Original Research Article

Nephrotoprotective Effect of *Vernonia amygdalina*(Bitter Leaf) Extract on Benign Prostatic

Abstract

Background: Benign prostatic hyperplasia (BPH) is a noncancerous enlargement of the prostate gland. The condition is associated with symptoms like frequency in urination, hesitancy, nocturia, weak urine stream and sexual dysfunction. The effect of *Vernonia Amygdalina* extract (VA) on kidney and liver function indices in BPH was investigated.

Methods: A total of 30 rats weighing 200-300 g were divided according to body weight into five groups (n=6). One group was used as a control and the other groups received subcutaneous injections of testosterone and estradiol for 3 weeks to induce BPH. Groups I and II were treated with different doses of VA extracts and group III received finasteride, all by gavages for thirty-five days. While group IV was left untreated, group V served as normal control. After thirty-five days of treatment with VA extract, the rats were anaesthetised by short contact with trichloromethane vapour. Blood was collected by cardiac puncture and the sera centrifuged and used for the determination of different biochemical indices. The prostates were harvested and weighed.

Results: The level of urea and creatinine were significantly (P<0.05) reduced when compared to the BPH control. No significant differences in serum concentrations of AST, ALT, ALP, and GGT were recorded in all treatment groups compared to the BPH control.

Conclusion: The extract of *Vernonia amygdalina* seed exhibited nephroprotective effect on the kidney of BPH induced rats, while there was no observable effect on the liver as benign prostate hyperplasia appeared not to have had any alteration on the liver enzymes.

Keywords: Creatinine, urea, aminotransferases, alkaline phosphatase, nephroprotective

1.0 Introduction

Benign prostatic hyperplasia (BPH) is a progressive noncancerous enlargement of the epithelial cells and smooth muscle of the prostate gland accompanied by lower urinary tract symptoms [1]. The enlarged prostate impinges on the urethra and therefore BPH is generally associated with impairment in urinary function [2, 3, 4]. The narrowing of the urethra and urinary retention—the inability to empty the bladder completely—cause many of the problems associated with benign prostatic hyperplasia. The prevalence of BPH is age dependent with approximately 50% of men developing BPH-related symptoms at 50 years of age but the condition is not common before age 40. At the age of 85, the prevalence is as high as 95% and 20-30% of men at the age of 80 years require surgical intervention to manage BPH [1, 5].

The mechanism underlying the pathogenesis of BPH remains largely unidentified, however, a number of overlapping and complementary theories have been proposed. Ageing and androgens are established risk factors for the development of benign prostatic enlargement, which may lead to lower urinary tract symptoms (LUTS) in elderly men [6, 7]. Androgens and dihydrotestosterone (DHT) play key roles in BPH development. DHT, an androgen derived from testosterone through the action of 5-α-reductase and its metabolite, 3-α-androstanediol, seems to be the major hormonal stimuli for stromal and glandular proliferation in men with nodular hyperplasia [8]. Experimental work has also identified age-related increases in estrogen levels that may increase the expression of DHT, the progenitor

of BPH [9]. The incrimination of DHT in the pathogenesis of BPH forms the basis for the current use of 5-α-reductase inhibitors in the treatment of symptomatic nodular hyperplasia. Several types of therapeutic agent, such as 5-α-reductase inhibitors, are currently available for treating BPH [8, 10, 11, 12, 13, 14, 15, 16, 17].

The natural history and evolution of benign prostatic enlargement ends up in urinary obstruction causing degradation of renal function over time. In older men, chronic kidney disease (CKD) is an important medical problem that can even be life-threatening [18]. It has been reported that an average of 13.6% of patients presented to urological clinics for the treatment of BPH had renal failure [19]. In the retrospective analysis of men having LUTS due to BPH, the observed incidences of CKD, as defined by elevated serum creatinine levels which is a biomarker of renal failure were similar to data reported by others [20, 21, 22, 23, 24, 25, 26]. From previous reports, various mechanisms have been proposed for renal failure among men with BPH, including vesico-ureteric junction (VUJ) obstruction from bladder remodeling [27].

During chronic retention, bladder wall thickening can occur, leading to a high bladder pressure. High intravesical pressure can lead to functional obstruction at the VUJ. Chronic urinary retention is thought to be the dominant mechanism by which BPH can cause chronic renal failure [27]. Rule *et al.* [20, 27] defined chronic urinary retention (CUR) as a post-void residual urine (PVR) higher than 100 mL, and reported that CUR was significantly associated in CKD in community-dwelling men. Anatomical obstruction at the VUJ can also occur due to bladder thickening and scarring.

