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Biochemical And Haematological Effects Of Ethanol Leaf-Extract Of Cnidoscolus Aconitifolius In Testosterone Propionate-Induced Benign Prostate Hyperplasic Rats

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Abstract
The study investigated the biochemical and hematological effects of the ethanol leaf extract of Cnidoscolus aconitifolius in testosterone propionate-induced benign prostate hyperplasia (BPH) rats. Thirty male albino rats were divided into six groups of 5 rats each. Group 1 received subcutaneous administration of 1 ml/kg body weight of olive oil and 1 ml/kg of distilled water orally. Groups 2 to 6 were induced with BPH through daily subcutaneous injection of 3 mg/kg testosterone propionate followed by oral administration of the extract. Group 2 received 1 ml/kg distilled water; Group 3 received 5/kg finasteride, while Groups 4 to 6 received 100, 200, and 400 mg/kg of the ethanol leaf extract respectively for 28 days. Biochemical and hematological parameters were determined using spectrophotometric techniques and an auto-hematological analyzer respectively. The results demonstrated that the administration of C. aconitifolius extract in BPH-induced rats significantly ($p<0.05$) restored the levels of prostate-specific antigen, alkaline phosphatase and dihydrotestosterone. Noticeable improvements in the hematological parameters of the treated animals were observed compared to the BPH control group. Histological assays revealed moderate healing of the prostate in the treated groups compared to the untreated BPH control group. These findings suggest that the leaves of C. aconitifolius may promote prostate function and could be recommended for BPH patients.

Keywords: *Cnidoscolus aconitifolius, benign prostate hyperplasia, haematology, dihydrotestosterone, ethanol leaf-extract.*

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I. Introduction

Benign prostatic hyperplasia (BPH) is characterized by the enlargement of the prostate gland, often resulting in lower urinary tract symptoms that substantially impact the quality of life of affected individuals (Franco *et al.*, 2023).

It is predominantly found in older men, with approximately 75% of men over 50 experiencing BPH-related symptoms. This condition can lead to various clinical symptoms such as difficulty in voiding and urinary retention (Praveen, 2013; Wu *et al.*, 2019). BPH occurs due to hyperplasia of both epithelial and stromal tissues, primarily affecting the transition zone of the prostate gland. Despite its high prevalence and significant social and economic impact, the full pathophysiology of BPH remains incompletely understood. However, a study by Ma and Dong (2023) suggested a link between poor sleep quality and the development of benign prostatic hyperplasia. Additionally, factors such as unhealthy lifestyle, imbalanced dietary habits, lack of exercise, and hormones like testosterone and dihydrotestosterone (DHT), which play crucial roles in prostate development, may contribute to the pathogenesis of this condition. Testosterone, produced by the testes and adrenal glands, is converted to DHT by the enzyme 5-alpha reductase (Kim *et al.*, 2016; Praveen, 2013).

The incidence of benign prostatic hyperplasia (BPH) is increasing daily (Madersbacher *et al.*, 2019). The global incidence and prevalence are expected to increase from 962.42 to 7878.68 per 100,000 populations in 2022 to 998.55 and 8620.60 per 100,000 populations in 2035, respectively (Wei *et al.,* 2025). While surgery and conventional synthetic drugs, such as alpha- 1-adrenergic receptor antagonists and 5-alpha-reductase inhibitors, have proven effective in BPH management, their usage is associated with adverse effects including dizziness, hypotension, somnolence, ejaculatory dysfunction (including retrograde ejaculation and reduction of

seminal ejaculated volume), decreased libido, impotence, gynecomastia, and erectile dysfunction. Moreover, factors such as non-availability, high cost, surgical risks, pain, and the potential for recurrence after surgery have prompted a growing search for alternative methods of preventing, treating, or managing this disease (Minciullo *et al.*, 2015).

As a result, there is need for more scientific research for readily available drugs of herbal origin with promising reduced adverse/side effects, low cost and low risks that will be used in the prevention, treatment and/or management of BPH.

Interestingly, the drawbacks and adverse effects from the posed by drugs and management processes of BPH prompted this study that aim at the use of natural product – *Cnidoscolus aconitifolius* in the management/treatment of BPH. *C. aconitifolius* have gained public interest in recent times due to its roles in the prevention and/or management of various diseases that implicate free radicals/reactive oxygen species. One of the major phyto- component of the plant leaf is 9-octadecenoic acid (C18H34O2). Just like finasteride, which is a known inhibitor of 5-α-reductase use in the management of BPH; 9-octadecenoic acid has been reported to possess an anti-inflammatory, anti-alopecic, 5-α-reductase inhibitory, lubricant, antitumor, immunostimulant, diuretic and antiandrogenic properties (Omotoso *et al.*, 2014). Owing to these properties in which the presence of anti- androgenic phytochemicals in the plant sources may provide an alternative to synthetic drugs with minimal drawbacks (Omotoso *et al.*, 2014; Olaniyan and Afolabi, 2017), the investigation on the possible roles of the plant leaves in the management of BPH was carried out.

C. aconitifolius, also known as Chaya, is a shrub cultivated in the Mayan region of Guatemala, Belize, southeastern Mexico, parts of Honduras, and is also found in Nigeria (Manzanilla-Valdez and Segura- Campos, 2020). It belongs to the family Euphorbiaceae and is characterized by evergreen, drought-deciduous shrubs with large leaves, growing up to 32 cm long and 30 cm wide, with petioles 28 cm long (Iwuji *et al.*, 2013). This plant holds economic importance as it is widely cultivated for food and medicinal purposes, particularly for its blood-boosting potential (Ogbuji and Ataga, 2022; Chikezie *et al.*, 2016).

Numerous studies have discussed the bioactivities of *C. aconitifolius* leaf extracts, including anti-diabetic, hepatoprotective, hypoglycemic, and anti-ulcerative effects (Onasanwo *et al.*, 2011; Ajiboye *et al.*, 2019; Oladeinde *et al.*, 2007; Njoku *et al.*, 2020). The medicinal properties of this plant have been attributed to the presence of phytochemicals such as flavonoids, tannins, and saponins (Bautista-Robles *et al.*, 2020). These phytochemicals are believed to enhance antioxidant enzyme activities, thus acting as antioxidant boosters (Ajiboye *et al.*, 2019; Oyagbemi *et al.*, 2011). Studies by Ezeigwe *et al.* (2020) and Atata *et al.* (2020) have reported that *C. aconitifolius* is both medicinally and nutritionally important, while also being non-toxic for consumption. Therefore, it is essential to investigate the biochemical and hematological effects of ethanol leaf-extract of *C. aconitifolius* in testosterone propionate- induced benign prostate hyperplasic rats.

II. Materials And Methods

Collection and extraction of plant materials

The leaves of *C. aconitifolius* were collected in March 2022 from Awka, Anambra State, Nigeria (Latitude: 6.210528, Longitude: 7.072277). They were identified by a taxonomist, Dr. B.O. Aziagba, from the Department of Botany, Faculty of Biosciences. The voucher number deposited in the herbarium of Nnamdi Azikiwe University, Awka, is 168, and the sample was preserved in the herbarium.

Preparation of ethanol leaf extract of *C. aconitifolius*

The leaves were thoroughly washed, air-dried at room temperature for three weeks to achieve a relatively constant weight, and pulverized using an electric grinding machine. One thousand eight hundred (1800) g of the ground leaf particles were macerated in 7.2 l (1:4) of 70% ethanol for 24 h with intermittent shaking. The ethanol extract 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 a water bath at 40°C and weighed (Ezeigwe *et al.*, 2020).

