Assessment of Lipid Profile of Testosterone and Estradiol induced Benign Prostatic Hyperplasia in Adult Male Rats on Administration of *Vernonia amygdalina*

*[1]Ugwu Melvin Nnaemeka, [1]Asuk, Atamgba Agbor and [2]Eteng, Mbeh Ubana

[1]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Cross River University of Technology, Calabar, Nigeria.
[2]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar Nigeria.
Email: melvincrux@yahoo.com

ABSTRACT

Benign prostatic hyperplasia (BPH) is the most common benign neoplasm of men. The stromal and, to a lesser degree, epithelial cells of the prostate become hyperplastic, causing the prostate to enlarge. Plant extracts belong to the most popular drugs in the medical management of lower urinary tract symptoms (LUTS) due to benign prostatic hyperplasia/benign prostatic enlargement. We investigated the effect aqueous extract of *Vernonia amygdalina* (VA) on lipid profile of BPH. BPH was induced in male rats weighing 200-350g through exogenous administration of testosterone and estradiol by subcutaneous injection. A total of 30 rats were divided into five groups. One group was used as a control and the other groups received subcutaneous injections of the hormones for 3 weeks to induce BPH. Groups 1 and 2 were treated with different doses of VA extracts and group 3 received finasteride, while group 4 was left untreated, group 5 served as normal control. After forty-five days of treatment with VA extract, the rats were sacrificed. Blood was collected by cardiac puncture and the sera centrifuged and used for the determination of lipid profile. The prostates were harvested and weighed. Administration of the extract caused a significant reduction in the size of the enlarged prostate (p<0.05) when compared with the BPH control group. There was significant reduction in the levels of triacylglycerol, cholesterol, low density lipoprotein cholesterol LDL-C), very low density lipoprotein cholesterol (VLDL-C) in the treated groups when compared to the BPH control. There was significant increase in HDL concentration in all treated groups when compared to BPH control group. The significant reduction of cholesterol, triacylglycerol, LDL, VLDL and increase in HDL indicates that hypolipidemic potential of VA might help in the management of complications that might arise due to increase in lipid of BPH patients. Furthermore, reduction of prostate weight in the treated groups suggests that the extract can be useful in the management of BPH.
Keywords: Benign prostatic hyperplasia, Lipid Profile, Wistar rat, finasteride, *Vernonia amygdalina*

INTRODUCTION

Benign prostatic hyperplasia (BPH) is the most common urological condition among elderly males, affecting approximately half of men over 80 years of age. It usually begins as a simple micronodular hyperplasia with a subsequent macroscopic nodular enlargement that may result in bladder outlet obstruction and the development of lower urinary tract symptoms (LUTS) [1], [2]. Macroscopic benign prostatic hyperplasia represents the enlargement of the

prostate arising from the stromal and epithelial proliferation; the main symptoms were lower urinary tract symptoms (LUTS); urodynamic manifestations and bladder outlet obstruction (BOO) [3], [4]. LUTS refers to various abnormal manifestations in the urination cycle caused by changes in the structure and function of the lower urinary tract including storage symptoms, voiding symptoms, post micturition symptom [5]. LUTS is most generally correlated with an increasing incidence of BOO. Many studies have shown that lower urinary tract symptoms (LUTS) are also associated with BPH [6]. Relevant epidemiological data shows that BPH may be closely related to metabolic disorders and cardiovascular and cerebrovascular diseases [7].

In males, aging, health and disease are processes that occur over physiologic time and involve a cascade of hormonal, biochemical and physiological changes that accompany the down-regulation of the hypothalamic-anterior pituitary-testicular axis [8]. As aging progresses there are relative increases of body fat and decreases in muscle mass. The increased adipose tissue mass is associated with the production of a number of newly generated factors. These include aromatase, leptin, PAI-1, insulin resistance, and the dyslipidemias, all of which can lead to tissue damage. The increase in adipose tissue is associated with an increase in the enzyme aromatase that converts testosterone to estradiol and leads to diminished testosterone levels that favor the preferential deposition of visceral fat [9]. The progressive insulin resistance leads to a high triglyceride-low HDL pattern of dyslipidemia and increased cardiovascular risk.

