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Effect of *Vernonia amygdalina* (VA) on Oxidative Stress Status of Benign Prostatic Hyperplasia Induced-wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MNU and MUE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors WAO and MNU managed the analyses of the study. Author MAE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: The most significant risk factor for developing benign prostatic hyperplasia (BPH) is an advanced age. As BPH and aberrant changes in reactive oxygen species become more common with ageing, oxygen species signalling may play an important role in the development and progression of this disease. In this study, we investigated the effect of Nigerian indigenous plant; *Vernonia amygdalina* (VA) on oxidative stress indices in BPH induced rats.
Methods: BPH was induced in male rats weighing 200-300 g by exogenous administration of testosterone and estradiol via subcutaneous injection at a dose of 400 µg/kg testosterone (T) and

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80 µg/kg estradiol (E_2) respectively. Thirty (30) rats were divided into five groups. One group was used as a normal control, and the other groups received subcutaneous injections of the hormones for 3 weeks to induce BPH. Groups I and II were treated with different doses of VA extract (50 and 100 mg kg^{-1} body weight respectively) and group III received finasteride (0.1 mg kg^{-1}), all by gavages for forty-two days, while group IV was left untreated, group V served as normal control. After forty-two 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 liver and kidney were harvested and homogenised and used for the assays of oxidative activities.

Results: The activities of catalase (CAT) and superoxide dismutase (SOD) in the extract treated rats were significantly increased when compared the BPH control which had a significant reduction in the activities of these enzymes. The concentration of reduced glutathione (GSH) in the extract treated group significantly (P<0.05) increased while thiobarbituric acid reactive substance (TBARS) concentration decreased when compared to BPH control group.

Conclusion: Human prostate tissue is vulnerable to oxidative damage due to more rapid cell turnover. Therefore *Vernonia amygdalina* can be used to reduce oxidative stress which was implicated in the pathogenesis of BPH.

Keywords: Oxidative stress; prostate; Vernonia amygdalina; finasteride; wistar rats.

1. INTRODUCTION

The prostate is a part of the male reproductive system which contributes to the formation of semen by producing alkaline fluid that maintains and nourishes sperm [1]. Benign prostatic hyperplasia (BPH) is the result of a gradual overgrowth of the prostate gland; a gland that lies at the base of the bladder and encircles the urethra [2]. BPH affects the quality of life of patients adversely, and alteration in the size of the prostate seen in BPH affects the bladder or constricts the urethra, resulting in lower urinary tract symptoms [3,4]. It is reported that 80% of men above an age of 80 suffer from BPH [5].

Potential molecular and physiologic contributors to increase the frequency of BPH occurrence in older individuals include the oxidative stress, chronic inflammation, and alterations in tissue microenvironment. Increased oxidative stress is a result of either increased reactive oxygen species generation or a reduced of antioxidant defence mechanisms. Oxidative stress is associated with several pathological conditions including inflammation and infection [6]. Oxygen species are byproducts of normal cellular metabolism and play vital roles in the stimulation of signalling pathways in response to changing intra and extracellular environmental conditions as well as extracellular activities.

Several parameters including inflammatory mediators, hormones, dietary factors, inflammatory genes, and oxidative stress (OS) have been considered to play a role in the development of BPH, but there is no consensus as to which is the primary cause [7,8]. These multifactorial and chronic conditions have been studied to prevent BPH progression [9]. Though it is not yet known exactly when and why chronic inflammation occurs, it has been hypothesised that BPH is an immune-mediated inflammatory disease and inflammation may directly contribute to prostate growth [9].

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and have remained relevant in both developing and the developed nations of the world for various chemotherapeutic purposes. Plants have the ability to synthesise a wide variety of chemical compounds such as resins, alkaloids, glycoside, saponins, lactose and essential oils [10]. Many of these phytochemicals have beneficial effects in human health and may be used to effectively treat human disease [11,12].

Lepidium meyeni, *Benincasa hispida Congn., Sphaeranthus indicus, Abrus precatorious, Urtica dioica and Vernonia amygdalina* have been established to have inhibitory effect on 5-alpha reductase enzyme activity, an enzyme that converts androgen to DHT [2,13,14,15,16]. These plants have demonstrated ameliorative effect on testosterone-induced prostate hyperplasia by reducing relative prostate weight in treated animals [1,13,14,15]. Also protective effect of *Echinops echinatus* and *Ganoderma lucidum* extracts [16,17,18,19] on testosterone-induced BPH have been reported.