Experimental animals

Thirty male Wistar albino rats, aged 16 weeks and weighing between 120 and 230 g, were procured from Chris Animal Farm and Research Laboratory, Awka, Anambra State, Nigeria, and utilized for the experiment. The animals were housed and maintained in cages within the same laboratory facility. After sorting, they were grouped and allowed a one-week acclimatization period to their new environment. Throughout the study, the rats were fed ad libitum with Vital Grower's Mash pellets obtained from a distributor in Awka, Anambra State, Nigeria. Ethical approval for the experiment was obtained from the Nnamdi Azikiwe University Animal Research Ethics Committee (Approval Number: NAU/AREC/2023/00004). Subsequently, the animals were weighed, sorted into six different groups consisting of five rats each, and appropriately labeled for identification purposes.

Acute toxicity (LD50) testing

The median lethal doses (LD50) for each of the extracts were determined using Lorke's method (1983). Twelve male rats were employed for assessing the median lethal dose of the extract. These twelve rats were randomly divided into 6 groups: 3 groups for the first phase, which received doses of 10, 100, and 1000 mg/kg body weight (b.w), and 3 groups for the second phase, which received doses of 1600, 2900, and 5000 mg/kg body weight (b.w) via oral intubation. The animals were closely monitored for changes in behavior and mortality within 2 h and subsequently for a period of 14 days following the single administration of the extract.

Weighing and induction of benign prostatic hyperplasia

The body weights of the study rats were measured weekly and on the 29th day using an electronic weighing balance. Benign Prostatic Hyperplasia (BPH) was induced subcutaneously in the rats using testosterone propionate, as described in studies by Ishola *et al.* (2017) and Yang *et al.* (2014b). The induction dose was formulated as 3 mg/kg body weight and administered by subcutaneous injection daily for 28 days, following the protocol outlined by Obisike *et al.* (2019). A stock solution was prepared by dissolving 25 mg of testosterone propionate in 8.33 ml of olive oil to obtain a concentration of 3 mg/ml, as described by Jeon *et al.* (2017).

Experimental design

Thirty male albino rats of the Wistar strain16 weeks old were used for this study. The rats were grouped accordingly 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 study lasted for 28 days, during which the body weights of the rats were recorded weekly. On the 29th day, the rats were euthanized. Blood samples were collected into appropriate containers for analysis, and their prostates were harvested, weighed, and processed for histopathological examinations. The prostate index was calculated as the ratio of the prostate weight to the total body weight, following the method described by Ishola *et al.* (2017).

Biochemical assays

PSA, ALP and Dihydrotestosterone (DHT) determination

Serum prostate-specific antigen (PSA) and dihydrotestosterone levels of the rats were determined using enzyme-linked immunosorbent assay (ELISA), following the methods described by 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, as described by Klin (1980).

Alkaline phosphatase (ALP) assay

Determination of ALP was according to the following reaction as described by Klin, (1980). ALP Para-nitrophenyl phosphate + H2O p-nitrophenol + inorganic phosphate

Procedure: Exactly 400 µl of alkaline phosphatase reagent 1 (mixture of 125 mmol/L Diethanolamine buffer at pH 10.2 and 0.625 mmol/L Magnesium chloride) and 100 µl reagent 2 (50 mmol/L p- Nitro phenyl phosphate) were mixed. The sample at volume of 10 µl was added mixed and incubated at 370C for 1 min and the absorbance was read at 405 nm at 1, 2 and 3 min, that is, measuring the change in absorbance per minute (Δ OD/min) during 3 min.

Calculation: ALP Activity (U/L) = (Δ OD/min) × 2750, 2750 = Calibration factor for estimation of ALP semi auto analyzers.

Dihydrotestosterone (DHT) assay

The assay for dihydrotestosterone (DHT) was performed using specific ELISA kits, following the manufacturer's instructions. The results for DHT were expressed in nmol/L. Working solutions of the DHT-HRP conjugate and wash buffer were prepared. The required number of microplate strips was removed from the bag and assembled into a plate frame. Unused strips were returned to the refrigerator and the bag resealed. Exactly 25 µL each of the calibrator, control, and specimen samples were pipetted into correspondingly labeled wells in duplicate. Additionally, 50 µL of the conjugate working solution was pipetted into each well. The plates were gently swirled for 10 s and incubated for 1 h at room temperature. After incubation, the wells were washed three times with 150 µL of diluted wash buffer per well.

Following washing, the plates were tapped firmly against absorbent paper to remove any residual liquid. Exactly 75µL of the substrate solution (buffered solution of tetramethylbenzidine and hydrogen peroxide) was added to each well. The plates were gently swirled for 10 s and then incubated for 15 min at room temperature. Subsequently, 25 µL of stopping solution was added to each well. Within 20 min, the plates were read on a microplate reader at 450 nm.

Haematological analysis

Hematological parameters including Hemoglobin (HGB), Packed Cell Volume (PCV), Red Blood Cells (RBC), Platelets (PLT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Cells (WBC), Neutrophils (NEUT), Lymphocytes (LYMPH), Monocytes (MON), Eosinophils (EOS), and Basophils (BAS) were analyzed using an automated hematology analyzer (Mindray-BC- 5300), following the manufacturer's instructions as adopted by Ugwu *et al.* (2019).

Histopathological examination

The prostate tissue samples were promptly preserved in 10% buffered formalin for histopathological processing. Subsequently, the prostate tissue samples were embedded in paraffin and sectioned at a thickness of 5 µm. Following dewaxing and rehydration, the prostate sections were mounted on slides and stained with hematoxylin and eosin (H&E) for routine histological examination under a light microscope, following the protocols outlined by Ishola *et al.* (2017) and 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 decisions and inferential evaluations were made. A probability (p) value of less than 0.05 was considered statistically significant.

III. Results

Acute toxicity (LD50) of ethanol extract

Acute toxicity (LD50) of ethanol extract in the experimental animals consisted of the first and second phases of treatment with varying doses ranging from 10 to 5000 mg/kg body weight. According to the findings, no mortality was recorded in the first and second phase of treatment. Also, there were no observable changes in the behavior of the animals after 24 h and 14 days of close monitoring.

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

The body weight of the study animals revealed that the induction of benign prostate hyperplasia could have diverse effects on the weight of the animals, as seen in the weight of the BPH control group, which exhibited a noticeable reduction as shown in Figure 1. There was a significant ($P<0.05$) increase in expression in the prostate weight of the BPH control animals when compared to other groups, whose measured prostate weights were reduced (Figure 2). The calculated observation of the prostate index is similar to the result of the prostate weight, as the latter is one of the factors of the former. Thus, the prostate index of the BPH control group was significantly ($P<0.05$) elevated, whereas other groups showed reduced prostate index values. The prostate index of the study animals is shown in Figure 3.

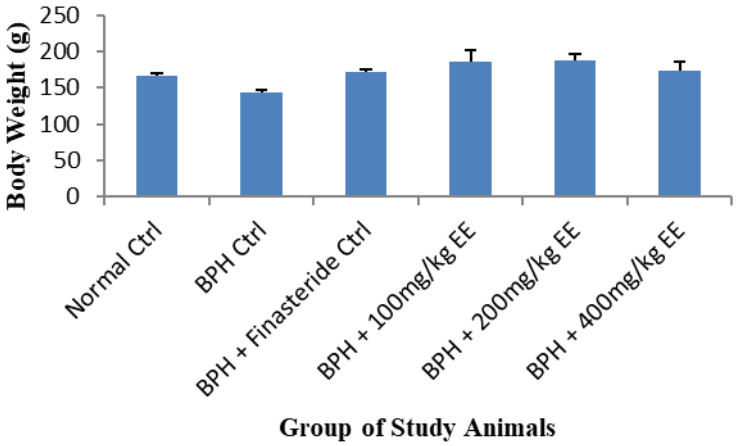

Figure 1. Final weight of BPH study animals. N.B: Ctrl= control, BPH= benign Prostate Hyperplasia, EE= Ethanol Extract.

