compounds can be used as therapeutic agents with very low adverse effects to treat human disorders. The frequent occurrence of Benign Prostate Hyperplasia in men as they approach the fourth decade of life has made it reasonable to search for a preventive measure that is affordable, readily available, and presents little or no side effects.

Cnidoscolus aconitifolius (family Euphorbiaceae), usually called Chaya, belonging to the family of perennial evergreen shrub containing milky sap from pinnate lobed leaves have proven to be of economic importance to man. The plant was first identified in the Southeast part of Mexico and in the Maya area of Guatemala where the plant was grown as household leafy green vegetable. *Cnidoscolus aconitifolius*is usually consumed in Southwestern of Nigeria as vegetable, where it is referred to as Iyana Ipaja, while within the Southeastern Nigeria; it is called "Ugwuoyibo" or “Ulo-ogwu di anya” due to its medicinal and health benefits. This plant is also called Hospital Too Far or spinach tree and is consumed as vegetable soups and salads across Nigeria. According to the findings of Somade *et al* (2021) *Cnidoscolus aconitifolius* leaves possess some bioactive compounds such as β-carotene, protein, calcium, riboflavin, niacin, ascorbate, phosphorus, iron, thiamin at an appreciable quantity. *Cnidoscolus aconitifolius* is a plant with medicinal benefits for different diseases such as diabetes mellitus, atherosclerosis and hypertension. Moreso, its usage in the treatment of protein energy malnutrition (Ajiboye *et al*. (2018) and anemia (Ezeigwe *et al.,* 2020) has been reported. The plant has been implicated in the treatment of paracetamol intoxication and as an antibacterial agent (Panghal *et al.*, 2021). However, this study was designed to investigate the improvement of biochemical parameters of *Cnidoscolus aconitifolius* in testosterone propionate-induced benign prostate hyperplasia rats.

MATERIALS AND METHODS

Collection, Identification and Preparation of plant materials

Mature leaves of *Cnidoscolous aconitifolius* were collected from Awka, Anambra State and were identified by a taxonomist, Dr. (Mrs.) B.O. Aziagba of the Department of Botany, Faculty of Biosciences, Nnamdi Azikwe University, Awka. The voucher number as deposited in the herbarium of Nnamdi Azikiwe University, Awka is 168. The leaves were thoroughly washed, air-dried at room temperature and pulverized using electric grinding machine.

Extraction of plant materials

One thousand eight hundred (1800) grams of the ground leaf particles were macerated in 7.2 litres (1:4) of 70% ethanol for 24 hours with intermittent shaking. The mixture was sieved using muslin cloth and filtered using Whatman no 1 (125mm) filter paper. The filtrate was concentrated using a rotary evaporator under vacuum pressure, then further dried using water bath at 40ºC and weighed.

Experimental animals and their grouping

A total of forty-two (42) male Wistar albino rats of 16 weeks weighing 120 to 230g were sourced from Chris Animal Farm and Research Laboratory, Awka, Anambra State, Nigeria and used for the experiment. The animals were maintained and housed in

cages in the same laboratory. Out of which, thirty (30) rats were sorted, weighed, labeled, grouped and allowed to acclimatize with their new environment for one week before use. The remaining 12 rats were used for acute toxicity study. The animals were fed on vital grower's mash pellets *ad libitum*. Request for ethical approval from Nnamdi Azikiwe University Animal Research Ethics Committee was made and obtained (NAU/AREC/2023/00004), and all procedures for animal studies were performed following guidelines for the use of laboratory animals for research and teaching based on the principles of 3Rs, reduce, refine or replace.

Median Lethal Dose (LD_{50}) Determination

The median lethal doses (LD_{50}) of the extract were determined using Lorke's method (1983). Twelve (12) male rats were used for the determination of the median lethal dose of the extract. They were randomized into 6 groups, 3 rats each for the first phase which was administered with 10, 100 and 1000mg/ kg body weight (b.w) and one rat each for the second phase which was administered with 1600, 2900 and 5000mg/kg body weight (b.w) via oral intubation. The animals were monitored for changes in behavior and mortality within 2hrs and subsequently till 14days after the single administration of the extract. The LD_{50} was calculated using the formula below:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality.

Induction of benign prostatic hyperplasia

Benign Prostatic Hyperplasia was induced in the rats using testosterone propionate (Ishola *et al.*, 2017; Yang *et al.*, 2014b). The stock was prepared by dissolving 25mg of testosterone propionate in 8.33ml olive oil which is 3mg/ml (Jeon *et al.*, 2013). Following pilot study and previous research. The dose for induction was formulated as 3mg/kg body weight that was administered by subcutaneous injection every day for 28days (Obisike *et al.*, 2019).