Vernonia amygdalina is a herb claimed to be very useful for the treatment of many diseases in many developing countries [20]. The plant has acquired special relevance in recent times, having been proven to possess several medicinal properties such as anticancer [21] antimalarial, anthelminthic properties [22], as well as antibacterial and antifungal [23], anti-amoebicidal [24] and antioxidant effect [25]. The biologically-active compounds of *Vernonia amygdalina* are saponins and alkaloids [26], terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones and anthraquinone [27], edotides [28] and sesquiterpenes [29]. The antioxidant activity of *Vernonia amygdalina* has been attributed to the presence of flavonoids, as reported by Igile et al. [30] and Farombi and Owoeye [31].

Antioxidants are substance or molecules that are capable of neutralizing the harmful effects of the reactive oxygen species (ROS). The antioxidant effect of a plant is mainly due to phenolic components like flavonoids, phenolic acids and phenolic diterpenes [32]. The antioxidants capacity of phenolic compounds is mainly due to their redox properties, which play an important role in absorbing and neutralising free radicals, quenching singlet and triplet oxygen and decomposing peroxides [33].

Acute toxicity of VA has been studied by several researchers and has proven to have no clinical symptoms of toxicity effect or adverse toxicological potentials at different doses. In a study by Okoduwa et al. [34], a single oral acute toxicity study of VA leaf extract at 5000 mg/kg b.w. did not show any noticeable toxic symptom or death at seven days of oral administration. This indicated that LD_{50} of VA was >5 g/kg b.w. Studies by Nwanjo [25] and Adiukwu et al. [35] showed that VA leaf extract had no adverse toxicological effects at doses of 500–2000 mg/kg/day for fourteen consecutive days. Several other researchers have also reported the non-toxic nature of the leaf extract either to the kidney or the liver [36,37,38,39].

2. 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 and authenticated by Dr. Michael Eko, a botanist in the Department of Biological Sciences, University of Calabar and a voucher specimens number (BOT/VA/010) deposited in a herbarium in the Department of Botany. 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 powered that was used for extraction.

2.1.1 Preparation of extract

The powered sample of *Vernonia amygdalina* 200 g was soaked into 200 ml of distilled water, this was filtered after 48 hours and filtrate was concentrated in water bath. The solutions were diluted with corn oil, to produce a solution 100 mg/ml. The administration of extract was entirely by gavage.

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 400 µg/kg and 80 µg/kg respectively [40]. 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 200-300 g were obtained from the animal house of the Faculty of Basic Medical Sciences, Cross River University of Technology, Okuku Campus, Nigeria. The rats were 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 42-day duration of the study. The animal room was well be ventilated with a temperature range of 27-29°C. The Institutional Animal Ethics Committee, Cross River University of Technology, Calabar, Nigeria, (IAEC/CRUTECH/17/101) 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 three times a week respectively for three weeks. The hormones were diluted with corn oil which served as the solvent. The dilution was done by taken 19 mL of corn oil and adding it to 1 mL (25 mg) of testosterone to form a 20 mL stock solution while 24 mL of corn oil was added to 1 mL of estradiol to make up a stock solution of 25 mL. From the stock solutions prepared, 200 g rat was injected with 400 µg/kg of testosterone and 80 µg/kg of estradiol separately at the different thighs [40] with modification by Mbaka et al. [41].

2.3.2 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 I to group IV). Groups I and II received 50 and 100 mg kg^{-1} body weight (bw) of *Vernonia amygdalina* extract; group III received finasteride (orthodox drug) at 0.1 mg kg^{-1}; all by gavages for forty two days, group IV was left untreated for forty two days before sacrifice to assess possible reversal of the exogenous induction 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.

Table 1. Animal grouping and treatment (daily for 42 days)

Group	Treatment
I	BPH + 50 mg/kg VA
II	BPH + 100 mg/kg VA
III	BPH + 0.1 mg/kg of Finasteride
IV	BPH control
V	Normal control

2.4 Determinations of Biochemical Parameters

After 42 days, the rats were anaesthetised by a brief exposure to trichloromethane vapour and bled by cardiac puncture. Blood samples were collected and transferred into vacutainers without anticoagulant, and serum was separated by centrifugation at 2,500 RMP for 15 min using bench top centrifuge (MSE Minor, England). After centrifugation serum samples were collected using dry Pasteur pipette and stored in the in a freezer at -20℃ until use. All analyses were completed within 24 h of sample collection. The liver and kidney were harvested and homogenized and used for the assays of oxidative activities.

2.4.1 Determination of thiobarbituric acid reactive substance (TBARS) concentration

Thiobarbituric acid reactive substance (TBARS) in tissues was determined by the procedure of Fraga et al. [42]. At low pH 3.5 and high temperature (100°C) Malondialdehyde (MDA) binds with thiobarbituric acid (TBA) to produce a pink colour that can be measured at 532 nm.

