comparative study of the effects of serial extracts of Solanum aculeastrum seeds on prostatic indices of testosterone propionate induced benign prostatic hyperplasia in male wistar rats.

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Abstract: This study investigated the effect of serial extracts of Solanum aculeastrum seeds on testosterone propionate induced benign prostatic hyperplasia in adult male Wistar rats. Solanum aculeastrum is a tropical plant whose berries are widely used in folk medicine for the treatment of various inflammatory diseases. Finely ground Solanum aculeastrum seeds (1000 g) were extracted with hexane, chloroform, benzene, ethylacetate and ethanol respectively using serial exhaustive extraction technique. Male Wistar rats weighing 280 ± 20g were injected with 10 mg/kg body weight of Testosterone Propionate through intraperitoneal route for twenty eight days to induce BPH. The animals were divided into eight (8) groups of six (6) rats each. Group 1 (Normal control) was not induced with BPH and served as normal control, group 2 was induced and not treated and served as BPH control, group 3 (Finasteride control) was induced and received standard drug, finasteride while groups 4 (Hexane extract treated group), 5 (Chloroform extract treated group), 6 (Benzene extract treated group), 7 (Ethylacetate extract treated group) and 8 (Ethanol extract treated group) were induced and treated orally with 300 mg/kg body weight of hexane, chloroform, benzene, ethylacetate and ethanol extracts respectively for twenty eight days. The animals were sacrificed and blood collected through cardiac puncture. Biochemical studies were conducted using standard procedures. The result revealed that there were significant decreases in prostate weights, prostatic index, serum PSA, DHT, 5αRD2 and TNF-α levels in all the test groups compared to the BPH and finasteride control groups. Catalase activity was significantly increased in all the treatment groups compared to the BPH and finasteride control groups. All the extracts showed significant anti-BPH and antioxidant properties. These results suggest that serial extracts may be safe for use in the management of BPH. However anti-BPH activity was found to be solvent dependent and compared significantly with the standard drug. These findings support its therapeutic use by the herbalists in treating inflammatory diseases.

Keywords: Solanum acculeastrum, BPH, Testosterone propionate, Finasteride, Wistar rats.

I INTRODUCTION

Benign prostatic hyperplasia is a non-cancerous increase in size of the prostate that progresses linearly with age in all ethnic groups and is clinically identifiable in at least 50 % of men above 45 years old (Iweala and Ogidigo, 2015a). It is characterized by the proliferation of prostatic tissues, prostate enlargement and lower urinary tract symptoms (Briganti *et al*., 2009). It is also associated with complex histological changes involving glandular and stromal hyperplasia, fibrosis and prostatitis (Chapple and Smith, 1994; Barnes, 2002). The prostate gland is a major secondary endocrine organ of males whose development and growth depends on androgen stimulation especially by dihydrotestosterone (DHT), an active metabolic product from the conversion of testosterone by steroid 5-alpha-reductase (SRD5α). It is documented that androgens and possibly estrogens constitute the primary factors responsible for prostate diseases (Shin *et al.*, 2012; De Nunzio and Tubaro, 2011; Farley, 2011). Benign prostatic hyperplasia is diagnosed by clinical examination, assessment of urination problems, rectal examination, ultrasound examination of prostate and serum level of prostate specific antigen (PSA) (Ejike and Eze, 2015). Symptoms include frequent urination, trouble starting to urinate, inability to urinate, weak stream, or loss of bladder control. Complications include urinary tract infections, bladder stones, and chronic kidney problems and these influences the patient's quality of life (Lee, 2019). Current methods of treatment include the use of hormonal products, androgen antagonists, 5-alpha reductase inhibitors (finasteride), α-1 adrenergic blockers (alfuzosin and terazosin) and surgery (Gravas and Oelke, 2010; Iweala and Ogidigo, 2015b). However, in aged people, there can be associated underlying conditions, thus surgical intervention cannot be performed in all cases. Some of these conventional medications are not only too costly but can cause severe side-effects such as erectile dysfunction and gynecomastia due to its structural similarities to steroidal hormones hence the shift in focus to herbal remedies with less severe or no side effects (Vaughan *et al*., 2002; Forley and Kirby, 2003; Saigal and Joyce, 2005; Chinedu *et al*., 2011; Nyamai *et al*., 2016; Ngulde *et al*., 2019; Madersbacher *et al*., 2019).

Several community-based epidemiological studies have documented varying prevalence of BPH. In developing countries, the prevalence of the disease reaches 86 % by the age of 81 - 90 years old. Ezeanyika *et al.* (2006) reported a prevalence of 25.30

% in Nsukka, South-Eastern Nigeria, which is similar to figures from the United Kingdom (25.3 %) and Spain (24.94 %). Adegun and Popoola (2011) reported a prevalence of 88 % in Ado-Ekiti, South-West Nigeria, which is comparable to 84.4 % in another hospital-based study in Ethiopia (Berhanu, 2008). In Port-Harcourt, South-South Nigeria, the prevalence of BPH was 72.2 % using the international prostate symptoms score (IPSS), and 60 % using digital rectal examination (DRE) (Bock-Oruma, 2013). In another study in Odi/Osi Local Government Area, another rural setting in South -West Nigeria, the prevalence rate of 23.7 - 45.3 % per 1000 men was reported (Ojewola *et al.*, 2017). In the US, it is estimated that each year about 1.7 million people visit to hospital is due to manifestations of this disease (Wei *et al*., 2005). It is a significant health care problem due to its high prevalence and the cost associated with its treatment. Walsh *et al*., (1974) established that analogous form of BPH can be induced in male rats using synthetic testosterone and estradiol.