Finasteride standard drug and the ethanol extract were dissolved in distilled water prior to their administration into the rats. The body weights of the study rats were determined weekly till the 29th day using an electronic weighing balance.

Experimental design

Thirty (30) male albino rats of the wistar strain of 16 weeks old were used for this study. The rats were grouped as follows with five rats in each group; Group 1(Normal control): this group received 1 ml/kg body weight of olive oil (subcutaneously) and 1 ml/kg of distilled water (oral intubation). Group 2 (Negative/BPH control): this group was induced with 3 mg/kg of Testosterone Propionate and 1 ml/kg of distilled water (oral intubation). Group 3 (Positive/Finasteride control): this group was induced with 3mg/kg body weight of Testosterone Propionate (subcutaneously) and treated with 5mg/kg finasteride (oral intubation). Group 4: this group was induced with 3 mg/kg of Testosterone Propionate and treated with oral intubation of 100 mg/kg body weight of ethanol extract. Group 5: this group was induced with 3 mg/kg of Testosterone Propionate and treated with oral intubation of 200 mg/kg body weight of ethanol extract. Group 6: this group was induced with 3 mg/kg of Testosterone Propionate and treated with oral intubation of 400 mg/kg body weight of ethanol extract.

The induction and treatment were done concurrently, the study lasted for 28 days, and the rats were sacrificed on the 29th day. Their blood samples were collected into appropriate containers for analysis and their prostates were harvested, weighed and processed for histopathological examinations.

Biochemical assays

PSA, ALP and dihydrotestosterone (DHT) determination

Serum Prostate specific antigen (PSA) and dihydrotestosterone levels of the rats were determined using Enzyme linked Immunoassay (ELISA) kit (AccuBind Monobind Inc. USA) according to methods of Chen *et al.* (1995) and Stamey *et al.* (1999) respectively. Alkaline phosphatase (ALP) activity was measured spectrophotometrically by monitoring the concentration of phenol formed when ALP reacts with disodium phenyl phosphate at 680 nm (ALP kit - Agappe Diagnostic Switzerland GmbH) as described by Klin (1980).

Inflammatory markers determination

Expression of Prostate inflammatory markers, Tumor Necrosis factor (TNF)-α, interleukin-(IL-) 8 and interleukin-(IL-) 10 in the serum were measured by enzyme-linked immunosorbent assay (ELISA) kits (Thermo Fisher ScientificTM Canine) according to the manufacturer's instructions as described by Schenk *et al. (*2010).

Histopathological Examination

The prostate tissue samples were preserved immediately in 10% buffered formalin for histopathological processing. The prostate tissue samples were embedded in paraffin and sectioned at thickness of 5µm.After dewaxing and rehydration, prostate sections were mounted on slides and stained with hematoxylin and eosin (H&E) for routine histological examination under a light microscope (Ishola *et al.,* 2017; Cai *et al.,* 2018).

Statistical analysis

Data generated were analyzed statistically using Statistical Package for Social Science (SPSS) version 25.0. The mean ± standard errors of means were determined. One way analysis of variance (ANOVA) with Turkey's Post Hoc test and bar charts were also done using SPSS and Microsoft Excel respectively. From the values obtained statistical decision and inferential evaluation were made. A probability (p) value of less than 0.05 was considered statistically significant.

RESULTS

Acute toxicity (LD_{50}) of ethanol extract

According to the findings, no mortality was recorded both in the first and second phase of treatment. Acute toxicity (LD_{50}) of ethanol extract in the experimental animals consisted of the first and second phase of treatment with varying doses ranging from 10 to 5000mg/kg body weight. However, there were no observable changes in the behavior of the animals after 24 hrs and 14 days of close monitoring (Table 1).

Table 1: Acute Toxicity of Ethanol Extract

Phase	Dose mg/kg	Death recorded in rats
First	10	0/3
	100	0/3

	1000	0/3
Second	1600	0/1
	2900	0/1
	5000	0/1

Number of rats per phase = 3 and 1 respectively. Number of deaths per group = 0.

Effect of leaf-extract of *Cnidoscolus aconitifolius* on body weight, prostate weight and prostate index of BPH induced rats

Induction of benign prostate hyperplasia showed diverse effects on the body weight of the study animals. The BPH control group had noticeable reduction in their body weight; conversely, the group expressed significant ($P<0.05$) increase in their prostate weight when compared to other groups whose measured prostate weight were reduced. Similarly, the calculated prostate index of the BPH group, being one of the factors of prostate weight were significantly ($P<0.05$) elevated when compared with other groups as depicted in Table 2.