Obesity a type of metabolic disorder increases cardiovascular risk through multiple mechanisms. In obesity, abdominal (visceral) adiposity is metabolically active and is largely responsible for the atherogenic dyslipidemia, hyperinsulinemia, hypertension, chronic inflammatory state, and prothrombotic state that constitute the metabolic syndrome [9]. Obesity resulting in a disruption of insulin and lipid metabolic pathways gives rise to metainflammation, targeting critical organs and adversely affecting homeostasis. Obesity acts as a precursor to the metabolic syndrome which is a crucial stage to many an ailments exhibiting inflammation and has been contributory in disorders like arthritis, cancer, benign prostatic hyperplasia, cardiovascular diseases (CVD), asthma, and Alzheimer's disease due to excessive and prolonged inflammatory responses [10], [11].

Obese men produce more oestradiol and oestrone than nonobese men through transformation of adrenal androstenodione in adipose tissue, a transformation that results in higher endogenous oestrogen levels [12]. [13] also reported that the degree of obesity appeared to have a direct effect on oestradiol levels through transformation of androgens in adipose tissue to oestrogens.

Obesity suggests higher stores of adipose tissue as a source of cholesterol and triglycerides [14]; therefore, a disturbed lipid profile may be seen in the patients of prostate cancer and BPH. Obesity is a morbid disorder associated with high serum lipid levels including total cholesterol, triglyceride, low-density lipids and reduced high-density lipid [15]. Animal studies indicated that the serum triglyceride, cholesterol concentration in obese dogs was significantly higher than in control dogs [16], [17]. Many studies have shown an association of dyslipedimia in BPH [18] and prostate cancer [19], [20]. The biologic effect of obesity on the prostate is complex. Lower body mass index is associated with higher serum testosterone levels whereas oestrogen levels are higher in obesity. Since obese men have higher serum levels and greater productions of oestrone and oestradiol than nonobese men do, more rapid development of BPH might be

62

expected among them. It has been established that dihydrotestosterone which is metabolic product of testosterone is the principal androgen responsible for both normal and hyperplastic growth of the prostate gland [21].

In addition, obesity, particularly abdominal obesity, may increase risk for BPH, presumably due to resultant hyperinsulinemia [22], [23]. Elevated levels of estrogens secondary to conversion from testosterone in adipose tissues may also play a role.

Conventional drug treatment regimens for BPH patients are mainly alpha-receptor blockers and 5-alpha-reductase inhibitors. Alpha -receptor blockers can improve bladder storage symptoms. However, there are still many patients who need surgical treatment. It is particularly important to explore the preventive measures of BPH.

Plants have been the companions of man since time immemorial and formed the basis of useful drugs since they are less toxic than synthetic drugs. *Vernonia amygdalina* (VA) is a shrub that grows predominantly in the tropical Africa. Leaves from this plant serve as food vegetable and culinary herb in soup [24], [25]. In Nigerian herbal homes, extracts of the plant are used as tonic, in the control of tick and treatment of cough, feverish condition, constipation and hypertension [26], [27], [28]. Phytochemical screening of VA revealed the presence of saponins, sesquiterpene, and flavonoids [29]. Strong antioxidant activities have been reported for flavonoids from VA and, its saponins have been reported to elicit antitumoral activities in leukemia cells [30]. Peptides from VA are known to be potent inhibitor of mitogen-activated proteins kinases, which are crucial for breast tumor growth and also represents a key regulatory point for the tumour [31], [32]. Hence the need to study the effect this plant on BPH.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Vernonia amygdalina* was harvested from a garden in Okuku in Yala Local Government of Cross river State, South-South, Nigeria. The plant was identified at the herbarium unit of the Department of Biological Sciences, University of Calabar. The fresh leaves were washed with clean water and dried under the shade for six days. The dried leaves were milled using pestle and mortar to get a powder that was used for extraction.

Preparation of extracts

The powered sample of *Vernonia amygdalina,* 100 g was soaked into 100 mL of distilled water, this was filtered after 48 h and the filtrate was concentrated in water bath. The extract was diluted with corn oil, to produce a solution 100 mg/ml. The administration of extract was totally by gavage via oral intubation tube. Proper concentrations were administered by the use of oropharyngeal canula and calibrated hypodermic syringe.