Recent advances in herbal therapies have listed plants and plants derived products that have shown some level of anti-BPH activities. *Pygeum africanum* extracted from the bark of the African plum tree has been used in Europe since 1969 in the treatment of symptomatic BPH (Wilt *et al*., 2002). The consumption of tomatoes and tomato products significantly reduced plasma prostate specific antigen (PSA) levels in patients with BPH (Edinger and Koff, 2006). The extract of *Urtica dioica* (Urticaceae) roots has been used for the treatment of BPH (Lopatkin *et al*., 2007; Pavone *et al*., 2010). Herbal preparations from saw palmetto are used to improve symptoms of BPH (Barnes *et al*., 2008). Cernilton, an herbal preparation from rye-grass pollen is a registered pharmaceutical product in Korea, Western Europe, Japan and Argentina used in the management of BPH (Shrivastava and Gupta, 2012). Also, Herbal remedies from *Saxifraga stolonifera*, *Zi-Shen Pill (ZSP)*, *Orbignya speciosa*, *Phellodendron amurense*, *Ganoderma lucidum*, *Serenoa repens*, *Lepidium meyenii* and *Telfairia occidentalis* extracts have shown some improvements on BPH (Ejike and Ezeanyika, 2011; Shrivastava and Gupta, 2012; Alhakmani *et al*., 2013; Cai *et al*., 2018). Extracts of *Prunus africana* bark are used to make capsules for BPH management (Nyamai *et al*., 2016). However, there is currently no available information on the effects of *Solanum aculeastrum* on the treatment or management of BPH, hence the need to explore these plant materials for possible pharmacological and biochemical influence on the pathology of BPH.

Solanum aculeastrum (Solanaceae) commonly known as *Omotobo* by the *Abagusii* community of Kenya is also known as soda apple or goat bitter apple or poison apple (Laban *et al*., 2015). In Nigeria, the *Efiks*/*Ibibios*, the fourth largest ethnic group in the country, it is commonly referred to as *Nditot Ekpo* or *Nkeŋhe nditot*. The species name *aculeastrum* refers to the thorns that adorn most parts of the shrub (Koduru *et al*., 2006b). It is a shrub or small tree native to tropical Africa down to South Africa. It grows in a wide range of soil terrain and climatic conditions (Iweala and Ogidigo, 2015a). *It* occurs naturally in grassland, woodland and in forest margins. It has also been recorded from gentle to steep slopes on various soil types such as sandy soils, reddish brown clay-loam and brown sandy loam (Aboyade *et al*., 2009; Aboyade *et al*., 2010). The petals are white to pale violet and the flower has a bitter, sour smell. At maturity, the fruits or berries are about 4 to 5 cm in diameter, egg-shaped, becoming greenish-yellow when ripe (Wanyonyi *et al*., 2003; Laban *et al*., 2015). The fruits, both matured and immatured, contain the alkaloid solanine (Hutchings *et al*., 1996). The leaves and berries of *Solanum aculeastrum* contain mainly straight-chain aliphatic hydrocarbons (Koduru *et al*., 2006a). Among the *Abagusii* community of Nyamira County of Kenya, the fruits and leaves of *Solanum aculeastrum* are used fresh, dried, boiled, or charred (ashed) for the treatment of jigger infestations and wounds (*Tungiasis*), swollen joints in fingers, gangrene, toothaches, gonorrhea, bronchitis, rheumatism and in ringworm in cattles (Koduru *et al*., 2006a; Koduru *et al*., 2007a; Laban *et al*., 2015). They are also used as eyewash (Laban *et al*., 2015). A decoction of the root bark is used in Kenya for the treatment of sexually transmitted bacterial diseases, including gonorrhea as well as acne (Kokwaro, 2009). The *Efik/Ibibios* of Nigeria use decoction of the ripe berries for the treatment of splenomegaly (Ubon, 2019). Ethnobotanical survey revealed that the berries are used in the treatment of breast cancer (Koduru *et al*., 2006a; Koduru *et al*., 2007a). Methanol and aqueous extracts of the berries have been shown to have moderate antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aureginosa* and *Bacillus subtilis* bacteria (Wanyonyi *et al*., 2002; Wanyonyi *et al*., 2003; Wabwoba *et al*., 2010).

In this study, we focused on serial-extracts of air-dried *Solanum aculeastrum* seeds obtain from the wild to examine their effects as anti-BPH agent against testosterone induced BPH. The effectiveness of herbal medicine depends on the synergicity and phytochemical load of the plant material (Engwa *et al*., 2013). The yield of phytochemicals however depends on the effectiveness of the extraction method; hence hexane, chloroform, benzene, ethyl acetate and ethanol solvent extraction methods were evaluated and compared.