Table 2: Body Weight, Prostate Weight, and Prostate Index

GROUP	Body Weight (g)	Prostate Weight (g)	Prostate Index (mg/g)
Normal Ctrl	166.16±4.50	0.53 ± 0.03^{b}	3.18 ± 0.09^{b}
BPH Ctrl	144.00±2.79	1.44 ± 0.09^{a}	10.00 ± 0.52^{a}
BPH + Finasteride Ctrl	172.56±3.53	0.64 ± 0.03^{b}	3.69 ± 0.17^{b}
BPH + 100mg/kg EE	185.94±16.24	0.68 ± 0.04^{b}	3.74 ± 0.26^{b}
BPH + 200mg/kg EE	187.40±9.33	0.68 ± 0.07^{b}	3.67 ± 0.46^{b}
BPH + 400mg/kg EE	173.44±11.76	0.62 ± 0.04^{b}	3.63 ± 0.32^{b}

Data are shown as Mean ± Standard Error of Mean (n=3). Mean values are significantly different at p≤0.05. N.B: a = significant with respect to Normal Control, b = significant with respect to BPH Control, Ctrl= control, BPH= benign Prostate Hyperplasia, EE= Ethanol Extract, mg= milligram, kg= kilogram.

Benign Prostate Hyperplasia (BPH) Specific Assays

The Prostate Specific Antigen (PSA) levels of testosterone induced BPH rats without treatment (negative control) was 27.68ng/ml. The results obtained indicates that this plant has dose dependent protective effect against the development of BPH as seen in the reduction of PSA levels in 100mg/kg ethanol extract (EE) which had a PSA level of 18.5ng/ml as against 200mg/kg and 400mg/kg with 22.72ng/ml and 21.08ng/ml respectively. That is, the administration of 100mg/kg dosage showed the best effect in the reduction of PSA levels.

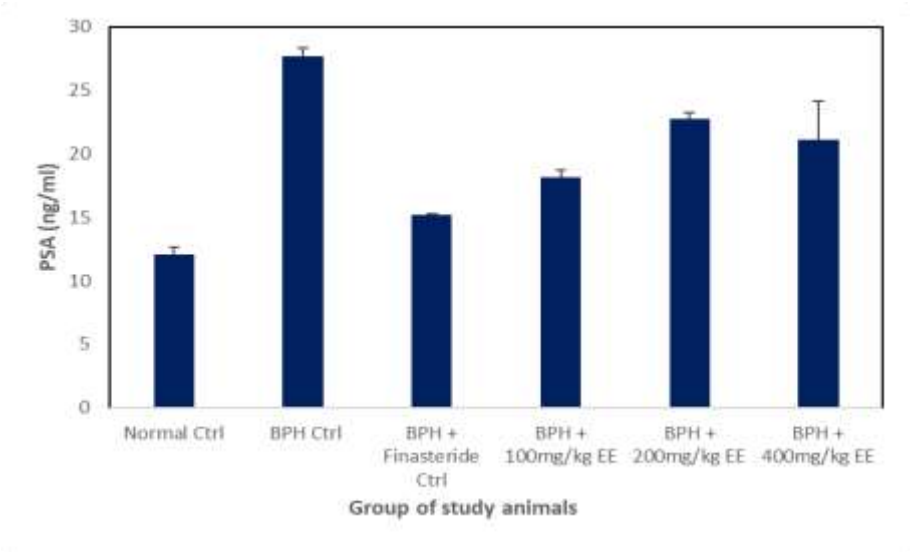


Figure 1: Prostate Specific Antigen levels in BPH induced rats**.**
Data are shown as Mean ± Standard Error of Mean (n=3). Mean values are significantly different at p≤0.05. N.B: Ctrl= control, BPH= benign Prostate Hyperplasia, EE= Ethanol Extract, mg= milligram, kg= kilogram.

Comparing the results in the assay as shown in figure 4, the rats induced with testosterone and no treatment (negative control) has an Alkaline phosphatase (ALP) level of 26.0(U/I) as against the normal control which was 6.39(U/I), and the known drug (finasteride control) which was 9.77(U/I). Considerable positive effects were recorded in the reduction of ALP levels. The 200mg/kgEE having the best effect with 11.14(U/I); 100mg/kgEE followed closely with 13.73(U/I) and 400mg/kgEE with 17.14(U/I).

