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Effect of *Anchomanes difformis* Ethanol Root Extract on Electrolyte Level of Testosterone-Induced Benign Prostatic Hyperplasia in Wistar Rats

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ABSTRACT

Benign prostatic hyperplasia is the most common male benign proliferative disease; characterized by overgrowth of prostatic tissue around the urethra constricting the urethral opening. The study investigated the effect of *Anchomanes difformis* ethanol root extract on electrolyte level of testosterone induced benign prostatic hyperplasis in Wistar rats. Forty two matured male Wistar rats weighing 150-180 g were divided into 6 groups of 7 animals each and acclimatized for 7 days. Prostate was induced with 5mg/kg of testosterone for 21 days. Two animals were sacrificed and blood collected for PSA assay. Groups 4, 5 and 6 were treated with 1000 mg/kg, 500mg/kg and 250 mg/kg of the extract respectively. The testosterone induced group 3 was treated with finesteride standard control and group 2 represented the BPH induced control group, while group 1 served as normal control. The administration of the extract lasted for 21 days. The results obtained showed a non significant ($P>0.05$) increase in Na^+ (mmol/L) concentration at 1000mg/kg of plant extract Bph C group vs normal control, while the finesteride group had no effect on Na^+ concentration treated with Bph C group. There was a significant ($p<0.05$) reduction in Cl^- concentration at 1000mg/kg vs 5mg/kg of testosterone and 5mg/kg and finesteride, with no significant ($p>0.05$) effect at 500 mg/kg Vs normal control. For K^+ (mmol/L) concentration in plant treated groups, 1000 mg/kg increase significantly at 250 mg/kg. Conclusively, the results suggested, *Anchomanes difformis* root extract has the potential to maintain serum electrolytes in benign prostatic hyperplasia management induced by testosterone and diarrhea patients.

***Keywords*:** Phytochemicals, Electrolytes, Testosterone, Prostate specific antigen, and 5-alpha Reductase Activity.

Introduction

Many edible and varieties of plants though neglected are indigenous to developing nations like Nigeria. From one group to another, different traditional uses for medicinal plants have been developed. Most people, especially those in underdeveloped nations, rely on using traditional plants for their daily health care needs,[1,2,] mostly because using drugs might have negative side effects or be very expensive. The leaves and tubers of *Anchomanes. difformis* are employed in folk medicine, and as food. Literatures have shown that its traditional used are not limited to these, the plant power of *A. difformis* can be integrated in pest management.[3] The use of *A diffomis* for treatment. According to studies, rhizome is used to treat a variety of conditions, including haemoglobinemia, edema, heartburn and anuresis. The roots of A are 4, 5. Diabetes, diarrhea, cough, and throat issues are all treated with *A difformis*. The stems were used to help with childbirth, as a diuretic, and as a purgative. Sixty percent of ethnomedical applications *A difformis* has been established and scientifically validated.[7] The prostate is one of the glands in the male reproductive system.

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The prostate is located in the pelvis and in adults, is about the size of a walnut,[8] weighing an average of around 11 g and normally ranging between 7 and 16 grammes.[9]It's underneath the bladder of pee and surrounds the urethra. The part of the urethra that travels through it is the prostatic urethra, which joins with the two ejaculatory ducts. The urethral dilator (musculus dilator urethrae) on the urethra's front side contracts during urination, shortening and tilting the prostate in its vertical dimension and widening the prostatic section of the urethral tube [10]. The muscle switches between contractions of the urethral dilator and the muscle switches between contractions of the muscle switches during ejaculation. The intricacies of the technique selected, the type of operation, and the characteristics of the operation all play a significant role in the degree to which these two muscle systems are damaged or conserved following surgery for benign prostatic hyperplasia (BPH). It varies depending on how postoperative ejaculation and urination are affected.[11]

Materials and Methods

Chemicals: Absolute ethanol (BBH), distilled water, uranyl acetate, magnesium acetate, ethyl acetate, dilute acetic acid, sodium chloride solution, mercuric nitrate, mercuric thiocyanate, mercuric chloride, feric nitrate, chloride calibrator, sodium tetraphenyl boron and wash buffer solution

Equipments: Electrical blender, rotary evaporator, water bath, refrigerator and spectrophotometer.