II MATERIALS AND METHODS

Plant Materials

Samples of ripe fruit berries of *Solanum aculeastrum* Dunal were obtained from locations in Itu Local Government Area of Akwa Ibom State in Nigeria between November, 2017 and January 2018, and authenticated by a taxonomist at the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. A voucher specimen with number'Ubon, UUH 2687 'Itu' was deposited in the herbarium of the University of Uyo, Uyo, Nigeria. The samples were washed under clean gently running tap water to remove dirt on the fruits. After the fruits were kept for 2 hrs for the water to dry off, a sharp stainless steel knife was used to cut open the fruits, in order to remove the seed. The seeds were freed from the mesocarp and pericarp and air-dried at room temperature (25 ± 2 ^{o}C) until a constant weight was obtained. After drying, the seeds were ground using a desk top grinder (Model No: QBL-18L40, Turinar Corp, Shang-Hai, China) into fine particles and stored in different plastic containers with screw cap.

Preparation of Extracts

The *Solanum aculeastrum* seeds extracts were prepared through serial exhaustive extraction technique using the modified methods of Nidal *et al*. (2015), Pandey and Tripathi (2014) and Azmir *et al*. (2013). The finely ground *Solanum aculeastrum* Dunal seeds (1000 g) were soaked in 1000 ml n-hexane at 25 ^{o}C for 24 hours in a 2000 ml separating funnel with continuous

shaking. After that, the filtrate was obtained by running the tap of the separating funnel. The sample residue in the separating funnel was re-extracted with another 1000 ml n- hexane. The combined filtrate was collected and kept in a labeled pre-weighed volumetric flask at room temperature. The residue was air-dried and the process of extraction was repeated as described four more times with chloroform, n-benzene, ethylacetate and finally with ethanol. The filtrates of each solvent extraction was collected and kept in labeled weighed volumetric flasks at room temperature. The different filtrates collected in weighed volumetric flasks were separately placed in a Büchi rotary evaporator at 40 ^{o}C in order to recover the solvents, and to obtain the crude extracts. The weights of the crude extracts were determined by calculating the difference in the weights. The extracts were kept in different sterile brown bottles and stored at – 4 ^{o}C in the refrigerator.

Animal Treatment

Forty eight (48) matured male Wistar rats weighing 200 - 280 ± 20.0 g were used in this work. The animals were obtained from the animal house, Biochemistry Department, University of Uyo, Uyo, Akwa Ibom State. The animals were housed in well ventilated cages in the experimental room at a temperature of 25 ± 4 °C and relative humidity of 65 ± 5 % with an alternating 12 hours light and dark cycle for three days to acclimatize. They were allowed access to food (grower's mash from Vital Feeds, Jos, Plateau State, Nigeria) and water *ad libitum*. All animals handling and experiments were carried out in line with the guidelines of institutional animals' ethical committee as approved by the Post-Graduate School, University of Uyo, Nigeria. Sacrifice of animals was performed under full anaesthesia and the carcasses were properly disposed by burying.

BPH Induction

Adult male Wistar rats weighing 200 - 280 ± 20.0 g were induced with BPH by intraperitoneal injection of testosterone propionate (10 mg/kg body weight) for twenty eight (28) days (Ejike and Ezeanyika, 2011; Iweala and Ogidipo, 2015b; Mbaka *et al*., 2017; Cai *et al*., 2018).

Experimental Design

The animals were weighed and randomly selected into eight (8) groups of six (6) animals each and treatment regimen conducted as shown in Table 1. All treatment lasted for twenty eight (28) days. The animals had free access to feed and water *ad libitum* throughout the period of experiment and their body weights were measured weekly throughout the period of the experiment.

Table 1: Animal Grouping and Treatment

Group	Name	Treatment
1.	Normal Control (NC)	Normal animals + 0.40 ml Olive oil
2.	BPH Control (BPHC)	BPH induced rats without treatment
3.	Finasteride Control (FinC)	BPH + finasteride (5 mg/kg b. wt.).
4.	Hexane Extract Treated group (HETG)	BPH + hexane extract (300 mg/kg body wt.).
5.	Chloroform Extract Treated group (CETG)	BPH + chloroform extract (300 mg/kg body wt.).
6.	Benzene Extract Treated group (BETG)	BPH + n-benzene extract (300 mg/kg body wt.).
7.	Ethylacetate Extract Treated group (EaETG)	BPH + ethylacetate extract (300 mg/kg body wt.).
8.	Ethanol Extract Treated group (OHETG)	BPH + ethanol extract (300 mg/kg body wt.).

Animal Sacrifice and Preparation of Sera for Analysis

All experimental animals were anaesthetized using chloroform fumes 24 hours after the last administration of the extract. Blood samples for sera preparation was collected by cardiac puncture into sterile plain tubes and EDTA (0.77M) bottles for haematological analysis. The liver, kidneys and prostates were harvested from scarified rats, washed with ice-cold saline solution (0.9% w/v), blotted, and weighed. Serum samples were extracted from the clotted blood into sterile plain tubes after centrifugation at 2000 rpm for 10 minutes using a bench top centrifuge (MSE Minor, England). The sera were stored in the refrigerator for analyses while the whole blood samples were used in determining haematological indices.