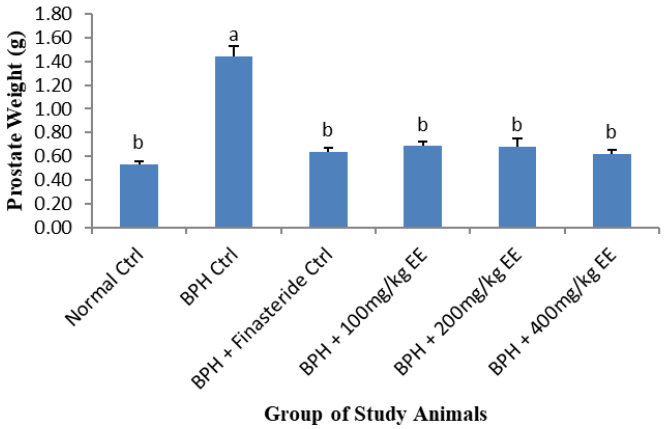

Figure 2. Prostate weight of BPH study animals. Data are shown as Mean ± Standard Error of Mean (n=3). Mean values are significantly different at p≤0.05. N.B: a = significant with respect to Normal Control, b = significant with respect to BPH Control, Ctrl= control, BPH= benign Prostate Hyperplasia, EE= Ethanol Extract, mg= milligram, kg= kilogram.

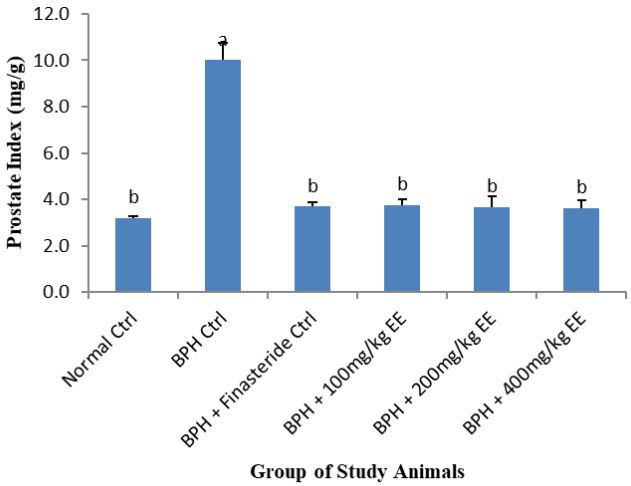

Figure 3. Prostate index of BPH study animals. Data are shown as Mean ± Standard Error of Mean (n=3). Mean values are significantly different at p≤0.05. N.B: a = significant with respect to Normal Control, b = significant with respect to BPH Control, Ctrl= control, BPH= benign Prostate Hyperplasia, EE=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

Effect of ELECA on prostate specific antigen (PSA), alkaline phosphate (ALP) and Dihydrotestosterone (DHT) levels in BPH-induced rats

Prostate specific antigen (PSA) activity

Figure 4 compared the PSA levels of the different groups of the study animals. The normal control group had the lowest PSA activity (12.07±0.53ng/ml), while the BPH control group recorded the highest PSA activity (27.68 ± 0.67ng/ml). The increased PSA activity recorded in theBPH control group is statistically significant with respect to the normal control group ($p \leq 0.05$).

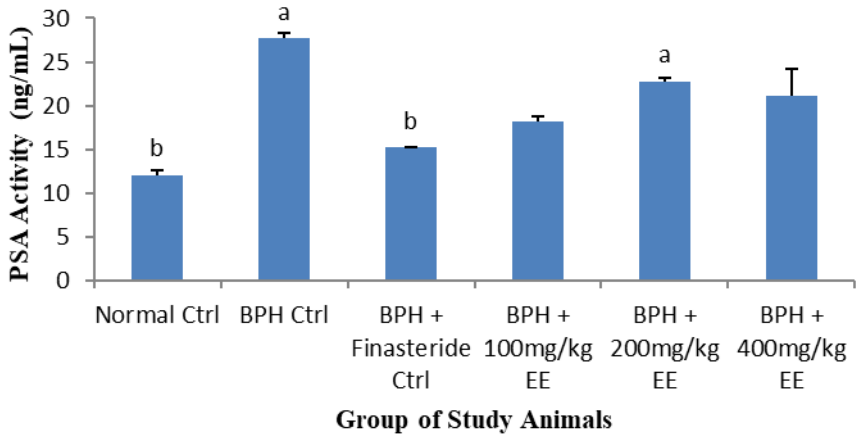

Figure 4. Activity of prostate specific antigen in BPH study animals.
Data are shown as Mean ± Standard Error of Mean (n=3). Mean values are significantly different at $p \leq 0.05$. N.B: a = significant with respect to Normal Control, b = significant with respect to BPH Control, Ctrl= control, BPH= benign Prostate Hyperplasia, EE= Ethanol Extract.

Alkaline phosphatase (ALP) activity

Figure 5 compared the ALP levels of the normal control group with the groups induced with BPH+ Finasteride control, BPH+100, 200 and 400 mg/kg EE. The normal control group had the lowest ALP activity (6.39 ±1.24 U/l), while the BPH control group recorded the highest ALP activity (26.00±3.63U/l). ALP activity recorded in the normal control group, BPH + Finasteride control, BPH + 100, 200 and 400 mg/kg EE was statistically significant with respect to BPH control group ($p \leq 0.05$).

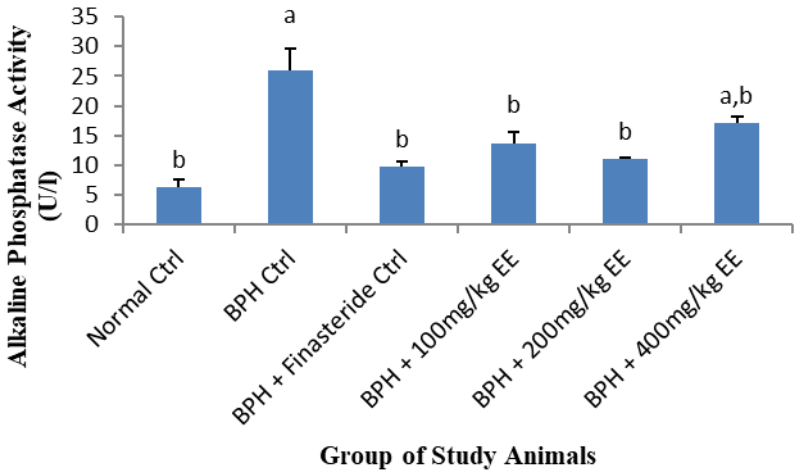

Figure 5. Activity of alkaline phosphatase in BPH study animals. Data are shown as Mean ± Standard Error of Mean (n=3). Mean values are significantly different at $p \leq 0.05$. N.B: a = significant with respect to Normal Control, b = significant with respect to BPH Control, Ctrl= control, BPH= benign Prostate Hyperplasia, EE= Ethanol Extract

Dihydrotestosterone (DHT)

Figure 6 compared the DHT levels of the different groups of the study animals. The normal control group had the lowest DHT activity (6.21 ± 0.48 nmol/L). There was dose dependent reduction in the level of DHT recorded in the different dosages of administered extract when compared to the BPH control group which recorded the highest DHT activity of 17.53±3.12 nmol/L. DHT activity recorded in the normal control group, group induced with BPH + Finasteride control and BPH +100 mg/kg EE was statistically significant with respect to BPH control group ($p \leq 0.05$).