Hormones

Testosterone propionate Brand name: Ricostrone; a product of Greenfield pharma, Jiangsu Co Ltd., China. Estradiol valerate (by Medipharm Ltd., 108-Kotlakhpat industrial Est; Lahore, India. Testosterone propionate (T) and estradiol valerate E 2 (puregynon depot) were used for the induction of prostate enlargement at a dose of 400 μg T and 80 μg E2 [33]. This was administered to the rats for three weeks subcutaneously in the inguinal region after which a few rats were sacrificed and inspected for gross examination of prostate enlargement. All Chemicals used in this study were of analytical grade and were obtained from reputable companies.

Animals

A total of thirty (30) Wistar rats weighing between 200-350 g were obtained from the animal house of the Faculty of Basic Medical Sciences, University of Calabar, Nigeria. The rats were used for the experiment. The rats were acclimatized for two weeks before the experiment

commences. The rats were exposed to approximately 12-hour light/dark cycles under humid tropical conditions, given tap water and feed *ad libitum*, and were housed in standard plastic cages (five per cage) throughout the duration of the study. The animal room was well ventilated with a temperature range of 27-29°C. The Institutional Animal Ethics Committee approved the study before the experiment and certified all experimental protocols.

Induction of BPH
BPH was induced by exogenous administration of testosterone and estradiol in staggered doses (three times a week respectively) for three weeks according to Bernoulli, [34] with modification by [35].

Animal grouping and treatment
The animals were divided into five (5) groups each comprised of six (6) male rats. Four groups were induced with BPH which were grouped as group 1 to group 4). Groups 1 and 2 received 50 and 100 mg kg⁻¹ body weight (bw) of *Vernonia amygdalina* extract; group 3 received finasteride (orthodox drug) at 0.1 mg kg⁻¹; all by gavages for forty five days, group 4 was left untreated for forty five days before sacrifice to assess possible reversal of the exogenous induction and group 5 served as normal control. The animals were weighed prior to the commencement of the experiment and subsequently every week till the end of the experiment. The fluid and water intake were taken daily till the end of the experiment.

Determinations of Biochemical Parameters
After 45 days, the rats were anaesthetised by a brief exposure to trichloromethane vapour and bled by cardiac puncture. The sera were carefully separated and used for the determination of various biochemical analyses. Each rat's carcass was promptly dissected and the prostates were carefully excised. Two prostates per group were randomly selected and their dorso-lateral lobes were dissected out and immediately processed for histology. The other three prostates pre group were freed of external fascias, washed in cold normal saline, blotted with filter paper and weighed on a sensitive balance.

Determination of total cholesterol, High density lipoprotein (HDL-cholesterol) and Triacylglycerol determination
Determination of total cholesterol, high density lipoprotein (CHOD-PAP method) as described by the National Cholesterol Education Program (NCEP) [36] while Triacylglycerol (GPO-PAP method) as described by Tietz [37]. Low density lipoprotein was determined as described by Assmann [38]. All were done using Randox assay kits.

Statistical Analysis
The experimental data were analysed for statistical significance by one-way analysis of variance and post hoc comparison using the SPSS version. All data were reported as mean ± SD and statistical significance was accepted at $P < 0.05$.

RESULTS

Weekly Body Weight
The BPH-control group exhibited a decline in body weight by 19% (270.4 g) when compared with normal control (without BPH, 322.2 g) and there was a declined appetite after three weeks of BPH induction. The 50 mg VA, 100 mg of VA and finasteride exhibited 1.45% (317.60), 0.19% (321.60) and 0.56% (320.40) decline in weight respectively when compared to the normal control, reaching the weight close to the normal control group (322.2 g). Finasteride is used as standard drug control. The administration of extract or standard drug (finasteride) improved the body weight of animals treated induced with BPH bringing it near the weight of normal control level.