Drugs and Chemicals

All chemicals and reagents used for this research were of analytical grade and were obtained from Sigma-Aldrich, St. Louis, USA. Testosterone Propionate (TP) was obtained from Tokyo Chemical Industry, Tokyo, Japan.

Assay for PSA, DHT, 5αRD2, TNF-α and catalase

The Enzyme-linked immunosorbent assay (ELISA) kits for the estimation of PSA, DHT, 5αRD2, TNF-α and catalase obtained from Uscn Life Science Inc. Wuhan 430056, Peoples Republic of China were adopted for their estimation following the procedure on the manufacturers' manual.

Statistical Analysis

Statistical analysis was carried out using window SPSS version 23.0. One way analysis of variance (ANOVA) was adopted for comparison and results were subjected to post hoc test using Turkey multiple comparison test. The data were expressed as means ± standard error of the mean (SEM) and values with $p < 0.05$ were considered significant.

III RESULTS

Effects of serial extracts of *Solanum aculeastrum* seeds on body weights, organ weights and prostatic index

The animals exhibited significant weight loss and decrease in appetite after four weeks of benign hyperplasia induction. Treatment with the extracts and finasteride resulted in progressive weight gain overtime with improvement in appetite. The results indicate that there was a significant ($p < 0.05$) increase in body weights of animals in all the treatment groups compared to the BPH

control as well as the normal control groups (Table 2). The results also indicate a significant decrease in liver weights in groups 5, 6, 7 and 8 compared to the BPH control and the normal control groups. The results in Figure 4 also reveals a significant increase in prostate weight in the BPH control compared to the normal control; and a corresponding significant decrease in prostatic weights in groups 3 and 6 compared to the BPH control group. The changes in the body, prostate and liver weights were however not significant compared to the finasteride control group. There were no significant changes in the kidney weights in all the test groups compared to both the BPH control and the normal control groups. The results also show a significant increase in the prostatic index (PI) of BPH control compared to the normal control. However, there was significant decrease in the PI all the test groups compared to the BPH control.

Table 2.Effects of serial extracts of *Solanum aculeastrum* seeds on organ weights and prostatic index of testosterone propionate induced BPH in male Wistar rats.

GROUP	GROUP NAME	Body wt. (g)	Liver wt. (g)	Kidney wt. (g)	Prostate wt. (g)	Prostatic Index
1.	Normal Control	229.67 ± 3.72	6.30 ± 0.37	1.09 ± 0.12	0.35 ± 0.02	1.50 ± 0.08
2.	BPH Control	226.00 ± 1.90	6.74 ± 0.26	1.17 ± 0.09	0.41 ± 0.03a	1.80 ± 0.12a
3.	BPH + Finasteride	274.00 ± 3.15a	6.03 ± 0.28	1.07 ± 0.01	0.35 ± 0.01b	1.29 ± 0.04b
4.	BPH + Hexane Extract	273.20 ± 3.15a	6.46 ± 0.26	1.06 ± 0.03	0.38 ± 0.02	1.41 ± 0.07b
5.	BPH + Chloroform Extract	281.60 ± 3.85ab	4.60 ± 0.22abcd	0.99 ± 0.00	0.37 ± 0.01	1.40 ± 0.09b
6.	BPH + Benzene Extract	289.50 ± 6.81ab	5.36 ± 0.23abd	0.99 ± 0.07	0.33 ± 0.02bd	1.13 ± 0.07abde
7.	BPH + Ethyl acetate Extract	285.17 ± 4.61ab	5.77 ± 0.34be	1.10 ± 0.03	0.38 ± 0.02f	1.32 ± 0.08b
8.	BPH + Ethanol Extract	292.00 ± 3.56ab	5.55 ± 0.26be	1.11 ± 0.09	0.38 ± 0.02f	1.30 ± 0.05b

Values are expressed as Mean ± SEM, n = 6
a = $p < 0.05$ (Test groups compared with normal control).
b = $p < 0.05$ (Groups 3, 4, 5, 6, 7 and 8 compared with group 2).
c = $p < 0.05$ (Groups 4, 5, 6, 7 and 8 compared with group 3).
d = $p < 0.05$ (Test groups compared with group 4).
e = $p < 0.05$ (Test groups compared with group 5).
f = $p < 0.05$ (Test groups compared with group 6).
g = $p < 0.05$ (Test groups compared with group 7).