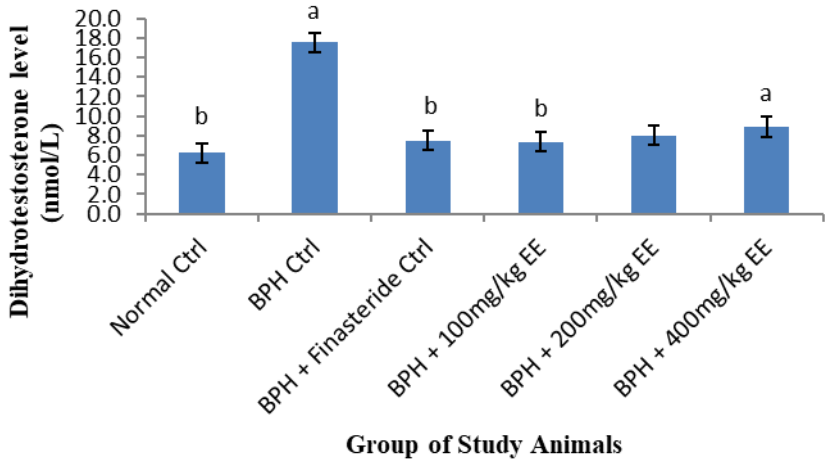

Figure 6. Activity of Dihydrotestosterone in BPH study animals. Data are shown as Mean ± Standard Error of Mean (n=3). Mean values are significantly different at $p \leq 0.05$. N.B: a = significant with respect to Normal Control, b = significant with respect to BPH Control, Ctrl= control, BPH= benign Prostate Hyperplasia, EE= Ethanol Extract.

Effect of ELECA on haematological parameters in BPH induced rats

Red blood cells (RBC)

Table 1 compared the results of red blood cell count (RBC), Haemoglobin (HGB), Packaged Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and Platelet Level (PLT) of BPH study animals. Red Blood Cell counts of the study animals maintained an average range that is within or higher than the recommended 3.50 - 5.50 (x10^12/L) range though the BPH control group showed the least RBC count. The test groups maintained appreciable RBC levels higher than the Normal control while level maintained by the animals treated with the extract were comparable to the BPH+Finasteride control group.

Table 1: RBC, Its Subordinates and Platelet

GROUP	RBC (x10^12/L)	HGB (g/dL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT (x10^9/L)
Range	**3.50 – 5.50**	**11.00 –16.00**	**37.00 –54.00**	**80.00 –100.00**	**27.00–34.00**	**32.00–36.00**	**150 – 400**
Normal Ctrl	5.08±0.22	10.20±0.15	35.03±0.75	58.03±0.96	17.27±0.32	29.73±0.27	844.67±48.49
BPH Ctrl	4.33±0.39	9.33±0.73	30.83±2.53	42.90±0.64	14.80±0.12	22.23±0.44	990.00±28.75
BPH + Finasteride Ctrl	7.58±0.22	12.63±0.30	41.63±1.05	54.97±0.26	16.70±0.10	30.30±0.10	715.67±34.23
BPH + 100mg/kg EE	6.51±0.29	11.10±0.74	37.47±1.78	57.70±2.46	17.03±0.49	29.60±1.24	945.33±107.53
BPH + 200mg/kg EE	7.11±0.35	13.40±0.36	45.57±1.19	64.20±2.08	18.87±0.39	29.43±0.52	679.67±27.03
BPH + 400mg/kg EE	5.87±0.51	11.00±0.44	36.97±1.01	63.63±3.89	18.90±0.91	29.77±0.35	897.67±36.13

Result expressed as Mean±Standard Error of Mean (n=3). N.B: Ctrl= control, BPH= benign Prostate Hyperplasia, EE= Ethanol Extract

White blood cell (WBC)

Table 2 compared the results of White Blood Cell (WBC), Neutrophil (NEU), Lymphocyte (LYM), Monocyte (MON), Eosinophil (EOS) and Basophil (BAS) of BPH study animals. The level of white blood cell was found to be elevated more than the normal range of 4.00 to 10.00 (x10^9/L) in the BPH control group compared to the test groups that showed general reduction in their white blood cell counts. Owing to the aforementioned, the animals treated with 400 mg/kg of the extract were found to maintain the least level of WBC when compared to the normal control.

Table 2: WBC and its Components

GROUP	WBC (x10^9/L)	Neu (%)	Lym (%)	Mon (%)	Eos (%)	Bas (%)
Range	4.00 – 10.00	50.00 – 70.00	20.00 – 40.00	3.00 – 12.00	0.50 – 5.00	0.00 – 1.00
Normal Ctrl	16.79±1.21	33.87±1.90	62.50±1.17	1.77±0.23	0.83±0.19	0.93±0.17
BPH Ctrl	20.43±1.27	21.23±4.29	71.01±8.94	3.21±0.12	1.93±0.11	2.63±0.20
BPH + Finasteride Ctrl	15.53±3.62	30.07±5.64	67.63±10.60	1.07±0.43	0.73±0.12	0.50±0.15
BPH + 100mg/kg EE	14.83±1.65	35.17±7.06	61.30±7.66	1.73±0.38	0.63±0.18	1.17±0.14
BPH + 200mg/kg EE	14.64±1.77	33.27±2.78	63.77±2.96	2.50±0.15	0.07±0.03	0.40±0.07
BPH + 400mg/kg EE	12.15±2.25	30.07±5.56	66.14±10.65	2.53±0.14	0.83±0.12	0.43±0.13

Result is expressed as a Mean±Standard Error of Mean (n=3). N.B: Ctrl= control, BPH= benign Prostate Hyperplasia, EE= Ethanol Extract.

Histological Findings

The plates below show the histopathological prostate findings of BPH induced rats from the effect of the administration of Ethanol extract of *Cnidoscolus aconitifolius* in the experimental animals after the various weeks of administration.