Prostate Weight
The average weight of the prostates was 2.21 g in the animal treated with BPH control group which increased 5.39 times more compared with normal control group with weight of 0.41 g. Therefore,

BPH control group showed a significant ($P<0.05$) enhancement in prostate weight when compared to normal control (Table 1). The animals treated with VA extract groups using 50 and 100 mg of VA showed a decrease in prostate weight by 0.83 and 0.72 g respectively, when compared with the BPH control group (2.21 g). Administration of VA extract or standard drug (finasteride) reduced partially the prostate weight to near normal. The animals groups treated with Finasteride served as standard drug control.

Effect of extract on serum cholesterol concentration of BPH-induced rats

Serum cholesterol concentrations (in mg/dl) were 180.92±21.81 for BPH control, 136.88±5.89 for normal control, 147.14±2.71 for 50mg VA, 145.69±14.72 for 100mg VA, and 160.00±7.93 for finasteride. There was a significant ($P < 0.05$) rise in the serum cholesterol level in BPH control group when compared with the treated groups. In all the treated groups there was an improved reduction of serum cholesterol levels when compared to the BPH control group.

Effect of extract on serum triacylglycerol (TG) concentrations of BPH-induced rats

Serum TG concentrations (in mg/dl) were 82.13±5.42 for BPH control group, 67.38±1.62 for normal control, 74.38±5.14 for finasteride, 74.23±1.93 for 50 mg VA and 72.75±2.00 for 100 mg VA. The results indicate that there was no significant reduction in the TG concentrations in the PA treated groups.

Effect of extract on serum high density lipoprotein (HDL-c) concentrations of BPH induced rats

Serum HDL-c concentrations (in mg/dl) were 49.80±1.76 for BPH control, 53.33±1.64 for normal control, 53.33±2.57 for 50mg VA, 52.24±2.69 for 100mg VA and 50.75±1.57 for finasteride. All groups treated were statistically similar with normal control.

Effect of extract on serum low density lipoprotein (LDL-c) concentrations of BPH induced rats

Serum LDL-c concentrations (in mg/dl) were 22.27±12.35 for BPH control, 7.35±2.69 for normal control, 8.90±2.54 for 50 mg VA, 9.41±6.59 for 100mg VA and 15.85±3.59 for finasteride. There was a significant ($P< 0.05$) increase in LDL-C level of BPH control group when compared with the treated groups. The administration of extract showed a significant decline in the levels of LDL concentrations when compared to the BPH control.

Effect of extract on serum very low density lipoprotein (VLDL-c) concentrations of BPH induced rats

Serum VLDL-c concentrations (in mg/dl) were 16.43±1.08 for BPH control, 13.48±0.32 for normal control, 14.85±0.39 for 50 mg VA, 14.55±0.40 for 100mg VA and 14.88±1.03 for finasteride. There was a significant ($P< 0.05$) increase in VLDL-C level of BPH control group when compared with the treated groups. The administration of extract showed a significant decline in the levels of VLDL concentrations when compared to the BPH control.

Table 1: Effect of extract of VA and finasteride body weight, prostate weight and protein content of prostate

GROUP	BODY WEIGHT (g)	PROSTATE WEIGHT (g)
BPH + 50mg VA	317.60±15.27[b]	0.83±0.52[ab]
BPH + 100mg VA	321.60±5.68[c]	0.72±0.36[ab]
BPH + FINASTERIDE	320.40±8.99[c]	0.63±0.23[ab]
BPH CONTROL	270.40±8.93[a]	2.21±0.28[c]
NORMAL CONTROL	322.20±13.99[c]	0.41±0.07[a]

Values are expressed as Mean ± SD. Benign prostate hyperplasia (BPH), *Vernonia amygdalina* (VA), body weight (BW) and prostate weight (PW). Identical superscript (i.e. a) means there is no significant difference between the comparing group P>0.05. Non- identical superscripts (i.e. a, b, c) means there is significance between the comparing groups at P < 0.05.