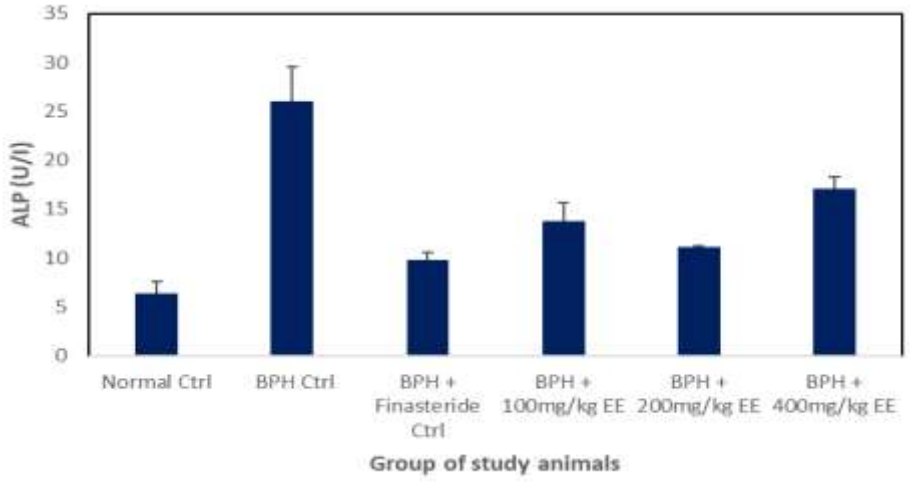


Figure 2: Alkaline phosphatase levels in BPH induced rats**.**
Data are shown as Mean ± Standard Error of Mean (n=3). Mean values are significantly different at p≤0.05.

The ethanol leaf-extract of *Cnidoscolous aconitifolius* also proved effective in the reduction of dihydrotestosterone (DHT) levels. From the results obtained in figure 5, the negative control had DHT level of 17.53(nmol/L) and the finasteride control had a DHT level of 7.50(nmol/L). 100mg/kgEE showed the best effect with DHT level of 7.35(nmol/L) which is closely related to that of the finasteride control.

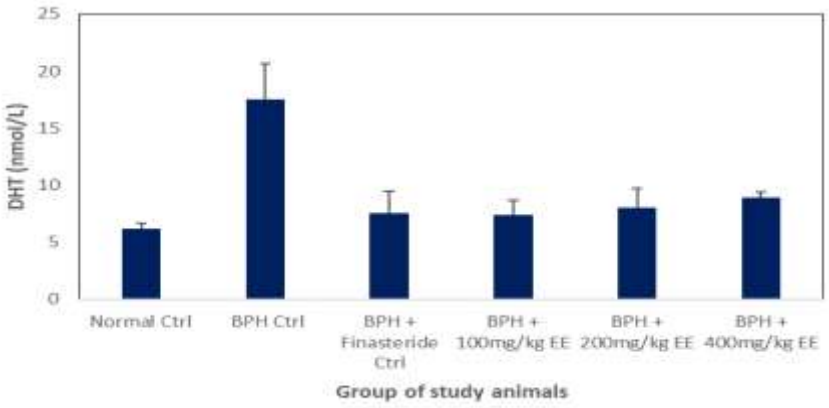


Figure 3: Dihydrotestosterone levels in BPH induced rats.
Data are shown as Mean ± Standard Error of Mean (n=3). Mean values are significantly different at p≤0.05.

Inflammatory Agents

Ethanol extracts of cnidoscolous aconitifolius also showed positive effect in the overall levels of the inflammatory markers as compared to that of the finasteride control. In the TNF-alpha assay, results showed that 100mg/kgEE had the best effect having levels of 43.11(pg/ml) which is closest to that of the finasteride control that is 46.28(pg/ml). The results showed close relation as opposed to the negative control level that rose up to 81.23(pg/ml). Similar observations was taken with the interleukin-8 and interleukin-10 markers which had 200mg/kgEE having the best effect.

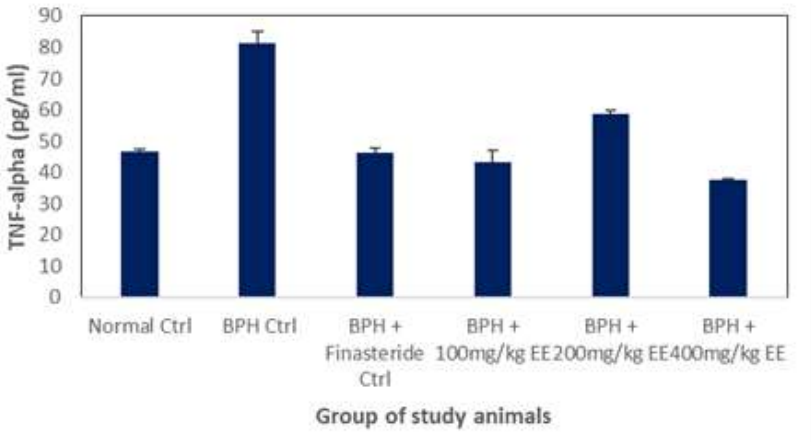


Figure 4: TNF-alpha levels in BPH induced rats.
Data are shown as Mean ± Standard Error of Mean (n=3). Mean values are significantly different at p≤0.05.