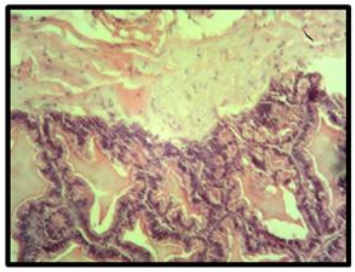

Plate 1: Normal Control

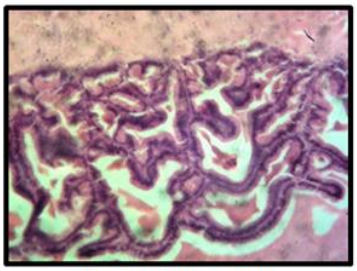

Plate 2: BPH Control

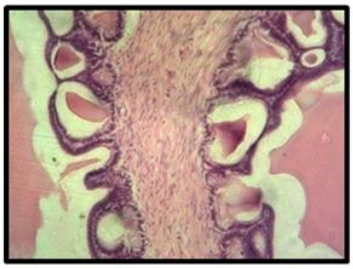

Plate 3: BPH + Finasteride Ctl

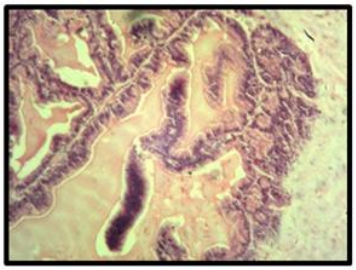

Plate 4: BPH + 100mg/kg EE

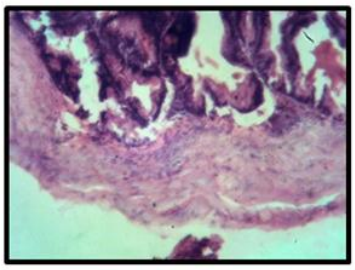

Plate 5: BPH + 200mg/kg EE

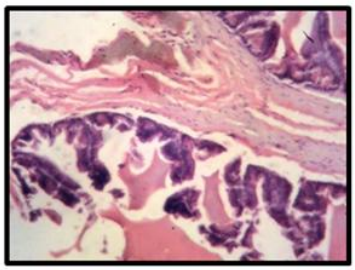

Plate 6: BPH + 400mg/kg EE

Plates 1-6: Histopathological prostate findings of study animals.

IV. Discussion

The results of the acute toxicity study revealed that mice orally administered with ethanol leaf extract of *C. aconitifolius* at doses ranging from 10 to 1600 mg/kg did not exhibit any signs of toxicity. However, mice administered with doses of 2900 and 5000 mg/kg showed signs of weakness post-administration of the extracts.

The LD50 result indicated that the plant leaf extract is safe for consumption, as it maintained an estimated LD50 value of more than 5000 mg/kg body weight of the test animals. These findings align with the published results of Ezeigwe *et al.* (2020) on *C. aconitifolius* leaf extracts, where no deaths of the test animals were recorded during the first and second phases of the tests.

Furthermore, Ijioma *et al.* (2014) reported that their experimental animals remained active and survived the 24-h study period with no signs of toxicity, even at an oral dose of 5000 mg/kg body weight. The extract demonstrated no acute or sub-chronic toxicity in animals at lower doses, although high doses may lead to liver and kidney damage (Bhattacharjee and Bhattacharyya, 2013). The LD50 value of a substance represents the dose required to kill half of the members of a tested population after specified test duration. LD50 figures are commonly used as a general indicator of a substance's acute toxicity, with a lower LD50 suggesting increased toxicity and potential safety concerns. Although the acute toxicity study revealed dose-dependent activities, the biochemical and hematological findings did not consistently exhibit dose-dependent activities in some instances. This discrepancy may be attributed to variations in body chemistry and metabolic processes among the animals.

PSA (kallikrein-3) is a glycoprotein secreted by both normal and malignant luminal epithelial cells in the prostate (Nnodim and Nwaokoro, 2020; Swerdloff *et al.*, 2017). Interestingly, the observations of this present study showed that the administration of testosterone propionate increased the prostatic testosterone concentration and the level of prostate-specific antigen in the BPH control group. This could result from the catalytic interactions of the 5α-reductase enzyme between the exogenously administered testosterone and the endogenously synthesized testosterone in their prostatic tissue, leading to a resultant increase in the level of dihydrotestosterone. According to the findings of Zhu *et al.* (2003), the intensity of PSA synthesis largely depends on the concentration of DHT in the prostatic tissue. The increase in PSA expression in the BPH rats supports the already published findings of many authors, including but not limited to Lee *et al.* (2011), Shin *et al.* (2012), and Eleazu *et al.* (2021).

Aminotransferase enzymes such as ALP are useful and one of the most frequently used enzyme markers of hepatotoxicity. According to Hayes *et al.* (2002), ALP in the cellular external membrane plays a major role in phosphate metabolism and prevents damage to the external membrane. Alkaline phosphatase (ALP) is one of the biomarkers that were earlier implicated in BPH study. The presented findings in Figure 5 showed that alkaline phosphatase level is more prominent in testosterone propionate-induced BPH control rats when compared to other control and extract-studied group animals, with significant ($p<0.05$) restorative serum ALP activity. This was in line with the findings of Orji *et al.* (2016), who reported that administration of ethanol extract of *C. aconitifolius* significantly decreases the activity of ALP in the serum. This finding was one of the important indices that showed that there were inductions of BPH in the study animals with a preventive effect of the standard drug and plant leaf extracts, which may be the cause of reduced levels of ALP in finasteride control and test animal groups, respectively.

Testosterone and DHT are steroid hormones related to BPH and play crucial roles in the development of internal male reproductive organs. Testosterone is converted into DHT by the 5α-reductase enzyme, which is responsible for prostate development and BPH pathogenesis. DHT, a derivative of testosterone, stimulates cell proliferation and growth in the prostate, making it a major contributor to rapid prostate enlargement (Jeon *et al.*, 2013). Thus, DHT is considered one of the most important prostatic hormones in BPH development and progression. Additionally, steroid hormones and growth factors regulate various cellular processes, including cell growth, proliferation, and differentiation (Atroshchenko *et al.*, 2022).

The results presented in Figure 4 demonstrated a significant decrease in DHT activity compared to the BPH control group following the administration of 100mg/kg of *C. aconitifolius* ethanol extract ($p<0.05$). This decrease suggests that the extract may possess an inhibitory effect against DHT activity, making it potentially valuable in BPH treatment, as increased DHT activity is associated with prostate BPH development (Carson and Rittmaster, 2003).

Blood indices, particularly red and white cells and hemoglobin concentration, are valuable clinical indicators of disease states (Ezebuiro *et al.*, 2020). From the results obtained in Tables 1 and 2, it was observed that there was a decrease in the RBC and HGB levels of the BPH control group and an increase in the groups administered various dosages of *C. aconitifolius* extract, though this was not statistically significant ($p>0.05$). This indicates that the administration of *C. aconitifolius* affects hematopoiesis in the body and is consistent with Onuoha *et al.* (2017), who reported that intake of *C. aconitifolius* for fourteen days improves erythrocyte count concentrations.

White blood cells serve as the body's defense system, fighting infections and protecting against foreign bodies such as germs. Results obtained in Table 2 revealed a decrease in WBC activity in the groups administered various dosages of ethanol extract of *C. aconitifolius* compared to the normal control, although this difference was not statistically significant ($p>0.05$). This suggests that the administration of *C. aconitifolius* extract may lack immune properties, although this conclusion may be limited by the short duration of the study. However, the levels of LYM, MON, and NEU showed an increase following the administration of the plant extract, though without significant difference recorded ($P>0.05$), indicating a potential immunomodulatory effect. Additionally, no significant differences were observed in the values of PCV, MCH, MCHC, EOS, and BAS ($P>0.05$) with the different treatments. These findings align with Lawal *et al.* (2010), who reported that most erythrocytic parameters do not respond to administration of *C. aconitifolius* extract.

Tables 1 and 2 validate the diverse results of various hematological parameters carried out in this present study, including red blood cells, hemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet, white blood cell, neutrophil, lymphocyte, monocyte, eosinophil, and basophil levels in the study animals. Previous scientific studies have shown that the administration of medicinal substances can alter the ranges of hematological parameters in either a positive or negative direction (Zhang *et al.*, 2022).