Collection of Plant material: The plant was harvested on the 10th of July 2021 from Udo village in Edo State, identified and authenticated with a voucher number UBD-A450 by a botanist (Prof. Emmanuel Izaka

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Aigbokan) in the department of Biology and Biotechnology, University of Benin.

*Preparation of plant material:*The tubers outer cover was carefully pilled off after which it was washed and dried under room temperature (25-29°C). The dried tubers were then cut into pieces, grounded into fine powder using electrical blender. The fine powder sample obtained was stored in a clean dry rubber container in preparation for extraction.

Solvent extraction of plant material: In a clean, dry 500 mL beaker with occasional agitation, the powdered material was suspended in absolute ethanol (BDH) in a 1:2 sample to solvent ratio and left to stand for about 48 hours at room temperature. It was filtered twice: once with Whatman No. 2 filter paper and once with a cheese substance. In a rotary evaporator set at 45 to 50 °C, the filtrate was concentrated to roughly 1/10th of its original volume, and then the concentrate was allowed to dry completely in the water bath. Weighing the dried extract, we estimated the yield as follows: Wt of extract/Wt of starting material 100%.

*Ethical approval:*Ethical approval for the treatment and handling of experimental animal was obtained from the Faculty Animal Research Ethics Committee on the Use and Care of Experimental animals. Faculty of Basic Medical Sciences, University of Calabar with approval number; 032BCH3319.

*Experimental animal:*The Department of Biochemistry at the University of Calabar provided 42 mature male Wistar rats, weighing between 150 and 180g, for the study. The experiment was carried out on animals that were divided into 6 groups of 7 animals each and acclimated for 7 days in the animal home of the Department of Biochemistry. Throughout the course of the investigation, they had unrestricted access to water and rat food

Induction and confirmation of prostrate: Prostrate was induced in 5 groups by injecting them with 5mg/kg body weight testosterone intraperitoneally for 21 days, while the last 1 group were fed with rat chow and water similarly to represent normal control group (NC group). After the 21 days of induction, 2 animals was randomly selected from all the groups and sacrificed under ketamine blood samples collected by cardiac puncture and used for PSA assay.

*Treatment:*Following the result of the PSA, three (3) (ie, groups 4,5,6) of the induced groups (with 5 rats left in each group) were treated with different doses of the plant extract orally for 21 days. The 4^{th} group received 1000mg /kg of the extract while the 5^{th} and 6^{th} groups were similarly treated with 500mg/kg and 250mg/kg respectively. The 3^{rd} testosterone induced groups (also with 5 rats left in each group) were similarly treated with Finesteride to represent the standard control (SC group) and the 2^{nd} testosterone induced group represent the BPH induced control group (BphC group) while the 1^{st} group that were not induced served as normal control group (NC group).

*Termination of experiment:*The rats underwent an overnight fast, were given anesthesia, and were then instantly slaughtered at the conclusion of the experiment.

Collection of blood samples for analysis: A 2 mL syringe and needle were used to draw blood from the patient's heart into simple sample tubes, which were then kept frozen in the refrigerator until they were utilized for biochemical analysis.

Measurement of serum electrolyte:
Determination of Sodium (Na^+): Sodium analysis was done according to the method provided by Teco Diagnostic, which involved mixing the reagent provided by the industry with serum of the animals, incubation and absorbance reading was taken using a spectrophotometer at 532nm wavelength

Determination of serum Chloride (Cl^-)
Chloride analysis was done according to the method provided by Teco Diagnostic, which involved mixing the reagent provided by the industry with serum of the animals, incubation and absorbance reading using a spectrophotometer at 532 nm wavelength

Determination of Potassium (K^-)
Potassium analysis was done according to the method provided by Teco Diagnostic, which involved mixing the reagent provided by the industry with serum of the animals, incubation and absorbance reading using a spectrophotometer at 532 nm wavelength

Determination of 5 –α- reductase [2(SRD5 -α-2)]
Principle:
The ELISA kit contain a micro-ELISA strip plate pre-coated with an antibody specific to SRD5a2.When the sample is mixed with an appropriate volume of micro-ELISA strip plate wells and combined a Horseradish Peroxidase (HRP) conjugated antibody specific for SRD5-α-2 and incubated.