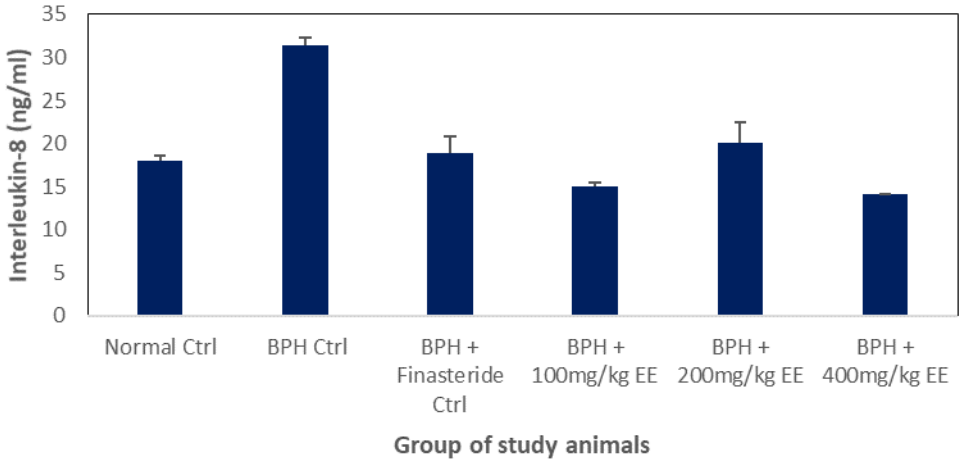


Figure 5: Interleukin-8 levels in BPH induced rats.
Data are shown as Mean ± Standard Error of Mean (n=3). Mean values are significantly different at p≤0.05.

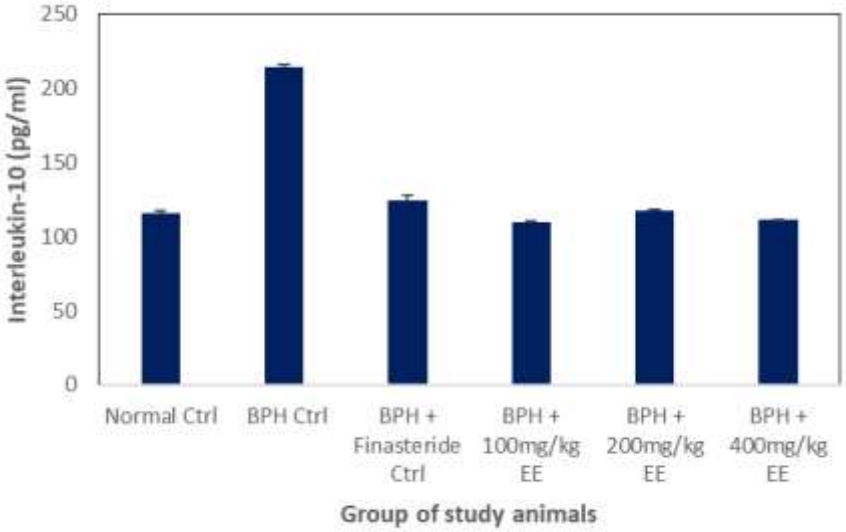
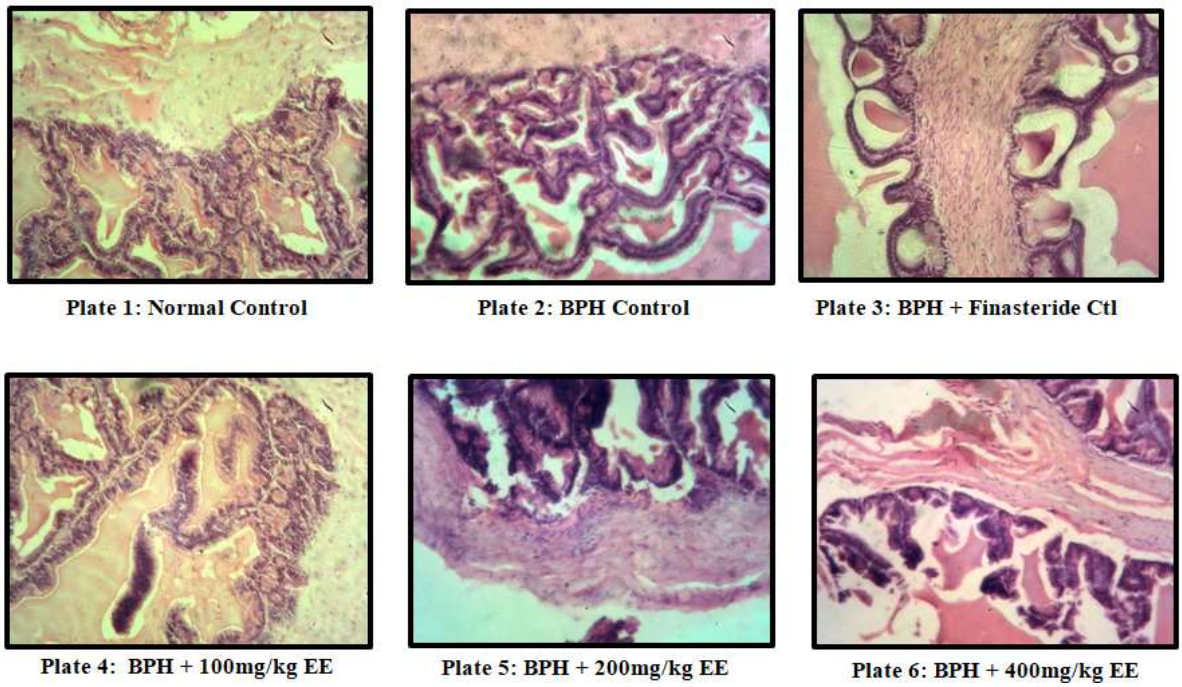