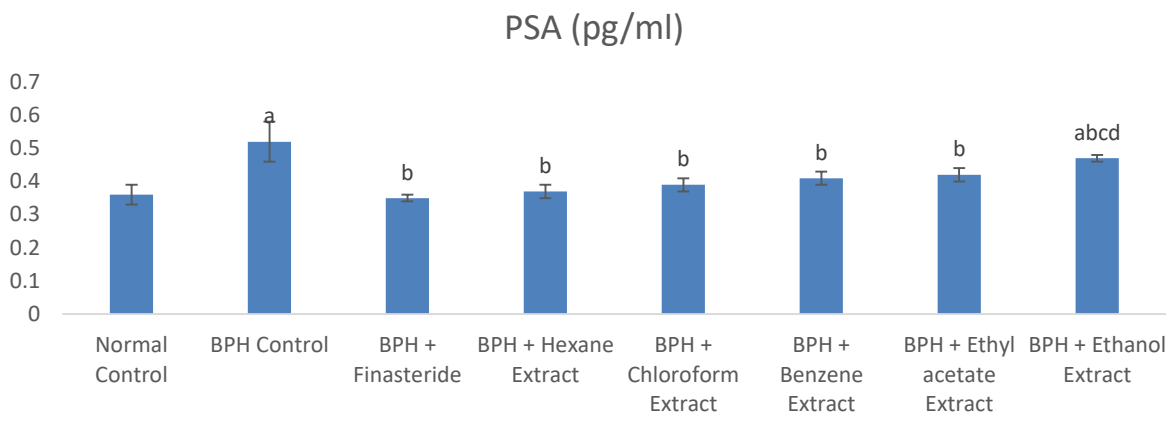
Effects of Serial Extracts of *Solanum aculeastrum* Seeds on Serum PSA Concentration

The results of the effects of serial extracts of *Solanum aculeastrum* seeds on serum PSA concentration of testosterone propionate induced BPH in male Wistar rats are presented in Figure 2. The results show that induction of BPH significantly ($p < 0.05$) increased the serum PSA levels compared to the normal control. In contrast, there were significant decreases in serum PSA levels of all the *Solanum aculeastrum* extracts and finasteride treatment groups compared to the BPH control. However, only the ethanol extract treated group was significant when compared to the finasteride treated group.

Effects of Serial Extracts of *Solanum aculeastrum* Seeds on Serum 5-alpha Reductase and Dihydrotestosterone Concentration of Testosterone Propionate Induced BPH in Male Wistar Rats

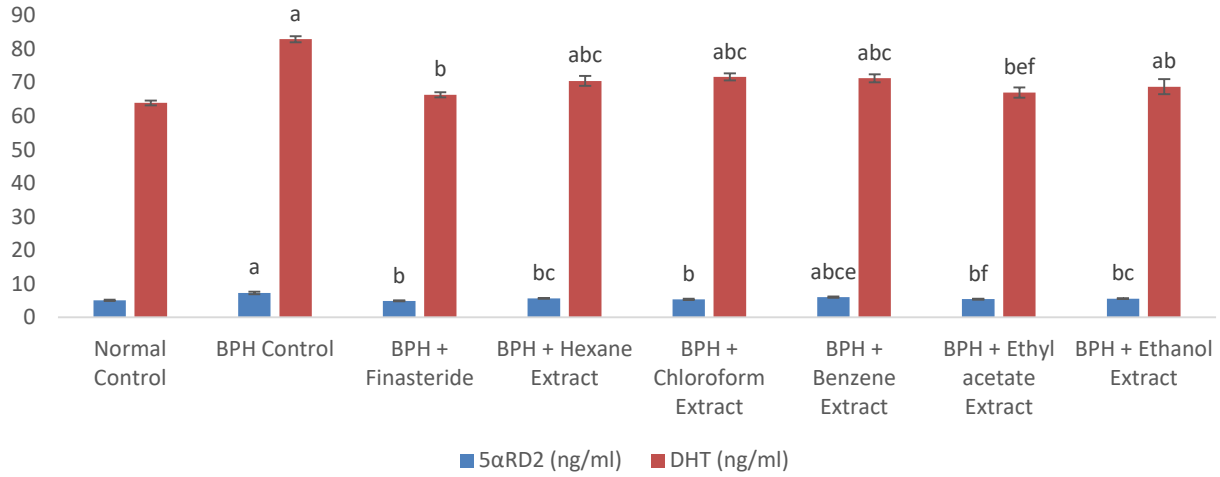
The results of the effects of serial extracts of *Solanum aculeastrum* seeds on serum 5-alpha reductase and dihydrotestosterone concentration of testosterone propionate induced BPH in male Wistar rats are presented in Figure 2. The results show that induction of BPH significant ($p < 0.05$) increased the serum 5αRD2 and DHT levels compared to the normal control. In contrast, there were significant decreases in serum 5αRD2 and DHT levels of all the *Solanum aculeastrum* extracts and finasteride treatment groups compared to the BPH control. However, only hexane, chloroform and benzene extracts treated groups showed significant decrease when compared with the finasteride treated group.

Figure 1: Effects of serial extracts of *Solanum aculeastrum* dunal seeds on serum PSA concentration of testosterone propionate induced BPH in male Wistar rats.



Values are expressed as Mean ± SEM, n = 6; a = $p < 0.05$ (Test groups compared with normal control); b = $p < 0.05$ (Groups 3, 4, 5, 6, 7 and 8 compared with group 2); c = $p < 0.05$ (Groups 4, 5, 6, 7 and 8 compared with group 3); d = $p < 0.05$ (Test groups compared with group 4); e = $p < 0.05$ (Test groups compared with group 5); f = $p < 0.05$ (Test groups compared with group 6); g = $p < 0.05$ (Test groups compared with group 7)

Figure 2: Effects of serial extracts of *Solanum aculeastrum* dunal seeds on serum 5-alpha reductase and dihydrotestosterone concentration of testosterone propionate induced BPH in male Wistar rats.