Procedure:
The microelisa strip plate's blanked control well was left unfilled. 100ml of sample and 40µl of dilution buffer were added to the sample wells [dilution factor is 3]. The sample was shaken and loaded onto the bottom of the well without touching it. The solution was sealed with a closure plate membrane after being incubated for 30 minutes at 27ºC. Using distilled water to wash the buffer, the concentration was diluted. After removing, the aspiration was performed; the container was then filled with a wash solution. After 30 seconds of relaxation, the wash solution was removed, and the washing process was performed five more times.

Determination of Total Prostate Specific Antigen (PSA)
Principle: A native antigen and a high affinity and specificity antibody that can recognize multiple unique epitopes in excess are necessary components of an immune-enzymatic assay. During the experiment, the immobilization occurs at the microplate well's surface due to the interaction between the well's streptavidin coating and an exogenously applied biotinylated anti-PSA antibody. When paired with an enzyme-labeled antibody, a monoclonal biotinylated antibody, and a serum containing the native antigen, the natural antigen and the antibodies react without competition or steric obstruction to generate a solution sandwich complex.

Table 1: Distribution of animals into experimental group

Group	Number of animals	Treatment
1	7	Rat chow and Water
2	7	5 mg/kg body weight testosterone plus normal saline for 21 days
3	7	5 mg/kg body weight testosterone plus standard (Finesterid) 500mg/kg body weight
5	7	5 mg/kg body weight testosterone plus 1000 mg/kg body weight plant extract
6	7	5 mg/kg body weight of testosterone plus 500 mg/kg body weight plant extract
7	7	5 mg/kg body weight of testosterone plus 250 mg/kg body weight plant extract.

NC = Normal Control, BphC = Prostrate Control, SD = Standard control

The antibody bound fraction was separated from the activity once equilibrium has been reached. The natural antigen concentration and the antibody bound fraction are directly proportional.

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Test procedure: Using two micro plate wells, exactly 25 µL of sample and reference calibrator was pipetted into each. And 100 µL of tPSA enzyme reagent was added to each well and the well was carefully welled for 30 seconds to mix properly and covered. The wells were then incubated at room temperature, 350 µL of buffer solution was added, 25 µL washed three times, and 100 µL of working substrate reagent was added to each well. These steps were followed by 15 minutes of incubation at room temperature and 25 minutes of gentle mixing. Within 30 minutes, 450 nm of absorbance was measured. The level of tPSA in the unknown sample was then determined using a dose response curve.

Determination Testosterone (TE)
*Principle:*An enzyme immunoassay requires three types of reagents: native antigen, enzyme-antigen conjugate, and antibody. Enzyme-antigen conjugate and enzyme-antigen combination compete for a limited number of antibody binding sites. The exchange is seen below.

$$^{Ezn}Ag + Ag + Ab_{Bin} \underset{k_b}{\overset{k_a}{\leftrightarrow}} AbBin + {}^{Enz}Ag_{Bin}$$

Placed react simultaneously,
$AgAb_{Bin}$ + $^{Enz}AgAb_{Bin}$ + Streptavidin = immobilized complex
In the antibody-bound fraction, there is an inverse relationship between the natural antigen concentration and the enzyme activity. The antigen concentration of an unknown can be calculated from a dose response curve utilizing a variety of serum references with known antigen concentrations.

Test procedure: Four (4) microplate wells were labeled as sample, reference, control and formatted, and exactly 10µl of the sample pipetted into each and 50 µL of working testosterone enzyme reagent was added to all the wells followed by gentle swirling of the microplate for 30 seconds to properly mix. 50 µL of testosterone biotin reagent was added to all the wells, mixed, covered and incubated for 60 minutes at room temperature after which the content was discarded. 350 µL and decanted, 2 more and 100 µL of working substrate solution was added to all the wells followed by incubation for 15 minutes and addition of 50 µl of stop solution and mixing for 25 minutes. Absorbance was then read at 450nm within 30minutes. A dose response curve is used to ascertain the concentration of testosterone in the unknown sample.