Figure 6: Interleukin-10 levels in BPH induced rats.
Data are shown as Mean ± Standard Error of Mean (n=3). Mean values are significantly different at p≤0.05.

Histopathological prostate findings of BPH induced rats

The normal control is a photomicrograph of a cross section of a normal prostate gland indicating inter glandular smooth muscles fibres, thick intraglandular epithelial convolution and glandular stroma as shown in plate 1. Plate 2 is a photomicrograph of a cross section of prostate gland of BPH control indicating a thin strip of interglandular smooth muscle fibres, extensive glandular hyperplasia and thin intraglandular epithelial lining.BPH + Finasteride control follows closely to that of the normal control, showing reduced glandular stroma and interglandular smooth muscle fibres. Treatment with aqueous extract of *Cnidoscolus aconitifolius* in plates 5, 6 and 7; indicated reduced glandular stroma, thick intraglandular epithelial and remarkable interglandular smooth muscles fibres as compared to the BPH control group.



Plates 1-6: Histopathological prostate findings of study animals

DISCUSSION

In the current study, we discovered that *Cnidoscolus aconitifolius* treatment could significantly inhibit the development of testosterone propionate induced BPH, which was confirmed by the extracts' positive findings on the tested parameters including PSA, DHT, ALP, Interleukin-8, Interleukin-10, Tumor Necrotic Factor alpha and histopathological changes. In previous studies using rat models, changes in prostate weight and histomorphology have been an important indicator for the inhibitory effects of substances on the development of BPH. *Cnidoscolus aconitifolius* showed improvement in the biochemical parameters, inflammatory agents and histopathological abnormalities in TP-induced BPH rats, consistently as previous studies, which supports inhibition BPH development by plant sources.

The value of LD_{50} for a substance is the dose required to kill half the members of a tested population after specified test duration. LD_{50} figures are frequently used as a general indicator of a substance's acute toxicity. A lower LD_{50} is indicative of increased toxicity and how unsafe a substance could be. The LD_{50} assay formed the baseline for the choice of the low, intermediate, and high doses of the extracts employed in the present study according to Lorke's report of 1983. The results of the acute toxicity study showed that rat orally administered with ethanol leaf extract of *C. aconitifolius* for doses of 10mg/kg – 1600mg/kg did not experience any strange signs of toxicity, but rats administered with doses of 2900mg/kg and 5000mg/kg experimental showed signs of weakness post administration of the extracts. There was indication that the leaf extracts of *C. aconitifolius* had higher LD_{50} values, this shows that the plant leaf could be less toxic when consumed in moderate quantity. This test was carried out to ascertain the effect of

a single dose of *C. aconitifolius* on test rat species. The LD_{50} results showed that the plant leaf extract could be safe for consumption having maintained estimated LD_{50} value of more than 5000mg/kg body weight of the test animals. These findings were in accordance with the published findings of Ezeigwe *et al.* (2020) on *C. aconitifolius* leaf extracts in which no death of the test animals was recorded at the first and second phases of the tests. Moreso, Ijioma *et al.* (2014) reported that their experimental animals were active and survived the 24 hours period of study with no signs of toxicity, even at an oral dose of 5000mg/kg body weight. The extract did not show any acute and sub-chronic toxicity in animals at lower doses, but high dose may cause liver and kidney damage (Bhattacharjee and Bhattacharyya, 2013).