The improvement in renal function seen after prostatic surgery in patients with BPH might also support the idea that BPH and CKD are significantly associated disease entities [24]. Kumar *et al.* [28] showed in their studies that acute renal failure in patients with obstructive uropathy were due to BPH (38%), neurogenic bladder (19%), obstructive

pyelonephritis (15%) which were similar to other studies [29, 30]. The most common renal pathology finding in men with obstructive nephropathy due to BPH is chronic interstitial nephritis [20, 27, 31] and 30% of cases have been attributed to obstructive uropathy.

In a research by Meludu *et al.* [32] the values of ALT and ALP of prostate cancer patients and benign prostatic hyperplasia were significantly higher compared to that of the control group. This corroborated with a study done by Harvey *et al.* [33] who reported that liver enzymes were significantly higher in BPH and prostate cancer subjects compared to the control group. However, AST mean values did not show any significant difference. Increase in enzyme activities suggests either hepato-cellular damage or cholestasis [34] this suggests that liver disease may be associated with patients with prostate disease.

Phytomedicine has been in existence for centuries ever before colonial administration and it is in use today with about 80% population depending on herbal medicine for its primary health values [35]. *Vernonia amygdalina* (bitter leaf) has been confirmed to have some vital phytochemical constituents [36]. Phytochemicals are plant secondary metabolites that plants naturally produce to protect themselves against viruses, bacteria and fungi. They are non-nutritive substance with potent biological activities that help in strengthening human immune system and help to lower the risk of many chronic diseases and infections [37].

Bitter leaf extracts may help suppress, delay or kill cancerous cell in many ways, such as induction of apoptosis as determined in cell culture and animal studies, enhance chemotherapy sensitivity, inhibition of the growth or growth signals of cancerous cells, suppression of metastasis of cancerous cells in the body by the inhibition of an anti-apoptotic transcription factors as demonstrated in animal studies and reduction of estrogen level in the body by the suppression of aromatase activity [38]. *Vernonia amygdalina* (VA) has demonstrated several medicinal properties enumerated above, hence the need to investigate

the possible ameliorative effect of *Vernonia amygdalina* extract on the kidney and liver of BPH induced rats.

2.0 Materials and Methods

2.1 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. Their fresh leaves were washed with clean water and dried under the shade for six days. The dried leaves were pulverized using pestle and mortar to get a powder that was used for extraction.

2.1.1 Preparation of extract

One hundred grams (100 g) of powdered sample of *Vernonia amygdalina* was soaked into 100 mL of distilled water and filtered after 48 hours and the filtrate was concentrated in a water bath. The concentrates were diluted with corn oil, to produce a solution 100 mg /mL.

2.2 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 400µg T and 80µg E2 [4, 7, 39, 40, 41]. 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.

2.3 Animals

A total of thirty (30) Wistar rats weighing between 200-300g were obtained from the animal house of the Faculty of Basic Medical Sciences, Cross River University of Technology, Okuku Campus, Nigeria and used for the experiment. The rats were acclimatized for two weeks before the experiment commenced. 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 (six per cage) throughout the 35-day duration of the study. The animal room was well ventilated with a temperature range of 27-29 ^{0}C. The Cross River University of Technology, Calabar, Nigeria, Animal Ethics Committee approved the study before the experiment and certified all experimental protocols.

2.3.1 Induction of BPH

BPH was induced by exogenous administration of testosterone and estradiol in staggered doses (Thrice weekly) for three weeks according to Bernoull [39] with modification by Mbaka *et al.* [42] and Ugwu *et al.* [4, 7, 40, 41].

2.3.2 Animal grouping and treatment

The animals were divided into five (5) groups each which comprised of six (6) male rats. Four groups were induced with BPH which were grouped I, II, III and IV). Groups I and II received 50 and 100 mg kg^{-1} body weight (bw) of *Vernonia amygdalina* extract while

group III received finasteride (orthodox drug) at 0.1mg kg^{-1}; all by gavages for thirty-five days, group IV was left untreated and group V 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 water intake was daily and lasted throughout the duration of the experiment.

2.4 Preparation and collection of samples for biochemical assay

After 35 days, the rats were anaesthetized 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. The prostates were freed of external fascias, washed in cold normal saline, blotted with filter paper and weighed on a sensitive balance. Afterward, they were homogenized in ice-cold normal saline and the homogenates was used for the estimation of the protein content of the prostate gland. The procedure used previously by Ugwu *et al.* [4, 7, 40, 41] was adopted.

2.4.1 Determination of Aminotransferases and Alkaline Phosphatase

The assay for alkaline phosphatase (ALP), asparate amino transferase (AST), alanine amino transferase (ALT) and γ-glutamyl transferase (GGT) were done using kits from Randox Laboratory, Ltd, United Kingdom. Urea and creatinine concentrations were done using Agape Diagnostic kits. All chemicals and reagents used in this research were of analytical grade.