Table 2: Effect of extracts of VA and finasteride on serum lipid profile

GROUP	CHOLESTEROL (mg/dl)	TRIACYL-GLYCEROL (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
BPH + 50mg VA	147.14 ± 2.71^{abc}	74.23 ± 1.93^{b}	53.33 ± 2.57^{a}	8.90 ± 2.54^{ab}	14.85 ± 0.39^{b}
BPH + 100mg VA	145.69 ± 14.72^{ab}	72.75 ± 2.00^{b}	52.24 ± 2.69^{a}	9.41 ± 6.59^{ab}	14.55 ± 0.40^{b}
BPH + FINASTERIDE	160.00 ± 7.93^{cd}	74.38 ± 5.14^{b}	50.75 ± 1.57^{a}	15.85 ± 3.59^{bc}	14.88 ± 1.03^{b}
BPH CONTROL	180.92 ± 21.81^{e}	82.13 ± 5.42^{c}	32.10 ± 1.10^{b}	22.27 ± 12.35^{c}	16.43 ± 1.08^{c}
NORMAL CONTROL	136.88 ± 5.89^{a}	67.38 ± 1.62^{a}	53.33 ± 1.64^{a}	7.35 ± 2.69^{a}	13.48 ± 0.32^{a}

Values are expressed as Mean ± SD. Benign prostate hyperplasia (BPH), High density lipoprotein (HDL), Low density lipoprotein (LDL), Very low-density lipoprotein (VLDL), *Vernonia amygdalina* (VA). Identical superscript (i.e. a) means there is no significant difference between the comparing group *P*>0.05. Non-identical superscripts (i.e. a, b, c, d, e) means there is significance between the comparing groups at *P* < 0.05.

DISCUSSION

Benign prostatic hyperplasia is a common disease in elderly men, with lower urinary tract symptoms (LUTS) caused by hyperplasia of the prostatic epithelium and stromal cells [39]. At present, the main therapeutic drugs for BPH are 5α-reductase inhibitors and α-blockers [40], [41], such as finasteride and terazosin. However, they can cause adverse reactions such as fatigue, hypotension, ejaculation disorders, sexual dysfunction, and an increased risk of prostate fibrosis [42], [43]. Surgical treatment of BPH requires strict surgical indications and carries with it the inevitable complications and risks of surgery and the possibility of recurrence. In fact, the number of patients undergoing surgery for BPH is gradually decreasing [44]. Therefore, it is particularly important to find a therapeutic drug that can effectively treat BPH with few adverse reactions.

The use of alternative therapy in BPH management is an ancient practice. In most developing economies, particularly in Africa, alternative therapy is the predominant mode of managing BPH because of the public belief in the efficacy of herbal therapeutic agents [45], [46].

In this study it was observed that there were increase in levels of cholesterol, triacylglcerol, low density lipoprotein and very low-density lipoprotein and decrease in high density lipoprotein in BPH control group signifies that lipid profile indices are affected in BPH disorder. But administration of the extract was capable of ameliorating this observed alteration in lipid profile.

Presence of dyslipidemia in the BPH patients is a frequently noted condition under clinical setups [47]. High level of total cholesterol, LDL-cholesterol, triglyceride, decreased level of HDL-cholesterol increases the risk of BPH, and cholesterol-lowering medication may reduce the risk [48]. [49] compared FA profiles in the serum of patients with prostate cancer and BPH and proposed that polyunsaturated FAs have certain relation with BPH and prostate cancer. Higher serum LDL is associated with greater risk of BPH [50], and physical activity, which is known to decrease the serum lipid level is associated with the decreased risk for BPH [51]. Hyperlipidemia is closely associated with the obesity, higher body mass index (BMI), and these parameters show a positive correlation with the BPH [52], [53]. [50] reported that the patients with more BMI tend to have larger prostate volume and higher International Prostate Symptom Score. Several studies indicate that obesity and sedentary lifestyle substantially increases the risk for BPH [20], [48], [49], [51]. Some studies have investigated the effect of plants extracts on lipid profile of experimentally induced BPH rats' models [41], [52], [53].

Reduction of the lipid levels of the body is critical in facilitating treatment process in BPH condition. The findings from this study shows that *Vernonia amygdalina* has the potential of regulating the metabolism of lipid which seems to be significant in the management of benign prostate hyperplasia.