Statistical analysis: Data obtained were analyzed using Microsoft Excel Office Excel 2007 and result expressed as Mean± SEM. The Statistical Package SPPSS version 16.0 was used to establish statistical significance at P<0.05.

Results and Discussion

The results of the effect of ethanol root extract of *Anchomanes differmis* of testosterone induced-benign prostatic hyperplasia in Wistar rats is presented as fellows in figures 3, 4, 5 and 6.

Effect of treatment on Na (mmol/L): Result obtained in Na^+ concentration (Figure 1) indicated an increase following BPH induction in BphC group vs NC group. The increase was restored only in the 1000 mg/kg group in a non-significant P>0.05 manner vs NC group. SC group also did not cause any effect on Na concentration following treatment vs BphC. While in the plant extract treated groups a dose dependent reduction were obtained with 1000mg/kg group indicating an effect of plant extract following treatment.
Figure 2 shows the effect of treatment on Chlorine ion concentration. The result showed that BPH induction caused a significant increase in Cl concentration shown by the increase in BphC group vs NC group. Also, from the result it was observed that the 21 days treatment caused a reverse in this effect in the treated groups with exception of the 250 mg/kg group which further increased after treatment. In the treated groups a significant P<0.05 reduction in Cl concentration were recorded in 1000 mg/kg group vs BphC and Finesteride treated groups. While in the plant extract treated groups a dose dependent effect in Cl concentration were obtained while non significant P>0.05 effect of treatment were obtained in 500mg/kg group vs NC group.

Results obtained on the concentration of K^+ showed that BPH induction caused a decrease in K^+ concentration indicated in the reduction obtained in BphC group vs NC group. Following 21 days treatment, the reduction were reversed in the treated groups significant P<0.05 in 1000mg/Kg group vs BphC group. In the extract treated groups1000 mg/kg group indicated a non significant effect vs 500 mg/kg group though increased significantly vs 250 mg/kg group. Finesteride treated group increased non significantly P>0.05 vs 250mg/kg group.A significant increase in testosterone level were recorded in all the BPH induced groups following 21 days testosterone administration indicated in BphC group vs NC group. The result of this study also showed that the level of testosterone were significantly *P<0.05* reduced in the treated groups majorly in the plant extract groups highly in the1000mg/kg group. In the extract treated groups the level of testosterone was reduced significantly P<0.05, vs SC group dose dependently with 1000 mg/kg group showing a higher effect followed by 500 mg/kg and 250 mg/kg groups respectively.
Result obtained on PSA showed that BPH induction caused a non significant P>0.05 increase in PSA level obtained in a slight difference obtained in BphC group vs NC group.

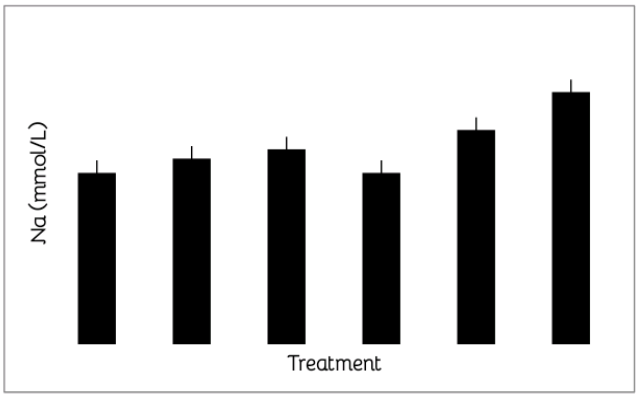

Figure 1: Effect of treatment on Sodium ion concentration (mmol/L).
Values are expressed as Mean ± SEM n=5. NC= normal control, BphC= Bph control, SC=standard control (finesteride),1000ng/kg, 500mg/kg and 250mg/kg.