Though there were no observed deaths of the animals during LD_{50} testing, during the first week of the study, the body weights of the test animals were observably reduced from their initial weights. This may be as a result of metabolic and physiologic changes in the body systems of the test animals occasioned by the stress and exposure of the test animals to the androgenic effects of the induced testosterone propionate and countering effects of the extracts. The second, third, fourth and final weights of the animals showed increments in the weights of the animals except for the benign prostate hyperplasia control group whose weights significantly reduced. The increments in the weights were observed more in test animals more than the control groups. The increase could be as a result of the preventive and recuperating effects of extracts on the test animals. Unlike the body weights of the animals, the relative prostate weights and prostate index showed increased expression in the BPH control animals when compared with other groups which had reduced expression of both parameters. Prostate weight and prostate index are commonly used to evaluate the development of BPH. As shown, the levels of prostate weight and prostate index in rats injected with testosterone propionate without treatment (BPH control group) had significantly ($P<0.05$) increased compared with rats in other groups, which suggested that BPH was successfully induced in the rats. The results showed that testosterone propionate at a concentration of 3mg/kg body weight administered daily through subcutaneous route for 28 days could induce BPH in male rats. Testosterone propionate induced BPH at 3mg/kg b.wt. sc. daily markedly increased the prostate weight and prostate index which is consistent with previous studies (Obisike *et al.,* 2019; Veeresh-Babu *et al.,* 2010; Yang *et al*., 2014a). Thus, changes in prostate index could indicate the percentage of inhibition of the progression of BPH disease.

One of the characteristic features of androgen responsive genes expressed in the prostate gland is the PSA. PSA is broadly recognized as a hallmark of BPH even though a little amount of PSA is usually found in the blood of healthy men. The transcriptional action of PSA is enhanced by the coupling of DHT to androgen receptors leading to its interaction with androgen response element (ARE) in the promoter region of PSA as found in BPH cases having high DHT activity (Vafa *et al*., 2020). This event results in the rise of PSA levels in the serum of BPH patients. Therefore, alleviation in PSA levels may be a representative of the mitigation of BPH. Our results showed that the administration of *Cnidoscolus aconitifolius* resulted in a decrease in the amount of PSA in serum of BPH treated rats.

DHT, an active metabolite of testosterone catalyzed by 5α-reductase, is an important

causative factor in BPH development. DHT can easily bind to ARs due to its higher affinity to the androgen receptor (AR), which stimulates the growth for the epithelial and stromal cells in the prostate. Therefore, DHT is basically responsible for prostatic epithelial and stromal cell hyperplasia (Cai *et al*., 2018).

Oluoch *et al.* (2023) identified that DHT accounts for about 90% of prostatic androgens and has autocrine, paracrine, and endocrine effects on prostatic tissue and adjacent cells, hence influencing cellular proliferation and cell death. Growth factors, such as FGF, EGF, TGF-beta, and so on, also impact the growth of the prostatic cells. BPH eventually develops due to the loss of control of cell proliferation and cell apoptosis, causing an imbalance in favor of cell proliferation (De Nunzio *et al.,* 2016).

Finasteride, as an elective drug targeting on 5α-reductase, has been reported to reduce DHT levels in the serum and prostate of BPH. However, because of the adverse effects, the use of 5α-reductase inhibitors (e.g., dutasteride and fnasteride) has been limited (Traish *et al*., 2011). In our current study, significant reductions in DHT levels of the serum and prostate were observed in *Cnidoscolus aconitifolius* treated groups compared with the BPH model group, as did the fnasteride-treated group. The liver which is responsible for xenobiotic metabolism and detoxification is also susceptible to hepatotoxic chemicals. The effect of aqueous leaf extract of *Cnidoscolus aconitifolius* on the liver of testosterone propionate-induced benign prostatic hyperplasia in male rats revealed a significant decrease in ALP concentration in Groups 3 to 6 of male rats compared to the negative control. This is in line with the study of Prakash *et al.* (2020), which showed that the rise in serum ALP levels reflects inflammation and the destruction of healthy tissues suggesting it as a biomarker. Specimens like Whole saliva, gingival crevicular fluid, plaque, and serum can be used as a source of sample for these markers. The normal level of alkaline phosphatase is 20-140IU/L (international unit per liter) in adults (Prakash *et al.*, 2020; Zaher *et al*., 2020).