REFERENCES

1. Briganti, A., Capitanio, U., Suardi, N., Gallina, A., Salonia, A. and Bianchi, M. (2009). Benign prostatic hyperplasia and its aetiologies. *Eur Urol Suppl.*, 8:865–71.
2. Rył, A., Rotter, I., Miazgowski, T., Słojewski, M., Dołęgowska, B., Lubkowska, A. and Laszczyńska, M. (2015). Metabolic syndrome and benign prostatic hyperplasia: association or coincidence? *Diabetol Metab Syndr.*, 7:94.
3. Yang, X., Zhang, Q., Jiang, G., Liu, J., Xie, C., Cui, S. and Wu, T. (2019). The effects of statins on benign prostatic hyperplasia and the lower urinary tract symptoms A Meta-analysis. *Medicine*, 98:18.
4. Abrams, P. (1999). LUTS, BPH, BPE, BPO: a plea for the logical use of correct terms. *Rev Urol.,* 1: 65.
5. Abrams, P., Cardozo, L. and Fall, M. (2002). The standardisation of terminology in lower urinary tract function: report from the standardisation subcommittee of the International Continence Society. *Neurourol Urodyn.,* 21:167–78.
6. Mancini, V., Balzarro, M., Illiano, E (2008). Lower urinary tract symptoms in elderly men: a simple yet comprehensive approach. *J Gerontol Geriatr.*, 66:245–52.
7. Ozden, C., Ozdal, O.L. and Urgancioglu, G. (2007). The correlation between metabolic syndrome and prostatic growth in patients with benign prostatic hyperplasia. *Eur Urol.,* 51: 199–203.
8. Cohen, P.G. (2001). Aromatase, adiposity, aging and disease. The hypogonadal-metabolic-atherogenic-disease and aging connection. *Med. Hypotheses,* 56(6):702-8.
9. Miller, W.M., Nori-Janosz, K.E., Lillystone, M., Yanez, J. and McCullough, P.A. (2005). Obesity and lipids. *Curr Cardiol Rep.*, 7 (6):465-70.
10. Serhan, C.N. (2005). "Lipoxins and aspirin-triggered 15-epi-lipoxins are the first lipid mediators of endogenous anti-inflammation and resolution." *Prostaglandins Leukotrienes and Essential Fatty Acids*, vol. 73, no. 3-4, pp. 141–162.
11. Nowak, J.Z. (2010). "Anti-inflammatory pro-resolving

derivatives of omega-3 and omega-6 polyunsaturated fatty acids." *Postepy Higieny i Medycyny Doswiadczalnej*, vol. 64, pp. 115–132.

12. Zumoff, B. (1982). Relationship of obesity to blood estrogens. *Cancer Res., suppl.*, 42: 3289.

13. Soygur, T., Kupeli, B., Aydos, K., Kupeli, S., Arikan, N. and Muftuoglu, Y.Z. (1996). Effect of obesity on prostatic hyperplasia: its relation to sex steroid levels. *Int Urol Nephrol.*, 28(1):55-9.

14. Tewari, R., Prabhat, P. and Natu, S.M. (2011). Association of benign prostatic hyperplasia (BPH) with metabolic syndrome (MS) and its components: A growing dilemma. *J Men's Health,* 8, 66-71.

15. Betteridge, D.J and Morrell J.M.: Risk assessment. In Lipids and Coronary Heart Disease, 1ˢᵗ ed. edited by Betteridge, D.J., Morrell, J.M. London: Chapman and Hall., 1998, chapter 6, pp. 117-144.

16. Nandeesha, H., Koner, B.C., Dorairajan, L.N. and Sen, S.K., 2006. Hyperinsulinemia and dyslipidemia in non-diabetic benign prostatic hyperplasia. *Clin Chim Acta*, 370, 89-93.

17. Gillitzer, R., Pahernik, S. and Hampel, C. (2005). Relationship between prostate cancer and serum dyslipidemia. *Urology,* 66, 13-14.

18. Clarke, W.N. and Brown, D.M. (2007). The Influence of Lipid Metabolism on Prostate Cancer Development and Progression: Is it Time for a Closer Look? *Euro Urol*, 52, 3-4.