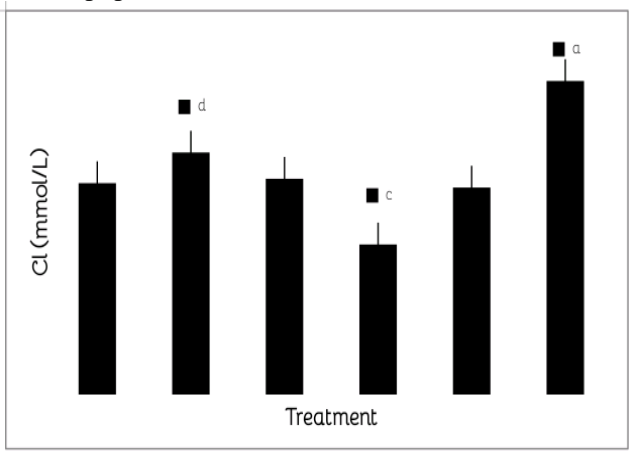

Figure 2: Effect of treatment on Chlorine ion concentration (mmol/L).
Values are expressed as Mean ± SEM n=5.b = P<0.05 vs d. d = P<0.05 vs c. f = P<0.05 vs a. NC= normal control, BphC = Bph control, SC= standard control (finesteride), 1000 mg/kg, 500 mg/kg and 250 mg/kg.
Furthermore, the result showed that 21 days treatment caused a reverse in the level of PSA significant in1000mg/kg group. In the treated groups 250mg/kg group a higher value showing no effect of plant extract on the level of PSA following caused by BPH induction and remained elevated after treatment. 1000 mg/kg and 500 mg/kg groups showed a dose dependent reduction significant P<0.05 vs Bph and finesteride treated groups respectively.

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A significant reduction in 5 alpha reductase activities was recorded in all the BPH induced groups following 21 days treatment vs NC group. In treated groups with exception of 250 mg/kg group, a significant increase in enzyme activity were recorded vs BphC higher in HD extract treated groups. Finesteride treated group also indicated a significant increase in enzyme activity vs 250 mg/kg, not significant P>0.05 vs 500mg/kg group but reduced significantly P<0.05 vs 1000 mg/kg group. In plant extract treated groups dose dependent reductions were obtained, 250 mg/kg group showed a higher reduction in enzyme activity significant vs 500 mg/kg and 1000mg/kg respectively.

Phytochemical screening: Table 2, shows the level of phytochemicals present in the extract of *Anchomanes difformis* tuber using different polar solvent. This table shows that alkaloids are present only in water fraction, tannins are present are high water fraction and low in ethanol fraction only, saponins and glycosides were higher in water fraction more than ethanol fraction but very low in ethylacetate fraction, phenols and lastly flavoniods were not present in any of the extract fractions, steroids were only present in water fraction of the extracts.

The presence of bioactive ingredients found in tubers of *Anchomanes difformis* Blume has justified its use in traditional medicine for the treatment of various clinical conditions such as, inflammation, ulcer, diabetes etc. [12,13] This study was aimed at understanding its potential in the treatment and management of benign prostatic hyperplasia and maintaining electrolyte level in male Wistar rats. Increase in PSA level in the blood has been show to be associated with BPH and prostate cancer. Studies on the relationship between testosterone administration and PSA level have indicated a slight or non significant increase in the PSA level following exogenous administration of testosterone this agrees with the data obtained in this study that indicated an increase in PSA level in a non significant manner. This effect may be as a result of disruption caused by testosterone on the epithelia cells of the prostrate resulting in diffusion of the antigen into the tissue around the epithelium causing its elevation in cases such as in BPH.[14]The result of this study further showed that extract of *Anchomanes difformis* effectively reduced the PSA level dose dependently indicating its potential for controlling the elevation of PSA following disruption of epithelia cells of prostate caused by benign prostate hyperplasia development.[14, 15]Recent studies has shown that increased testosterone level are related to the development of BPH cells which has place a restricting exogenous use of testosterone mostly in patients with prostate cancer and BPH history.[16] The role elevated level of testosterone play in BPH development has been well established,[17] the balance between proliferation and programmed cell destruction (apoptosis) in prostrate is regulated by androgens. In androgen sensitive tissues including prostate testosterone secreted in the testes by Leydis cells is irreversibly converted to dihydrotestosterone (DTH) by 5-alpha reductase (5-AR).[17] The result obtained in this study indicated that exogenous administration of testosterone in BPH induction caused an elevation in the level of testosterone above the normal level comparing the level obtain in control with that of the administered groups, this agrees with the report of which indicated that testosterone administration resulted in an elevation in serum testosterone and in the development of BPH.Dihydrotestosterone bindings to androgen receptor in the prostatic cells influence androgen responsive genes causing prostate growth as well as secretion and elevation of PSA level.[15] According to the data obtained in this study slight increase in PSA level following administration of testosterone is as a result of 5-AR activity which increased significantly following days of testosterone administration and were further reduced in *Anchomanes difformis* treated groups in a dose dependent manner significantly in HD group.

