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

*Ugwu Melvin Nnaemeka, Asuk, Atamgba Agbor and Eteng, Mbeh Ubana*

1Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Cross River University of Technology, Calabar, Nigeria.
2Department 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 200-350 g 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*
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 androstenedione 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
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.
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 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.

**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, 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

<table>
<thead>
<tr>
<th>GROUP</th>
<th>BODY WEIGHT (g)</th>
<th>PROSTATE WEIGHT (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPH + 50mg VA</td>
<td>317.60±15.27$^a$</td>
<td>0.83±0.52$^{ab}$</td>
</tr>
<tr>
<td>BPH + 100mg VA</td>
<td>321.60±5.68$^c$</td>
<td>0.72±0.36$^{ab}$</td>
</tr>
<tr>
<td>BPH + FINASTERIDE</td>
<td>320.40±8.99$^c$</td>
<td>0.63±0.23$^{ab}$</td>
</tr>
<tr>
<td>BPH CONTROL</td>
<td>270.40±8.93$^a$</td>
<td>2.21±0.28$^c$</td>
</tr>
<tr>
<td>NORMAL CONTROL</td>
<td>322.20±13.99$^a$</td>
<td>0.41±0.07$^a$</td>
</tr>
</tbody>
</table>
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>
<thead>
<tr>
<th>GROUP</th>
<th>CHOLESTEROL (mg/dl)</th>
<th>TRIACYL-GLYCEROL (mg/dl)</th>
<th>HDL-c (mg/dl)</th>
<th>LDL-c (mg/dl)</th>
<th>VLDL-c (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPH + 50mg VA</td>
<td>147.14±2.71abc</td>
<td>74.23±1.93b</td>
<td>53.33±2.57a</td>
<td>8.90±2.54ab</td>
<td>14.85±0.39a</td>
</tr>
<tr>
<td>BPH + 100mg VA</td>
<td>145.69±14.72ab</td>
<td>72.75±2.00i</td>
<td>52.24±2.69a</td>
<td>9.41±6.59ab</td>
<td>14.55±0.40b</td>
</tr>
<tr>
<td>BPH + FINASTERIDE</td>
<td>160.00±7.93cd</td>
<td>74.38±5.14a</td>
<td>50.75±1.57a</td>
<td>15.85±3.59bc</td>
<td>14.88±1.03b</td>
</tr>
<tr>
<td>BPH CONTROL</td>
<td>180.92±21.81c</td>
<td>82.13±5.42c</td>
<td>32.10±1.10b</td>
<td>22.27±12.35c</td>
<td>16.43±1.08c</td>
</tr>
<tr>
<td>NORMAL CONTROL</td>
<td>136.88±5.89a</td>
<td>67.38±1.62b</td>
<td>53.33±1.64a</td>
<td>7.35±2.69a</td>
<td>13.48±0.32a</td>
</tr>
</tbody>
</table>

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, triacylglycerol, 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 potential of regulating the metabolism of lipid which seems to be significant in the management of benign prostate hyperplasia.

REFERENCES