Values are expressed as Mean ± SEM, n = 6; a = $p < 0.05$ (Test groups compared with normal control); b = $p < 0.05$ (Groups 3, 4, 5, 6, 7 and 8 compared with group 2); c = $p < 0.05$ (Groups 4, 5, 6, 7 and 8 compared with group 3); d = $p < 0.05$ (Test groups compared with group 4); e = $p < 0.05$ (Test groups compared with group 5); f = $p < 0.05$ (Test groups compared with group 6); g = $p < 0.05$ (Test groups compared with group 7)

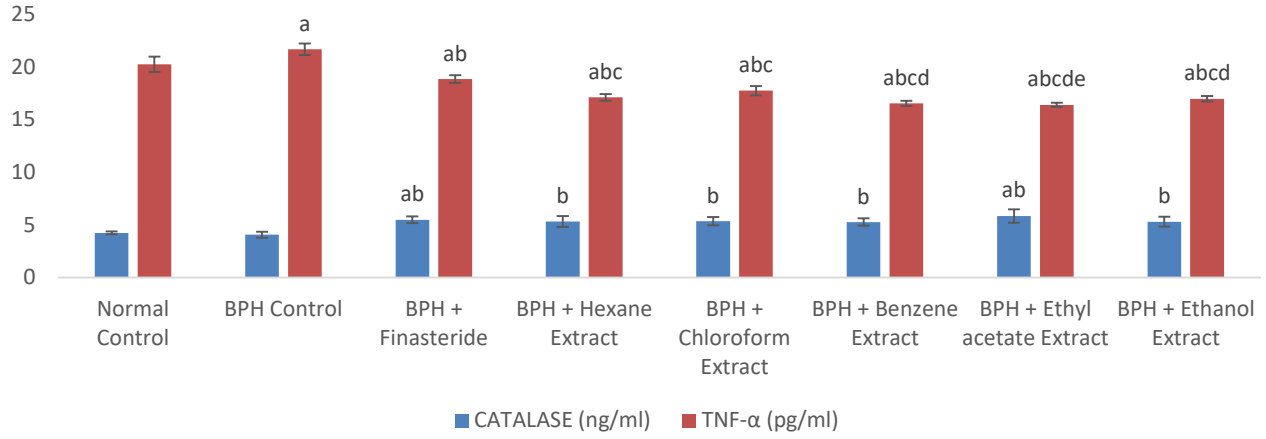
Effects of serial extract of *Solanum aculeastrum* seeds on serum TNF-α conentration of testosterone propionate induced BPH in male Wistar rats

The results of the effects of serial extracts of *Solanum aculeastrum* seeds on serum TNF-α concentration of testosterone propionate induced BPH in male Wistar rats are presented in figure 3. The results show that induction of BPH in rats caused a significant ($p < 0.05$) increase in serum TNF-α concentration compared to the normal control. There was a significant decrease in serum TNF-α level in the finasteride treated group compared to both the BPH control and normal control groups. In a similar manner, there was a significant decrease in serum TNF-α level in all the *Solanum acculeastrum* extracts treated groups compared to the BPH and normal control groups. All the *Solanum aculeastrum* extracts treated groups compared significantly with the finasteride (standard drug) treated group hence, could be adjudge to have anti-TNF-α activity.

Effects of serial extract of *Solanum aculeastrum* seeds on serum catalase activity of testosterone propionate induced BPH in male Wistar rats

The results of the effects of serial extracts of *Solanum aculeastrum* seeds on serum catalase activity of testosterone propionate induced BPH in male Wistar rats are presented in figure 3. The results show that BPH induction in rats resulted in a non-significant ($p < 0.05$) decrease in serum catalase activity compared to the normal control. There was however a significant increase in catalase activity in the finasteride treated group compared to both the BPH control and normal control groups. Interestingly, there was a significant increase in catalase activity in all the *Solanum acculeastrum* treatment groups compared to the BPH control; but not significant when compared to the finasteride treated group.

Figure 3: Effects of serial extracts of *Solanum aculeastrum* dunal seeds on serum catalase and TNF-α levels of testosterone propionate induced BPH in male Wistar rats.



Values are expressed as Mean ± SEM, n = 6; a = $p < 0.05$ (Test groups compared with normal control); b = $p < 0.05$ (Groups 3, 4, 5, 6, 7 and 8 compared with group 2); c = $p < 0.05$ (Groups 4, 5, 6, 7 and 8 compared with group 3); d = $p < 0.05$ (Test groups compared with group 4); e = $p < 0.05$ (Test groups compared with group 5); f = $p < 0.05$ (Test groups compared with group 6); g = $p < 0.05$ (Test groups compared with group 7).

IV. DISCUSSION

Over the last few years, plant phytochemicals have gained extensive attention because their constituents are believed to have numerous therapeutic activities such as anti-HIV, anti-plasmodial, anti-diarrheal, anti-septic, anti-bacterial, anti-viral, anti-inflammatory, anti-microbial, hypoglycemic, antioxidant, analgesic and hepatoprotective properties as well as other physiological activities (Sofowora, 1993; Cushnie and Lamb, 2005; Ebana *et al*., 2005; Evans, 2005). They exhibit structure related biochemical and pharmacological actions capable of reducing the risk of multiple diseases (Savage, 1993; Karimi *et al*., 2013). Their effectiveness depends on the synergicity and phytochemical load (Engwa *et al*., 2013) and the yield depends on the effectiveness of the extraction method.