In accordance with the reports of Ugwu *et al.* (2019), the red blood cell counts of the study animals maintained an average range that is within or higher than the recommended 3.50 to 5.50 (x10^12/L) range, except for the BPH control group, which exhibited the lowest RBC level. This could be attributed to the

oxidative stress caused by the debilitating effect of the induced BPH, left untreated, unlike the positive and test groups whose oxidative stresses were ameliorated by finasteride and the leaf extract treatments, respectively. Another potential explanation for the observed decrease in RBC could be linked to red cell distribution width (RDW). The RDW blood test measures the amount of red blood cell variation in volume and size and can help determine the underlying cause of anemia. In a previous study, Patel *et al.* (2010) reported that BPH patients have high RDW values, reflecting an underlying inflammatory state that can impair erythrocyte maturation and subsequent inadequate production of the hormone erythropoietin. A decrease in the number of red cells in the blood, as observed, is often associated with the development of anemia. Red blood cells (RBC) or erythrocytes are the most common type of blood cells and the principal means of delivering oxygen (O2) to the body tissues through the circulatory system. This decrease could be due to the stimulation of the lipid peroxidative system by toxins or disease, resulting in the production of lipid peroxides that hemolyze the red blood cells (Kiseleva *et al.*, 2021; Igbinaduwa *et al.*, 2019). The decrease in hematocrit (PCV) of rats observed in the BPH control suggests that they may induce anemia and the inability of cells to deliver oxygen to body tissues that require them (Patel *et al.*, 2010).

Packed Cell Volume (PCV) or Hematocrit is clinically used to indicate known or suspected anemia. It measures red blood cell mass, with an increase indicating erythrocytosis and a decrease indicating anemia. Some medicinal plants are known to cause red blood cell destruction leading to anemia, contrary to the findings of Ezeigwe and colleagues (2020), who identified that the combined leaf extracts of *C. aconitifolius* and *Ficus capensis* could improve the anemic condition of study Wistar albino rats.

White blood cells (WBCs), also called leukocytes, are immune cells found in the body involved in protecting against infectious diseases and foreign invaders (Chiang *et al.*, 2022). The present findings showed a higher recruitment of white blood cells in the BPH control groups compared to other control or test groups. As noted by Ugwu *et al.* (2019), neutrophils, lymphocytes, monocytes, and eosinophils are different types of white blood cells that combat disease and infection. Neutrophils, predominant in human blood, patrol and protect the body from pathogens and diseases. Our findings suggest that, apart from the BPH control group, the neutrophil components of white blood cells in other groups were more mobilized to counter the effects of induced BPH in the study animals. It is possible that BPH-induced inflammation stimulated the bone marrow to produce and release neutrophils into systemic circulation, but the preventive measures of the standard drug and the extracts may have enhanced neutrophil production and multiplication in the treated groups to mobilize more fighting forces against induced BPH molecules compared to the untreated BPH control group. Neutrophils are major granulocytes activated when the body is invaded by bacteria, providing the first line of defense against microorganisms. The granules of neutrophils contain many enzymes, making them powerful and effective in killing pathogens (Ugwu *et al.*, 2019).

In response to various stimuli, eosinophils are recruited from circulation into inflammatory foci where they modulate immune responses through various mechanisms. Triggering eosinophils via engagement of receptors for cytokines, immunoglobulins, and complement can lead to the secretion of inflammatory cytokines, such as interleukin (IL)-10, which have proinflammatory effects including upregulation of adhesion systems, modulation of cellular trafficking, and activation and regulation of vascular permeability, mucus secretion, and smooth muscle constriction. This may explain the observed increase in eosinophils in the BPH control group, with the extract aiming to restore this observed increase. Similarly, basophils levels were highest in the BPH control group. Basophils play crucial roles in combating bacterial, fungal, and viral infections alongside other white cells, constituting one of the components of the immune system.

Furthermore, the histological examination of the prostatic tissues from experimental rats that received 200 mg/kgEE demonstrated partial recovery in prostate cellular structure, while those administered with the 400 mg/kg dose exhibited better prostatic integrity. This indicates the potential of plant extracts to restore and ameliorate the damaging effects of certain disease conditions (Eng-Tat *et al.*, 2022; Nwobodo *et al.*, 2020). In the histological examination of prostatic tissues from rats in the BPH control group, extensive glandular hyperplasia and significant thickening and hypertrophy were observed. Additionally, widening of the diameter of the lumen without remarkable expansion in the stroma was evident. The histological examination revealed thickening of inter- and intra-glandular epithelial linings in

the BPH control group, indicative of rapid cell multiplication and enlargement characteristic of BPH, contrasting with the observed histological examination of the treatment groups. Additionally, the histological findings demonstrated that the rat ventral lobes used in the study responded to testosterone treatment, consistent with a study by Eleazu *et al.* (2021), where they reported that the rat prostate can respond to hormone treatment. However, it's important to note that only the dorsal lobe of the rodent prostate was ontogenetically comparable to the human prostate. Hormonal treatment has been reported to not only induce prostate growth but also harden the ventral lobe of the prostate (Obisike *et al.*, 2019). This present study has shown that testosterone induced the epithelial cell layer and stromal cell space in both the ventral lobe of the rat prostate. Although apoptotic markers were not studied in this present research, it is worth noting that while the leaf ethanol-extract of *C.*

aconitifolius attenuates BPH as demonstrated in its properties, finasteride, which was adopted as a positive anti-BPH control drug in this study, has also been reported to attenuate BPH through the induction of apoptosis (Eleazu *et al.*, 2017). However, finasteride is a known anti-BPH drug with some possible side effects.

V. Conclusion

C. aconitifolius, an underexplored plant with significant therapeutic potential, belongs to the Euphorbiaceae family and possesses notable nutritional properties and health benefits. Traditionally utilized by various cultures as a remedy for diverse ailments, its active components hold promise for nutraceutical and pharmacological studies. Administration of *C. aconitifolius* plant extract at different dosages may exert significant effects on PSA, ALP, DHT, and other biochemical and hematological parameters. This study suggests that ethanol leaf extract of *C. aconitifolius* may offer ameliorative effects against BPH and proposes its potential as a novel therapeutic agent for BPH treatment. Nonetheless, further studies are warranted to elucidate its possible mechanisms.

Conflict Of Interests

The authors have not declared any conflict of interests.

References

[1]. Ajiboye BO, Oyinloye BE, Agboinghale PE, Ojo OA. Cnidoscolus Aconitifolius (Mill.) I. M. Johnst Leaf Extract Prevents Oxidative Hepatic Injury And Improves Muscle Glucose Uptake Ex Vivo. Journal Of Food Biochemistry, (2019); 43(12): 1-8.

[2]. Atata JA, Ayoola TO, Ajadi AA, Adamu S, Olatunji AO, Biobaku KT. Anti-Anaemic Effect Of Ethanol Leaf Extract Of Cnidosculusaconitifolius On Cyclophosphomide-Induced Anemia In Rats. Journal Of Complementary And Integrative Medicine, (2020); 18(1): 87–92.

[3]. Atroshchenko MM, Shitikova AM, Krokhotina LV, Engalycheva MG, Dmitrieva MN. Effect Of Testosterone, Dihydrotestosterone, Estradiol And Cortisol On The Quality Of Fresh And Cryopreserved Stallion Sperm.Journal Of Experimental Biology And Agricultural Sciences, (2022); 10(3): 619 – 627.

[4]. Bautista-Robles V, Guerrero-Reyes G, Sánchez-Torres GI, Parada-Luna F, Barrios-Gutiérrez JJ, Vázquez-Cerero D, Keita H. Cnidoscolus Aconitifolius: Usosterapéuticos Y Propiedadesfitoquímicas. Revisión De La Literatura. Revista De La Facultad De Medicina, (2020); 68(3): 1-8.