Interestingly, epidemiologic studies have found elevated levels of TNF-α, IL-8 and IL-10 in men with BPH as compared to normal prostate (Steiner *et al.*, 2002; Schenk *et al.,* 2010; McLaren *et al.,* 2011), just like Eleazu *et al.* (2021) previously reported increased expression levels of the anti-inflammatory (IL-10) and proinflammatory cytokines in the prostatic tissues induced BPH in experimental rats, with the expression level of IL-10 being higher in the rats with more severe cases of BPH. These were consistent with our finding. In the present study, testosterone propionate induced BPH increased the level of TNF-α, IL-8 and IL-10 in study groups especially in BPH control group, whereas treatment with finasteride, ethanol extract, n-hexane and ethyl acetate fractions of leaf extract of *C. aconitifolius* showed significant decrease in the cytokine levels. Therefore, anti-BPH effect of the leaf extracts of *C. aconitifolius* may be related to the observed anti-inflammatory properties of the plant (Yang *et al.,* 2014b). Inflammation is commonly presented in BPH, which may cause tissue injury; and cytokines, secreted from infammatory cells, can drive angiogenesis and local growth factor production in the tissues as a self-protection response (Kim *et al*., 2016). IL-8, IL-10 and TNF-α, as proinfammatory cytokines, which are considered as potent growth factors for prostatic epithelial and stromal cells, increases in BPH models according to previous studies. Interleukin 8 is a pro-

inflammatory cytokine produced by macrophages and other cell types such as microglia. IL-8 has a number of pro-inflammatory effects including promotion of neutrophil adhesion, chemotaxis and lysosomal discharge (Tsai, 2021). Clinically, IL-8 plays crucial roles in several inflammatory and autoimmune diseases.

Tumor necrosis factor- alpha (TNFα) is a cytokine that has pleiotropic effects on various cell types. It has been identified as a major regulator of inflammatory responses and is known to be involved in the pathogenesis of some inflammatory and autoimmune diseases (Jang *et al.,* 2021). Structurally, TNFα is a homotrimer protein consisting of 157 amino acids, mainly generated by activated macrophages, T-lymphocytes, and natural killer cells. It is functionally known to trigger a series of various inflammatory molecules, including other cytokines and chemokines (Jang *et al*., 2021).

It's worthy to note that tumor necrosis factor-alpha (TNFα) is a pro-inflammatory mediator which plays a pivotal role in inflammation associated with benign prostate Hyperplasia. A high dose of TNFα has anti neoplastic properties and has even been used as a cytotoxic agent. However, exposure to chronic low level TNFα endows hyperplasic cells with more aggressive behaviors, such as increased growth, invasion, and metastasis (Wang *et al.,* 2020).

Thus, agents with the properties of anti-inflammation in BPH have been reported by some research (Cai *et al*., 2018). In our study, significant reduction of, IL-8 and IL-10 levels was determined in both serum and prostate in *Cnidoscolus aconitifolius* treated groups, compared to control groups. This might suggest that anti-inflammation is involved in the mechanisms of *Cnidoscolus aconitifolius* in treating BPH, although more investigation of other cytokines would further strengthen our findings.

The histological findings of this study showed that the rat ventral lobes that were used responded to treatment with testosterone. This is in line with a study by Eleazu *et al*. in 2021 where they reported that rat prostate can respond to hormone treatment. However, they noted that only the dorsal lobe of the rodent prostate was ontogenetically comparable to the human prostate. It has been reported that hormonal treatment not only induces prostate growth but also hardens the ventral lobe of the prostate (Obisike *et al.,* 2019). Our findings have shown that the epithelial cell layer and stromal cell space in both the ventral lobe of the rat prostate were induced by testosterone. Thus, Administration of extract of *C. aconitifolius* in BPH induced rats histologically reduced the proliferation of glandular cells of the prostate.

Conclusion

This study firstly demonstrates that dose dependent treatment of *Cnidoscolus aconitifolius* can reduce PSA, DHT, ALP levels and protect the morphology of prostate tissue by the mechanisms of anti-infammation, downregulation of DHT and antiproliferation of experimental BPH rats. Therefore, these results reveal that *Cnidoscolus aconitifolius* effectively inhibits the development of TP-induced BPH rat model, which strongly supports the application of *Cnidoscolus aconitifolius* therapeutically in the treatment of BPH in future.

Ethical Approval

This research work was approved and supervised by Nnamdi Azikiwe University-Animal Research Ethics Committee (NAU-AREC) in accordance with Animal Care and

Use in Research, Education and Testing (ACURET). The ethical approval number as issued by the Nnamdi Azikiwe University-Animal Research Ethics Committee is NAU/AREC/2023/00004.

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