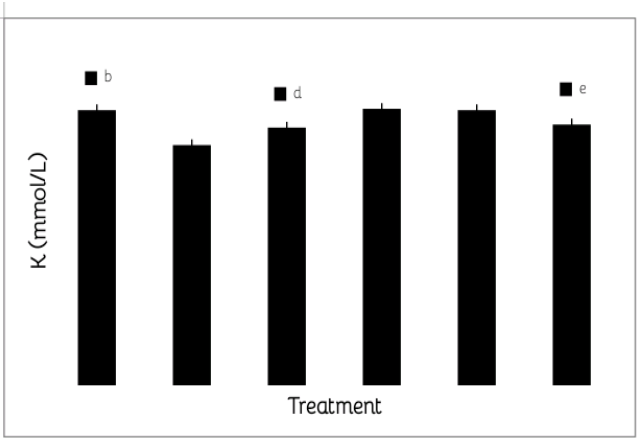

Figure 3: Effect of treatment on Potassium ion concentration (mmol/L).
Values are expressed as Mean ± SEM n= 5.a = P<0.05 vs b. = P<0.05 vs d. f = P<0.05 vs e. NC = normal control, BphC = Bph control (Finesteride), 1000 mg/kg, 500 mg/kg and 250 mg/kg.

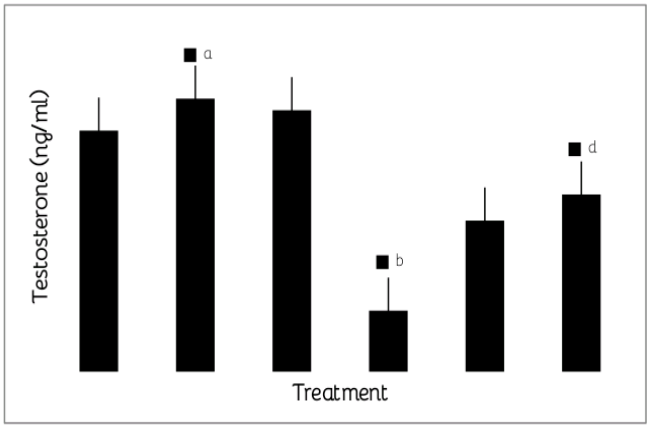

Figure 4: Effect of treatment on testosterone (TE) level (nm/mL).
Values are expressed as Mean ± SEM n= 5. b = P<0.05 vs a. d = P<0.05 vs b. f = P<0.05 vs d. NC= normal control, BphC= Bph control, SC = standard control (Finesterid), 1000 mg/kg, 500 mg/kg and 250 mg/kg.

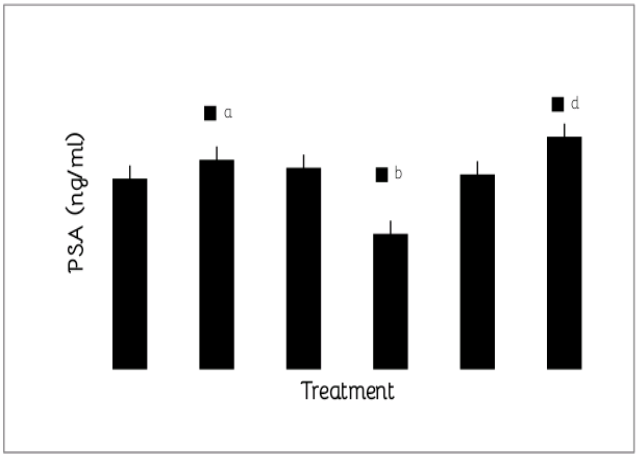

Figure 5: Effect of treatment on Prostate Specific Antigen (PSA) (ng/mL).
Values are expressed as Mean ± SEM n= 5. b = P<0.05 vs a. d = P<0.05 vs b. f = P<0.05 vs d. NC= normal control, BphC= Bph control, SC= standard control (finesteride), 1000 mg/kg, 500 mg/kg and 250 mg/kg.