2.4.2 Determination of Protein Content of the Prostate

Cupric ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of coloured complex. The protein content of the prostate was determined using the modified Biuret method [43] and [4]. Briefly, 3.9ml of deionized water and 4.0 ml of Biuret reagent were added to 0.1ml of the aliquot and allowed for 30 minutes at room temperature to develop. A standard and blank were also prepared by adding 4.0ml of Biuret reagent and 3.9ml of deionized water to 0.1ml of standard albumin and water respectively. Subsequently, the absorbance of the test and standard were read against the blank at 540nm using a UV/VIS spectrophotometer.

2.5 Statistical Analysis

The data obtained from the experiment was presented as mean ± SD after calculation using Microsoft Office Excel 2007. The data was also subjected to a one-way analysis of variance (ANOVA) and post hoc (LSD) for levels of significance using SPSS version 16.0. The level of significance was accepted at $P< 0.05$

3.0 RESULTS

3.1 Body weight

Reduction in body weight was observed in the BPH-control group when compared with normal control (Table 1). The extract and standard drug treated groups showed significant ($P< 0.05$) increase in body weight when compared with the BPH control group. Administration of extract and finasteride enhanced the body weight when compared with normal control.

3.2 Prostate gland and Prostate/body weight (P/PW)

The average weight of the prostate gland and prostate/body weight ratio were significantly increased in the BPH control group compared with normal control group (Table 1). The extract and finasteride treated groups showed a decrease in prostate gland and prostate/body weight ratio when compared with the BPH-control group.

3.3 Kidney indices of BPH-induced rats

There were significant ($P<0.05$) increase in level of serum urea concentration and creatinine in BPH control group when compared with normal control and test groups. The value of the doses of VA and finasteride were similar to the normal control. The results showed that all the treated groups exhibited reduction in the level of urea and creatinine concentration (Table 2).

3.4 Liver function enzymes activities of BPH-induced rats

Serum ALT, AST, ALP and GGT concentrations are given in (Table 3). The result of the investigation showed no significant difference ($P>0.05$) in all the test groups compared with both the BPH control and normal control. There was also no significant difference ($P>0.05$) among the test groups.

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

GROUP		BW (g)	PW (g)	PW (mg)	P/BW ratio (mg/g)	PC (g/dl)
BPH + 50mg VA	I	275.40±5.68[b]	0.39±0.05[a]	388.00±45.50[a]	1.41±0.14[a]	5.30±0.20[a]
BPH + 100mg VA	II	271.60±5.68[b]	0.36±0.06[a]	360.00±57.01[a]	1.33±0.21[a]	5.09±0.21[a]
BPH + FINASTERIDE	III	271.80±2.77[b]	0.35±0.05[a]	352.00±50.70[a]	1.30±0.18[a]	5.27±0.89[a]

BPH CONTROL	IV	220.40±8.9b[a]	0.96±0.03[b]	962.00±32.71[b]	4.37±0.20[b]	7.41±0.96[b]
NORMAL CONTROL	V	279.20±4.97[b]	0.36±0.03[a]	356.00±33.62[a]	1.28±0.12[a]	5.08±0.73[a]

Values are expressed as mean ± SD.

[a, b] Values with different superscripts are significantly different at $P<0.05$

BPH (Benign prostate hyperplasia) and VA (*Vernonia amygdalina*).

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206 **Table 2: Effect of extract VA and finasteride on kidney function parameters**

GROUP		UREA (mg/dl)	CREATININE (mg/dl)
BPH + 50mg VA	I	19.49±1.07[a]	0.92±0.21[a]
BPH + 100mg VA	II	18.46±1.46[a]	0.87±0.16[a]
BPH + FINASTERIDE	III	18.97±1.07[a]	0.83±0.15[a]
BPH CONTROL	IV	26.41±2.81[b]	1.96±0.33[b]
NORMAL CONTROL	V	17.69±1.07[a]	0.67±0.35[a]

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208 Values are expressed as mean ± SD.

209 [a, b] Values with different superscripts are significantly different at $P<0.05$

210 BPH (Benign prostate hyperplasia) and VA (*Vernonia amygdalina*).

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212 **Table 3: Effect of extract of VA and finasteride on serum enzyme activities**

GROUP		ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)
BPH + 50mg VA	I	23.49±0.58[a]	33.35±0.51[a]	241.15±3.01[a]	17.88±1.40[a]
BPH + 100mg VA	II	22.99±1.33[a]	33.31±0.46[a]	241.20±2.36[a]	18.17±1.21[a]
BPH + FINASTERIDE	III	23.07±1.14[a]	32.55±3.18[a]	241.14±2.62[a]	18.17±1.71[a]
BPH CONTROL	IV	23.56±1.50[a]	33.82±1.27[a]	241.58±2.40[a]	18.15±0.60[a]
NORMAL CONTROL	V	23.32±1.66[a]	33.01±0.99[a]	241.12±2.97[a]	18.15±0.97[a]

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214 Values are expressed as mean ± SD.