19. Tewari, R., Chhabra, M., Natu, S.M., Goel, A., Dalela, D., Goel, M. M. and Rajender, S. (2014). Significant Association of Metabolic Indices, Lipid Profile, and Androgen Levels with Prostate Cancer. *Asian Pac J Cancer Prev,* 15 (22), 9841-9846.

20. Dahle, S.E., Chokkalingam, A.P., Gao, Y.T., Deng, J., Stanczyk, F.Z. and Hsing, A.W., 2002. Body size and serum levels of insulin and leptin in relation to the risk of benign prostatic hyperplasia. *J Urol*, 168, 599-604.

21. Hammarsten, J., Hogstedt, B., Holthuis, N. and Mellstrom, D., 1998. Components of the metabolic syndrome-risk factors for the development of benign prostatic hyperplasia. *Prostate cancer and prostatic dis*, 1, 157-162.

22. Adaramoye, O.A., Akintayo, O., Achem, J. and Fafunso, M.A. (2008). Lipid-lowering effects of methanolic extract of *Vernonia amygdalina* leaves in rats fed on high cholesterol diet. *Vascular Health and Risk Management:* 4(1) 235–241.

23. Argheore, E.M., Makkar, H.P.S. and Becker, K. (1998). Feed value of some browse plants from central zone of Delta State of Nigeria. *Trop Sci*, 38:97–104.

24. Regassa, A. (2000). The use of herbal preparation for tick control in Western Ethiopia. *J S Afr Vet Assoc*, 71:240–3.

25. Kambizi, L. and Afolayan, A.J. (2001). An ethnobotanical study of plants used for the treatment of sexually transmitted diseases (njovhera) in Guruve District, Zimbabwe. *J Ethnopharmacol*, 77:5–9.

26. Amira, C.A. and Okubadejo, N.U. (2007). Frequency of complementary and alternative medicine utilization in hypertensive patients attending an urban tertiary care centres in Nigeria. *BMC Complementary and Alternative Medicine*, 7:30–48.

27. Igile, G.O., Oleszek, W. and Jurzysta, M. (1994). Flavonoids from *Vernonia amygdalina* and their antioxidant activities. *J. Agric. Food Chem*, 42:2445–8.

28. Jisaka, M., Ohigashi, H. and Takagawa, K. (1993). Steroid

glucosides from *Vernonia amygdalina.* A possible Chimpanzee medicinal plant. *Phytochemistry*, 34:409–13.

29. Izevbigie, E.B. (2003). Discovery of water-soluble anticancer agents (edotides) from a vegetable found in Benin City, Nigeria. *Exp Biol Med*, 228:293–8.

30. Izevbigie, E.B. and Ernest, O. (2005). Phytochemotherapy for cancer. US Patent No 6,849,604.

31. Bernoulli J. (2008). An Experimental Model of Prostatic Inflammation for Drug Discovery. Finland: University of Turku, 139 p.

32. Mbaka, G.O., Ogbonnia, S.O., Olarewaju O.T. and Duru, F.I. (2013). The effects of ethanol seed extract of *Raphia hookeri* (Palmaceae) on exogenous testosterone and estradiol induced benign prostatic hyperplasia in adult male rats. *Journal of Morphological Science*, 30 (4): 235-243.

33. NCEP. (2001). The Expert Panel. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *The Journal of the American Medical Association (JAMA)*, 285, 2486-2497.

34. Tietz N.W. (1995). *Clinical guide to laboratory tests* (3rded). Philadelphia: WB Saunders Company, PA, pp. 518-519.

35. Assmann, G., Jabs, H.U, Kohnert, U., Nolte, W. and Schriewer, H. (1984). LDL-cholesterol determination in blood serum following precipitation of LDL with polyvinylsulfate. *Clinica Chimica Acta*, 140:77-83.

36. Saito, M., Tsounapi, P. and Oikawa R. (2014). "Prostatic ischemia induces ventral prostatic hyperplasia in the SHR; possible mechanism of development of BPH," Scientific Reports, vol. 4, article 382.

37. Saez, C., Gonzalez-Baena, A.C. and Japon, A.C. (1998). "Regressive changes in finasteride-treated human hyperplastic prostates correlate with an upregulation of TGF-beta receptor expression." *Prostate,* vol. 37, pp. 84–90.