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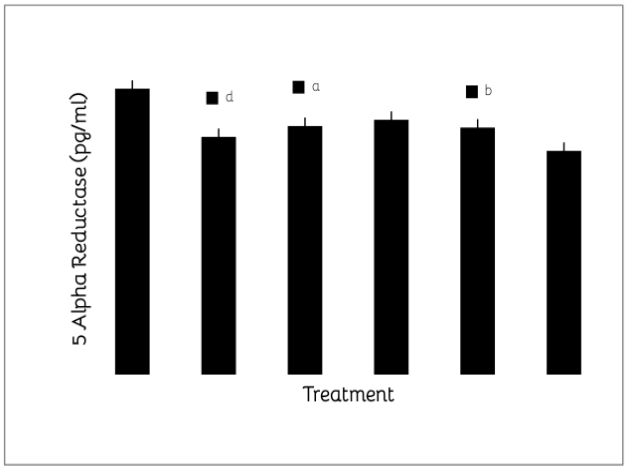

Figure 6: Effect of treatment on 5 Alpha Reductase (5AR) (pg/ml)activity.
Values are expressed as Mean ± SEM n=5.b = P< 0.05 vs d. c = P<0.05 vs a, e = P<0.05 vs b NC= normal control, BphC= Bph control, SC= standard control (finesteride), 1000 mg/kg. 500 mg/kg and 250 mg/kg.

Table 2: Phytochemical screening of plant sample

Phytochemical	water	ethanol	EACT	N-Hexane
Alkaloids	++	-	-	-
Tannins	+++	+	-	-
Saponins	+++	++	+	-
Flavonoids	-	-	-	-
Phenols	-	-	-	-
Steroids	++	-	-	-
Glycosides	+++	++	+	-

+++ = represent the level of phytochemical present in the respective solvent extracts, ─ = represent absence of the phytochemical in the respective solvent extracts.

Studies have also demonstrated that DTH is a more potent stimulating factor in prostate growth more than testosterone, thus in theory DTH production by 5-AR result in prostate enlargement and possible BPH development.[18,15] Also poor prostate growth has been reported as a result of low level of testosterone and DTH seen in men with 5-AR deficiency using medication such as finesteride,[17] a report in line with the results of this study which reordered elevation in PSA, testosterone and 5-AR in the BphC group (BPH induced group) but in the group treated with finesteride as standard drug (SC group) and in different doses of *Anchomanes difformis* extract the different increases were effectively reversed respectively.The body's electrolytes, notably sodium, the main positive (cation) in fluid outside of cells that controls the body's water content, must be in balance for cells and other vital organs to function normally.[4] The flow of Na^+ produces the electrical signals that are necessary for many bodily activities, particularly those involving the brain, neurological system, and muscles. [(5)] Therefore, too much or too little of these electrolytes causes cell malfunction and extremes in the blood may also prove to be fetal, resulting in conditions such as hyponatremia as a result of liver (or kidney) disease, hyperkalemia and hypokalemia following seriously abnormal increase or decrease in potassium and hyperchloremia and hypochloremia respectively in abnormal level of chloride in serum.[19]In this present study administration of testosterone caused a significant disruption in electrolyte balance agreeing with the report of.[3] Sodium ion Na^+ and Cl^- cation and anion of extracellular compartment were reduced following BPH induction while K^+ and HCO_3^- cation and anion of the intracellular compartment were increased respectively, this effect was reversed in the groups treated with *Anchomanes difformis* extract in a dose dependent manner significant in HD group, suggesting ability of the plant in maintaining fluid or electrolyte imbalance as a result of BPH development.

Conclusion

The findings showed that the extract of *Achomanes difformis* root were effective in reversing the effect of testosterone used to induce BPH on electrolytes and PSA level studied in matured Wistar rats. In maintenance of electrolyte balance the extract indicated an effective potential, and also in reversing the effect of BPH development. Thus, it may be concluded that the plant extract is an effective remedy in the treatment BPH in matured Wistar rats.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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