[5]. Bhattacharjee P, Bhattacharyya D. Characterization Of The Aqueous Extract Of The Root Of Aristolochia Indica: Evaluation Of Its Traditional Use As An Antidote For Snake Bites. Journal Of Ethnopharmacology, (2013);145(1):220-226.

[6]. Cai H, Zhang G, Yan S, Shang X. The Effect Of Xialiqi Capsule On Testosterone-Induced Benign Prostatic Hyperplasia In Rats.Evidence-Based Complementary And Alternative Medicine, (2018); 18: 1-9.

[7]. Carson C, Rittmaster R. The Role Of Dihydrotestosterone In Benign Prostatic Hyperplasia.Urology, (2003); 61(4):2–7.

[8]. Chen Z, Prestigiacomo AF, Stamey TA. Purification And Characterization Of Prostate Specific Antigen (PSA) Complexed To A_1-Chymotrypsin: Potential Reference Material For International Standardization Of PSA Immunoassays. Clinical Chemistry, (1995); 41(9): 1273-1282.

[9]. Chiang MH, Yang CY, Kuo YJ, Cheng CY, Huang SW, Chen YP. Inverse Relationship Between Mean Corpuscular Volume And T-Score In Chronic Dialysis Patients.Journal Of Medicina, (2022); 58:497-502.

[10]. Chikezie NU, Chijioke NA, Adjeroh AL, Ogbulie ET, Udensi UJO, Chialuka K. An Evaluation Of The Phytochemical And Nutritional Compositions Of Fresh Leaves Of Cnidoscolus Aconitifolius [Miller] I.M. International Journal Of Research Studies In Biosciences, (2016); 4:21-28.

[11]. Eleazu C, Eleazu K, Kalu W. Management Of Benign Prostatic Hyperplasia: Could Dietary Polyphenols Be An Alternative To Existing Therapies? Frontiers In Pharmacology, (2017); 8: 234.

[12]. Eleazu K, Aja PM, Eleazu CO. Cocoyam (Colocasiaesculenta) Modulates Some Parameters Of Testosterone Propionate-Induced Rat Model Of Benign Prostatic Hyperplasia. Drug And Chemical Toxicology, (2021); 10:1-12.

[13]. Eng-Tat A, Liu P, Jitesh R, Chandrika M, Satish RL, Lian WD, Huppertz B, Khamuani M, Minarcik J. The Challenges Of Histology Education.Clinical Anatomy, (2022); 36: 1-7.

[14]. Ezebuiro I, Obiandu C, Saronee F, Weleh II, Obiandu AC. Effects Of Cnidoscolus Aconitifolius On Some Hematological Parameters Of Male Wistar Rats.Asian Journal Of Biotechnology And Bioresource Technology, (2020); 6(3):1-5.

[15]. Ezeigwe OC, Okpala CO, Ogana J, Akonam NE, Aziagba BO, Nnadi NN, Anekwe OJ, Nwobodo VOG. Comparative Phytochemical And Nutritional Profiles Of Ficus Capensis And Cnidoscolus Aconitifolius Leaves. International Journal Of Research And Innovation In Applied Science, (2020a); 5(1): 16-21.

[16]. Franco JVA, Tesolin P, Jung JH. Update On The Management Of Benign Prostatic Hyperplasia And The Role Of Minimally Invasive Procedures. Prostate International, (2023); 11(1):1-7.

[17]. Hayes PC, Simpson KJ, Garden OJ. Liver And Biliary Tract Disease.In Davidson's Principles And Practice Of Medicine. (2002); Pp. 350-364.

[18]. Igbinaduwa PO, Kabari KM, Chikwue TC. Phytochemical And Anti-Anaemic Properties Of Ethanol Leaf Extract Of Justicia Carnea Vahl (Acantheceae).Nigerian Journal Of Pharmaceutical And Applied Science Research, (2019); 8(2): 55-61.

[19]. Ijioma SN, Nwosu CO, Emelike CU, Okafor AI, Nwankwo AA. Antinociceptive Property Of Costus Afer Ker Stem Juice And Ethanol Leaf Extract In Albino Rats. Comprehensive Journal Of Medical Sciences, (2014); 2(2):14-19.

[20]. Ishola IO, Yemitan KO, Afolayan OO, Anunobi CC, Durojaiya TE. Potential Of Moringaoleifera In The Treatment Of Benign Prostate Hyperplasia: Role Of Antioxidant Defense System. Journal Of Medical Principles And Practice, (2017); 27:15-22.

[21]. Iwuji SC, Nwafor A, Egwurugwu J, Ejeta K, Akpan U. Comparative Characterization Of The Phytomedicinal Constituents Of Cnidoscolus Aconitifolius Leaf Extracts. American Journal Of Pharmtech Research, (2013); 3(1): 1-7.

[22]. Jeon W, Kim OS, Seo C, Jin SE, Kim J, Shin H, Kim Y, Lee M.. Inhibitory Effects Of Poncirifructus On Testosterone-Induced Benign Prostatic Hyperplasia In Rats.BMC Complementary And Alternative Medicine, (2017); 17:384-394.

[23]. Jeon YJ, Kim BH, Kim S, Oh I, Lee S, Shin J, Kim TY. Rhododendrin Ameliorates Skin Inflammation Through Inhibition Of NF-Kb, MAPK, And PI3K/Akt Signaling. European Journal Of Pharmacology, (2013); 714:7-14.