Solanum aculeastrum is one of the plants reported to contain many of these bioactive phytochemical compounds with a high medicinal and nutritional values (Wanyonyi *et al*., 2003; Laban *et al*., 2015). The fruits, both matured and immatured, contain the alkaloid solanine (Hutchings *et al*., 1996). The leaves and berries of *Solanum aculeastrum* contain mainly straight-chain aliphatic hydrocarbons (Koduru *et al*., 2006a). Ethnobotanical survey revealed that the berries are used in the treatment of breast cancer (Koduru *et al*., 2006a; Koduru *et al*., 2007a). Methanol and aqueous extracts of the berries have been shown to have moderate antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aureginosa* and *Bacillus subtilis* bacteria (Wanyonyi *et al*., 2002; Wanyonyi *et al*., 2003; Wabwoba *et al*., 2010). However, the therapeutic properties of *Solanum aculeastrum* for BPH seeds have not been widely reported. In this study, we focused on serial extracts of air dried *Solanum aculeastrum* seeds obtain from the wild to examine their effects as anti-BPH agent against testosterone propionate induced BPH.

The present study shows that induction of BPH resulted in body and organ weights loss. Treatment with *Solanum aculeastrum* seeds extracts and finasteride resulted in marked gain in body and organ weights (Table 2). The extract seemed to have stimulated increase in appetite and feeding efficiency ratio which appeared to have been suppressed during BHP induction. This shows that the BPH induction protocol in the experimental model was successful because relative prostate weight loss is a common indicator of BPH development in experimental animals (Jeon *et al*., 2017). The prostate gland weight and prostatic index (PI) were significantly decreased in the finasteride control and *Solanum aculeastrum* treated groups compared to BPH control. This indicates that the extract might have caused a marked decrease in prostate weight of BPH induced rats comparative to the orthodox drug. Cho *et al*., (2010); Iweala and Ogidipo, (2015b) and Maryam *et al*., (2016) concluded that decrease in prostate weight and prostatic index are indicative of BPH amelioration. Therefore *Solanum aculeastrum* may be effective for treatment of prostate enlargement.

Serum PSA correlates with prostate volume and is a reliable marker for BPH and prostate cancer. It is and usually elevated in prostate disorders. A decrease in PSA is associated with reduced prostate hyperplasia as a direct consequence of 5α-reductase inhibition or anti-BPH actions (*Sing et al*., 1991; Afriyie *et al*., 2014). Dihydrotestosterone (DHT) has an important role in the development of BPH. Testosterone, the precursor of DHT, is synthesized in the testes and adrenal glands, and is converted to DHT via the action of the enzyme, 5-alpha reductase which is mainly present in prostate, epididymis, hair follicle, and liver tissue. DHT has substantially greater affinity for androgens receptors (AR) than testosterone does, and binding of DHT to AR in the prostate results in the production of proteins such as PSA as well as regulatory proteins that induce cell proliferation, resulting in BPH (Park *et al*., 2013). DHT is important for the development of the prostate. However, it is also responsible for the pathologic growth of the prostate. DHT binds to androgen receptors with subsequent modulation of target genes causing BPH and its related cancer (Bartsch *et al*., (2002). To arrest BPH and the further development of cancer, 5-α-reductase inhibitors are administered to act as pathologic substrates of the disease, thereby arresting the disease, reducing the prostate volume, and improving symptoms (Andriole *et al*., 2004).

In this study, the results (figures 1 and 2) show that induction of BPH significant ($p < 0.05$) increased the serum PSA, 5αRD2 and DHT levels compared to the normal control. In contrast, there were significant decreases in serum PSA, 5αRD2 and DHT levels of all the *Solanum aculeastrum* extracts and finasteride treatment groups compared to the BPH control. These observed decreases in serum PSA, 5αRD2 and DHT levels in the *Solanum aculeastrum* extracts treated groups were however not significant compared with the finasteride treated group.

Solanum aculeastrum is rich in a wide variety of phytochemicals - saponins, steroids, alkaloids, flavonoids, polyphenols and phenols which may be responsible for the reduction in PSA level (Sanchez-Mata *et al*., 2010; Halinski *et al*., 2012). Phytochemicals reported to cause a significant reduction in serum PSA levels constitute a class of phytoestrogens which are biologically active compounds with weak estrogenic or anti-estrogenic, antioxidant, and antitumor properties, mediated through inhibition of growth signal factors and modulation of enzymes involved in the hormone metabolism that can influence prostate metabolism (Koduru *et al*., 2006b; Iweala and Ogidipo, 2015; Ishola, 2018). These findings further buttresses that the *Solanum acculeastrum* seed extracts might possess anti-BPH properties.