38. Foo, K.T., Ho, H.S.S. and Wong, M.Y.C. (2017). "Singapore urological association clinical guidelines for male lower urinary tract symptoms/benign prostatic hyperplasia." *Singapore Medical Journal,* vol. 58, no. 8, pp. 473–480.

39. Kim, H.K., Zhao, C. and Choi, B.R. (2013). "Is transforming growth factor-beta signaling activated in human hypertrophied prostate treated by 5-alpha reductase inhibitor?" *Disease Markers*, vol. 35, no. 6, Article ID 783287, pp. 679–685.

40. Minutoli, L., Rinaldi, M. and Marini, H. (2016). "Apoptotic pathways linked to endocrine system as potential therapeutic targets for benign prostatic hyperplasia." *International Journal of Molecular Sciences,* vol. 17, article 1311.

41. Mbaka, G., Ogbonnia, S., Sulaiman, A. and Osiagwu, D. (2019). Histomorphological effects of the oil extract of Sphenocentrumjollyanumseed on benign prostatic hyperplasia inducedby exogenous testosterone and estradiol in adult Wistar rats. *Avicenna J Phytomed.,* 9(1): 21-33.

42. Odugbemi T. (2006). Outlines and Pictures of Medicinal Plants in Nigeria. Nigeria: University of Lagos Press, pp. 55–71.

43. Moyad, M.A. and Lowe, F.C. (2008). Educating patients about lifestyle modifications for prostate health. *Am J Med*, 121, S34-42.

44. Yang, Y.J., Lee, S.H., Hong, S.J. and Chung, B.C., (1999). Comparison of

fatty acid profiles in the serum of patients with prostate cancer and benign prostatic hyperplasia. *Clin Biochem*, 32, 405-409.

45. Parsons, J.K., Bergstrom, J. and Barrett-Connor, E. (2008). Lipids, lipoproteins and the risk of benign prostatic hyperplasia in community-dwelling men. *BJU Int*, 101, 313-318.

46. Parsons, J.K. and Kashefi, C. (2008). Physical activity, benign prostatic hyperplasia, and lower urinary tract symptoms. *Eur Urol,* 53, 1228-1235.

47. Hammarsten, J. and Hogstedt, B. (1999). Clinical, anthropometric, metabolic and insulin profile of men with fast annual growth rates of benign prostatic hyperplasia. *Blood Press*, 8, 29-36.

48. Parsons, J.K., Carter, H.B., Partin, A.W., Windham, B.G., Metter, E.J., Ferrucci, L., Landis, P. and Platz, E.A. (2006). Metabolic factors associated with benign prostatic hyperplasia. *J Clin Endocrinol Metab*, 91, 2562-2568.

49. Parsons, J.K., Sarma, A.V., McVary, K. and Wei, J.T. (2009). Obesity and benign prostatic hyperplasia: clinical connections, emerging etiological paradigms and future directions. *J Urol*, 182, S27-31.

50. Kim, B.H., Kim, C.I., Chang, H.S., Choe, M.S., Jung, H.R., Kim, D.Y. and Park, C.H. (2011). Cyclooxygenase-2 overexpression in chronic inflammation associated with benign prostatic hyperplasia: is it related to apoptosis and angiogenesis of prostate cancer? *Korean J Urol*, 52, 253-259.

51. Parsons, J.K. (2011). Lifestyle factors, benign prostatic hyperplasia, and lower urinary tract symptoms. *Curr Opin Urol*, 21, 1-4.

52. Ugwu, M.N., Asuk, A.A., Eteng, M.U. and Amaku, E.E. (2018). Effect of *Prosopis africana* Seed Extract on Lipid Profile of Experimentally Induced Prostatic Hyperplasia Animal Model, *The Pharmaceutical and Chemical Journal*, 5(1):10-16.

53. Ugwu, M.N., Mgbekem, M.A. and Eteng, M.U. (2018). Evaluation of *Ocimum gratissimum* Leaf Extract on Lipid Profile of Experimentally-Induced Prostatic Hyperplasia Animal Model, *Journal of Advances in Medical and Pharmaceutical Sciences,* 17(3): 1-8.