[24]. Kiseleva O, Kurbatov I, Ilgisonis E, Poverennaya E. Defining Blood Plasma And Serum Metabolome By GC-MS. Metabolites, (2021); 12: 1-31.
[25]. Klin Z. Chemistry.Klin Biochemistry, (1980); 8: 658.
[26]. Lawal TT, Adebowale B, Saba AB, Ewuola EO, Iyiola-Tunji AO. Haematological Responses Of Adult Male Rabbits To Prolonged Administration Of Chaya Plant (Cnidoscolus Acotinifolus) Extract. Africa Journal Of Animal And Biomedical Sciences, (2010); 5(3):118-122.
[27]. Lee CS, Lee SA, Kimyj, Seo SJ, Lee MW. 3,4,5-Tricaffeoylquinic Acid Inhibits Tumor Necrosis Factor-A-Stimulated Production Of Inflammatory Mediators In Keratinocytes Via Suppression Of Akt- And NF-Kb-Pathways. International Immunopharmacology, (2011); 11: 1715–1723.
[28]. Ma K, Dong Q. Association Between Sleep Quality And Benign Prostate Hyperplasia Among Middle-Aged And Older Men In India. BMC Public Health, (2023); 23: 1147.
[29]. Madersbacher S, Sampson N, Culig Z. Pathophysiology Of Benign Prostatic Hyperplasia And Benign Prostatic Enlargement: A Mini-Review. Gerontology, (2019); 65:458-464.
[30]. Manzanilla-Valdez ML, Segura-Campos MR. Renal And Hepatic Disease: Cnidoscolus Aconitifolius As Diet Therapy Proposal For Prevention And Treatment. Journal Of The American College Of Nutrition. (2020); 6:1–19.
[31]. Minciullo PL, Inferrera A, Navarr M, Calapi G, Magno C,Gangemi S. Oxidative Stress In Benign Prostate Hyperplasia: A Systemic Review. Urology International (2015); (94):249-254.
[32]. Njoku UO, Uzoagulu H, Ogugofor MO. Protective Effect Of Cnidoscolus Aconitifolius Leaves Against Diclofenac Induced Gastric Mucosal Damage. Pakistan Journal Of Pharmaceutical Sciences. (2020); 33(2):1-8.
[33]. Nnodim J, Nwaokoro JC. Alteration Of C-Peptide And Prostate Specific Antigen On People Living With Diabetes In Owerri".EC Diabetes And Metabolic Research, (2020); 4(12):4-7.
[34]. Nwobodo VOG, Udedi SC, Ezeonwumelu IJ, Ezeigwe OC, Oguazu CE, David EE. Effect Of Methanol Leaf Extract Of Costusafer On Rats Injected Withnajanigricollisvenom. IOSR Journal Of Pharmacy And Biological Sciences, (2020); 15(6): 13-21.
[35]. Obisike UA, Nwachukwu EO, Boisa N, Nduka N. Determination Of Exogenous Testosterone Propionate Dose For Induction Of Benign Prostatic Hyperplasia In Rat Model.European Journal Of Biomedical And Pharmaceutical Sciences, (2019); 6(13): 141-147.
[36]. Ogbuji NG, Ataga AE. Mycobiota Of Cnidoscolus Aconitifolius (Mill.)Phyllosphere.Journal Of Advances In Microbiology, (2022); 22(4): 25-36.
[37]. Oladeinde FO, Kinyua AM, Laditan AA, Michelin R, Bryant JL, Denaro F, Makinde JM, Williams AL, Kennedy AP, Bronner Y. Effect Of Cnidoscolus Aconitifolius Leaf Extract On The Blood Glucose And Insulin Levels Of Inbred Type 2 Diabetic Mice. Cell Molecular Biology (Noisy-Le-Grand), (2007); 53(3):34-41.
[38]. Olaniyan MF, Afolabi T. Possible Inflammatory Responses In The Traditional Application Of Raw Liquid Extract Of Cnidoscolus Aconitifolius Leaf (Iyana Ipaja-Chaya) In The Treatment Of Anaemia. Saudi Journal Of Medical And Pharmaceutical Sciences, (2017)3(10): 1031-1037.
[39]. Omotoso AE, Ezealisiji K, Mkparu KI. Chemometric Profiling Of Ethanol Leaf Extract Of Cnidosculosaconitifolius (Euphobiaceae) Using UV-VIS, FTIR And GC-MS Techniques. Peak Journal Of Medicinal Plant Research, (2014); 2: 6-12.
[40]. Onasanwo SA, Oyagbemi AA, Saba AB. Anti-Inflammatory And Analgesic Properties Of The Ethanolic Extract Of Cnidoscolus Aconitifolius In Rats And Mice. Journal Of Basic Clinical Physiology And Pharmacology, (2011); 22(2):37-41.
[41]. Onuoha NO, Okafor AM, Eme PE, Odo EC. Haematinic Effect Of Raw And Boiled Leaf Juice Of Cnidoscolus Aconitifolius Using Cyclophosphamide-Treated Adult Male Albino Rats.EC Nutrition, (2017); 5:187–194.
[42]. Orji OU, Ibiam UA, Aloke C, Awoke J, Aja PM, Okechukwu UPC, Ezeani N, Nzubechukwu E. Biochemical Assessment Of Ethanol Leaf Extract Of Cnidoscolus Aconitifolius On Liver Intergrity Of Albino Rat Treated With Lead.Global Journal Of Pharmacology, (2016); 10(4): 108-113.
[43]. Oyagbemi AA, Odetola AA And Azeez OI.Phytochemical Investigation And Proximate Analysis On The Leaves Of Cnidoscolus Aconitifolius. Journal Of Medicinal Food, (2011); 14(3):322–324.
[44]. Patel KV, Semba RD, Ferrucci L, Newman AB, Fried LP, Wallace RB, Bandinelli S, Phillips CS, Yu B, Connelly S, Shlipak MG, Chaves PH, Launer LJ, Ershler WB, Harris TB, Longo DL, Guralnik JM. Red Cell Distribution Width And Mortality In Older Adults: A Meta-Analysis. Journals Of Gerontology.Series A, Biological Sciences And Medical Sciences, (2010); 65:258-265.
[45]. Praveen R. Benign Prostatic Hyperplasia: Updated Review. International Research Journal Of Pharmacy, (2013); 4(8):45–51.
[46]. Shin IS, Lee MY, Jung DY, Seo CS, Ha HK, Shin HK. Ursolic Acid Reduces Prostate Size And Dihydrotestosterone Level In A Rat Model Of Benign Prostatic Hyperplasia. Food Chemistry And Toxicology, (2012); 50: 884–888.
[47]. Stamey TA, Mcneal JE, Yemoto CM, Sigal BM, Johnstone IM. Biological Determinants Of Cancer Progression In Men With Prostate Cancer.Journal Of American Medical Association, (1999); 281:1395-1400.
[48]. Swerdloff RS, Dudley RE, Page ST, Wang C, Salameh WA. Dihydrotestosterone: Biochemistry, Physiology, And Clinical Implications Of Elevated Blood Levels. Endocrine Reviews, (2017); 38(3):220–254.
[49]. Tosoian J, Loeb S. PSA And Beyond: The Past, Present, And Future Of Investigative Biomarkers For Prostate Cancer. The Scientific World Journal, (2010); 10: 1919–1931.
[50]. Ugwu MN, Asuk AA, Eteng MU, Emuru EO. Evaluation Of Impact Of Some Selected Plants On Haematological Parameters Of Testosterone And Estradiol Induced Benign Prostatic Hyperplasia Adult Male Rats. International Blood Research And Reviews, (2019); 10(1): 1-15.
[51]. Ugwu MN, Asuk AA, Utu-Baku AB, Eteng MU. Tissue-Protective Effect Of Prosopisafricana Seed Extract On Testosterone And Estradiol Induced Benign Prostatic Hyperplasia Of Adult Male Rats. International Journal Of Innovative Research And Advanced Studies (IJIRAS), (2018); 5(3):1-7.
[52]. Wei, H., Zhu, C., Huang, Q., Yang, Y., Li, Y., Zhang, Y., Li B., And Zi H.. Global, Regional, And National Burden Of Benign Prostatic Hyperplasia From 1990 To 2021 And Projection To 2035. BMC Urology, (2025); 25: 34-46.
[53]. Wu S, Haigin H, Wang Y, Xu R, Zhu B, Zhou X. Association Between Benign Prostate Hyperplasia And Metabolic Syndrome In Men Under 60 Years Old: A Meta-Analysis. Journal Of International Medical Research, (2019); 47(11): 5389-5399.
[54]. Yang X, Yuan L, Xiong C, Yin C, Ruan J (2014b).Abacopteri Spenangiana Exerts Testosterone-Induced Benign Prostatic Hyperplasia Protective Effect Through Regulating Inflammatory Responses, Inducing Oxidative Stress And Anti-Proliferative. Journal Of Ethnopharmacology, 157:105-113.
[55]. Zhang J, Liu Z, Chen R, Ma Q, Lyu Q, Fu S, He Y, Xiao Z, Luo Z, Luo J, Wang X, Liu X, An P, Sun W. A MALDI-TOF Mass Spectrometry-Based Haemoglobin Chain Quantification Method For Rapid Screen Of Thalassaemia. Annals Of Medicine, (2022); 54(1): 293–301.
[56]. Zhu Y, Cai L, You X, Cordero JJ, Huang Y, Imperato-Mcginley J. Androgen-Induced Prostate-Specific Antigen Gene Expression Is Mediated Via Dihydrotestosterone In Lncap Cells. Journal Of Andrology, (2003); 24(5):681−687.