Studies on plant extracts have reported that changes in SRD5a2, DHT, and PSA can be used as indicators of BPH status (Kim *et al*., 2017). It is clear that treatment with finasteride results in a decrease in DHT but not testosterone. Mc Connell *et al*. (1998) reported that decrease in serum DHT concentration relieves the symptoms of BPH by increasing urine flow and decreasing prostatic volume. The mechanism is through stimulation of androgen receptors within the prostatic stromal cells (Forley and Kirby, 2003). These results were consistent with our earlier observations showing lower prostate weights, thus strongly buttressing that the *Solanum acculeastrum* seed extracts might possess anti-BPH properties.

Tumour necrosis factor (TNF-α) is a key proinflammatory cytokine rapidly released after inflammatory stimuli. It prompts several intracellular events that results in the activation of the transcription nuclear factor kappa β (NF-κ β), leading to the production of other proinflammatory cytokines, chemokines, and proteases (Choy and Panayi, 2001; Scott and Kingsley, 2006). The overproduction of this cytokine is associated with different inflammatory diseases (Sethi *et al*., 2008). Therefore, TNF-α is considered a valid target for the development of new drugs to treat chronic inflammatory diseases (Henriques *et al*., 2016).

The results of the present study (figure 3) show that induction of BPH in rats caused a significant ($p < 0.05$) increase in serum TNF-α concentration compared to the normal control. There was a significant decrease in serum TNF-α level in the finasteride treated group compared to both the BPH control and normal control groups. In a similar manner, there was a significant decrease in serum TNF-α level in all the *Solanum acculeastrum* treated groups compared to the BPH and normal control groups. All the *Solanum aculeastrum* extracts treated groups compared significantly with the finasteride (standard drug) treated group. This suggests that all the five extracts may possess anti-TNF-α activity. Phytochemicals like flavonoids, terpenoids, alkaloids, and phytosterols have been reported to inhibit the upstream signaling molecules that are involved in TNF-α expression (Verma *et al*., 2012b; Iqbal *et al*., 2013). The mechanism involves a decrease in the expression of genes that code the expression of proinflammatory cytokines, including TNF-α and IL-1β or inhibition of inflammatory mediators such as cyclooxygenase isoenzymes COX-1 and COX-2 (Crouvezier *et al*., 2001; Wang *et al*., 2014). Moreover, some natural products with TNF-α inhibiting property also exhibit antioxidative stress activity (Zhang *et al*., 2012). These findings suggest that the *Solanum acculeastrum* seed extracts might possess anti-BPH properties.

Oxidative stress has been considered to be one of the mechanisms that trigger the chain of reactions involved in the development and progression of BPH (Iweaala and Ogidipo, 2015; Eleazu *et al*., 2017a). It is defined as an imbalance between prooxidant and antioxidant factors that can lead to the generation of reactive oxygen species (ROS) and electrophiles with potential cellular and tissue damage (Eleazu *et al*., 2013; Eleazu *et al*., 2017b). The cause of oxidative stress could be the overproduction of free radicals or decrease in the activities of free radical scavenging enzymes like superoxide dismutase (SOD), glutathione-s-transferase (GST), glutathione peroxidase (GPx), catalase (CAT) or both. This is especially true as the human prostate tissue is vulnerable to oxidative DNA damage due to more rapid cell turnover and fewer DNA repair enzymes (Minciullo *et al*., 2015). Levels of antioxidants in the prostatic tissue are significantly decreased in prostatic hyperplasia (Udensi and Paul, 2016).

In the present study, the results (figure 3) show that BPH induction in rats resulted in a non-significant ($p < 0.05$) decrease in serum catalase activity compared to the normal control. There was however a significant increase in catalase activity in the finasteride treated group compared to both the BPH control and normal control groups. Interestingly, there was a significant increase in catalase activity in all the *Solanum acculeastrum* treatment groups compared to the BPH control; but not significant when compared to the finasteride treated group. These suggest that the *Solanum acculeastrum* seed extracts might possess antioxidant properties. Catalase is a very important enzyme that protects the cell from oxidative damage by ROS (Chelikani *et al*., 2004). These enzymes work in synergy to counteract the deleterious effect of free radicals. The antioxidant properties of *Solanum acculeastrum* seed extracts may be due to the presence of flavonoids, a polyphenol which have been shown to play greater roles in the prevention of oxidative stress through mechanisms such as: inhibition of redox-sensitive transcription factors, down-regulation of pro-oxidant enzymes, induction of Phase II enzymes and directly or indirectly strengthening the degradation of misfolded and damaged proteins (Frei and Higdon, 2003; Siddiqui *et al*., 2008; Eleazu *et al*., 2017b; Hajieva, 2017).

V. CONCLUSION

The findings of this study revealed that induction of BPH caused a significant decrease in body, liver, kidney and prostate weights as well as prostatic index and catalase activity. In contrast, the serum concentrations of PSA, 5αRD2, DHT and TNF-α were significantly increased. However, these untoward effects were significantly attenuated by all the five serial extracts of *Solanum acculeastrum* seedscomparative with the standard drug, finasteride. Therefore, our findings suggest that the extracts may be safe for use in the management of BPH. These beneficial effects of the extract justified its use in traditional medical practice in treatment of spleenomegaly and other related cases. However, further studies on dose dependent effects of each extract should be conducted. Investigations on the hepatotoxicity, nephrotoxicity and genotoxicity of the extracts are also recommended.

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The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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