2.4.2 Assay for catalase activity

Calatase was assayed according to the method of Machly and Chance [43]. Catalase can act on H_2O_2 to yield H_2O and O_2. The concentration of H_2O_2 was taken with spectrophotometer after 10 min and was used to determine the catalase activity which was expressed in terms of units/mg protein. The absorbance was measured at 230 nm.

2.4.3 Determination of superoxide dismutase (SOD) activity

Superoxide Dismutase activity assay was carried out according to the method described by Martin [44]. Exactly 920µL of assay buffer (Phosphate buffer pH 7.8) of 0.05M was added into clean test tube containing 40 µL of sample; they were mixed and incubated for 2mins at 25°C. 40µL of hematoxylin solution was added, mixed quickly and the absorbance was measured at 560nm. Auto-oxidation of hematoxylin is inhibited by SOD at the assay pH, the percentage of inhibition is linearly proportional to the amount of SOD present within a specific range [38].

2.4.4 Estimation of glutathione concentration

The method of Rukkumani et al. [45] was followed in estimating the level of reduced glutathione (GSH). The reduced form of glutathione comprises in most instances the bulk of cellular non-protein sulfhydryl groups. This method is therefore based upon the development of a relatively stable yellow colour when 5, 5 – dithiobis – (2-nitrobenzoic acid) (Ellaman's reagent) is added to sulfhydryl compounds. The chromophoric product resulting from the reaction of Ellaman's reagent with the reduced glutathione, 2- nitro-5-thiobenzoic acid possesses a molar absorption at 412 nm.

Reduced GSH is proportional to the absorbance at 412 nm.

2.5 Statistical Analysis

The experimental data were analysed for statistical significance by one-way analysis of variance and post hoc comparison using the SPSS version. The Independent Samples t-test was used to compare the means of two independent groups. All data were reported as mean ± SD and statistical significance was accepted at $P < 0.05$.

3. RESULTS

3.1 Effect of Extract of VA and Finasteride on Body Weight, Prostate Weight and Prostate/Body Weight Ratio

The effect of oral administration of extract and finasteride (Group I, II and III which received 50 mg/kg of VA, 100 mg/kg of VA and 0.1 mg/kg of finastride respectively) on body weight is shown in Table 2. The BPH-control group exhibited a decline in body weight when compared with normal control. The extract and standard drug (finasteride) treated groups exhibited an increase in body weight when compared with the BPH control group.

The weight of the prostates and prostate/body weight ratio were at the highest in the BPH control group when compared with normal control group (Table 2). BPH control group exhibited a significant ($P< 0.05$) increase in prostate weight and prostate/body weight ratio when compared to normal control. The extract and standard drug-treated groups showed a decrease in prostate weight and prostate/body weight ratio when compared with the BPH-control group (Table 2).

3.2 Liver and Kidney Superoxide Dismutase (SOD) Activity

There was a significant ($P< 0.05$) decrease in the activity of superoxide dismutase in the liver and kidney of the BPH control group when compared with the normal control. Treatments with extract and standard drug exhibited a significant increase in the activity of superoxide dismutase when compared with the BPH control (Tables 3 and 4).

3.3 Concentration of Glutathione (GSH)

There was a significant reduction in the concentration of glutathione in the BPH control compared to normal control ($P< 0.05$) and treated groups. Group I (50 mg/kg VA) and III (0.1 mg/kg of finastride) were statistically similar when compared to each other but statistically different when compared to IV (BPH control) and V (normal control) (Table 3). Group IV is statistically different ($P< 0.05$) from the group that received 50 mg/kg VA, 100 mg/kg VA, and 0.1mg/kg standard drug (finastride).

3.4 Liver and Kidney Catalase (CAT) Activity

The activity of catalase decreased significantly ($P< 0.05$) in the liver and kidney of BPH control group when compared with normal control. Administration of the extract and the standard drug significantly increased the catalase activity in the liver and kidney of treated groups when compared to the BPH control group (Tables 3 and 4).