215 Values with identical superscript (a) are not significantly different at $P>0.05$.

216 BPH (Benign prostate hyperplasia) and VA (*Vernonia amygdalina*)

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4. Discussion

Given the many side effects of surgery and pharmacological therapy and the long latency of BPH, phytotherapy based on products derived naturally from plants has emerged as an alternative treatment for BPH because it is thought to be less toxic [44]. Despite the many possible causes of obstructive uropathy, in studies of elderly patients with acute renal failure, the most common cause among all patients was BPH [45]. Previous studies showed that acute renal failure in patients with obstructive uropathy were due to BPH [46, 47]. This necessitated the evaluation of the effect of *Vernonia Amygdalina* on the kidney and liver integrity of rats induced with BPH.

The prostate weight is used as one important marker of BPH development [12, 48, 49]. In previous studies, animals with BPH have shown an increased prostate weight indicating increase in cell number [13, 14]. Finasteride or other agents used to treat BPH decrease the prostate weight [11, 16, 40]. In the present study, the animals with BPH showed an increased prostate weight compare to the control group. In contrast, the animals treated with *Vernonia amygdalina* showed a reduction in prostate weight compared to BPH group. These results indicate that *Vernonia amygdalina* attenuated the prostatic enlargement induced by testosterone. Increase in cell number (hyperplasia) of the prostate would come with a collateral increase in its weight (especially its relative weight) [8, 15]. Also increase in cell number in a tissue also goes with a collateral increase in the protein content of the tissue [4, 50]. The protein content of the prostate was significantly high in BPH untreated group compared with the treated groups.

The liver enzymes found within organs and tissues are released into the bloodstream following cellular necrosis and cell membrane permeability and are used as diagnostic

measure of liver damage [51, 52]. Tissue cells contain characteristic enzymes which enter the blood only when the cells to which they are confined are damaged or destroyed [53]. The tissue activities of the transaminase (ALT and AST) enzyme are markers for the functions and integrity of the liver and heart [54, 55]. The present study was therefore conducted to provide scientific data on the effect of aqueous extract of *Vernonia Amygdalina* on alanine transaminase (ALT), aspatate transaminase (AST), alkaline phosphatase (ALP), γ-glutamyltransferase (GGT), creatinine and urea levels in male Wistar rats induced with BPH.

The extract did not affect the activities of ALT, AST, ALP and GGT indicating normal liver function. This implied that benign prostatic hyperplasia may not have exhibited adverse effect on the liver function and that the extract had no toxic effect on this organ [24, 56]. Earlier studies showed that oral administration of the aqueous extract of some plant could accelerate the reversion of liver damage through reduction of liver marker enzymes, including aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP), glutamate-oxaloacetate transaminase, glutamate- pyruvate transaminase, lactate dehydrogenase and bilirubin indices in liver biochemical tests [41, 57].

The phytochemicals found to be present in the leaf extract are the flavonoids, terpinoids, alkaloids, tannins, saponins sesquiterpene lactones like vernodalin and vernoamygdalin and steroid glycosides like vernonioside B1 and vernoniol B1. Among them tannins, terpinoids, flavonoids and saponins could be responsible for antioxidant property as these phytoconstituents are already reported to have antioxidant activity [58, 59, 60, 61]. The possible mechanisms for the nephroprotective effect of *Vernonia amygdalina* extract could be due to the antioxidant action. Atangwho *et al.* [62] reported that *Vernonia amygdalina* leaves possess strong electron/hydrogen –donating bioactive compounds, which can serve as effective antioxidants. The importance of protecting the antioxidants pool in preventing renal

damage has been emphasized by Singh *et al.* [63]. Also tannins have vasodilatory activity [64]. Renal vasodilatation can improve the glomerular filtration rate (GFR) and renal blood flow, reduce the ischemic changes and also increase urine output. Hence terpinoids, tannins and flavonoids present in the leaf extract may have contributed to the protection from renal damage induced as a result of BPH by their antioxidant and vasodilatory actions.

5. Conclusion

The extract of *Vernonia Amygdalina* leaf exhibited nephroprotective effect on the kidney of BPH induced rats, while there was no observable effect on the liver as benign prostate hyperplasia appeared not to have had any alteration on the liver enzymes.

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