Table 2. Effect of extract of VA and finasteride body weight and prostate weight

Group	BW (g)	PW (mg)	P/BW (g/mg)
BPH + 50 mg VA	292.40±9.27^{b}	550±0.60^{b}	1.88±0.06^{b}
BPH + 100 mg VA	296.40±4.68^{b}	540±0.45^{b}	1.82±0.09^{b}
BPH + Finasteride	295.20±6.49^{b}	533±0.35^{b}	1.81±0.05^{b}
BPH control	244.20±9.13^{a}	980±0.38^{c}	4.02±0.10^{c}
Normal Control	297.10±6.99^{b}	230±0.52^{a}	0.77±0.07^{a}

Values are expressed as Mean ± SD. Benign prostate hyperplasia (BPH), Vernonia amygdalina (VA), Body weight (PW), Prostate weight (PW), Prostate/Body weight ratio (P/BW).Groups bearing the same superscripts are not significantly different from one another at P > 0.05. Groups bearing different superscripts are significantly different from one another at P < 0.05

Table 3. Effect of aqueous extract of *Vernonia amygdalina* (VA) on catalase activity and MDA concentration in the liver of BPH induced wistar rats

Group	SOD liver (mg protein)	CAT liver (mg protein)	MDA liver (mg protein)	GSH (mg/g)
BPH + 50 mg VA	9.70±0.90^{bc}	65.66±1.87^{b}	30.12±0.89^{ab}	65.87±1.71^{bc}
BPH + 100 mg VA	10.84±1.46^{d}	73.75±3.27^{cd}	29.91±1.89^{ab}	68.48±4.39^{c}
BPH + Finasteride	9.14±0.77^{bc}	68.55±1.91^{bc}	30.71±0.58^{ab}	65.71±2.97^{bc}
BPH control	4.24±0.86^{a}	31.62±4.98^{a}	42.41±1.41^{c}	37.86±1.61^{a}
Normal control	12.30±1.20^{e}	79.52±10.54^{d}	27.58±4.45^{a}	73.91±0.76^{d}

Values are expressed as Mean ± SD. Benign prostate hyperplasia (BPH) Vernonia amygdalina (VA).Groups bearing the same superscripts are not significantly different from one another at P > 0.05. Groups bearing different superscripts are significantly different from one another at P < 0.05

Table 4. Effect of aqueous extract of *Vernonia amygdalina* (VA) on catalase activity and MDA concentration in the kidney of BPH induced wistar rats

Group	SOD kidney (mg protein)	CAT kidney (mg protein)	MDA kidney (mg protein)
BPH + 50 mg VA	8.75±0.66^{bc}	72.99±4.67^{bc}	36.51±2.55^{bc}
BPH + 100 mg VA	9.65±0.95^{c}	75.98±4.29^{cd}	35.70±2.62^{ab}
BPH + Finasteride	8.95±1.44^{bc}	76.50±7.69^{cd}	36.31±2.26^{bc}
BPH Control	4.67±0.83^{a}	38.37±3.58^{a}	44.49±4.36^{d}
Normal Control	11.31±0.83^{d}	80.83±7.58^{d}	34.54±3.56^{a}

Values are expressed as Mean ± SD. Benign prostate hyperplasia (BPH) Vernonia amygdalina (VA).Benign prostate hyperplasia (BPH) Vernonia amygdalina (VA).Groups bearing the same superscripts are not significantly different from one another at P > 0.05. Groups bearing different superscripts are significantly different from one another at P < 0.05

3.5 Concentration of Liver and Kidney Malondialdehyde (MDA)

Malondialdehyde (MDA) concentrations increased significantly (P<0.05) in the liver and kidney of BPH control group when compared with the normal control. Group I, II and III which received 50 mg/kg of VA, 100 mg/kg of VA and 0.1 mg/kg of finastride respectively, had a significant (P< 0.05) reduction in the MDA concentrations in the liver and kidney of treated groups respectively (Tables 3 and 4).

4. DISCUSSION

Increase in prostate weight is used as one of essential markers of BPH [2,13,17]. BPH is characterized by stromal and epithelial cells hyperplasia, resulting in prostate enlargement [2]. Our studies showed a significant increase in prostate weight and prostate/body weight ratio in BPH control when compared with normal control whereas those animals treated with finasteride and extract had significant reduction in prostate weight and prostate weight ratio when compared with BPH animals. Several studies on herbal management of BPH have shown a similar trend [2,12,13,14,15,18,19,41].

Oxidative stress (OS) 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 [46,47]. In living cells ROS are generated as byproducts of cellular metabolism whereby hydrogen peroxides and superoxide anions constitute the major sources of endogenous ROS [48].

OS has already been established as a culprit in the BPH pathogenesis [49,50,51,52]. 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 [51,53]. A handful of studies in the literature have demonstrated higher levels of oxidants or lower levels of enzymatic or nonenzymatic antioxidants in patients with BPH compared to normal persons. Several studies have demonstrated the presence of oxidative stress in BPH [8]. The cause of enhanced oxidative stress could be the overproduction of free radicals or decrease in the activities of free radical scavenging enzymes like SOD, GST, GR and glutathione levels in their circulation, or both [8,9]. Some studies on animal models of BPH showed significant elevation of prostatic lipid peroxidation with concomitant significant

reduction of the prostatic levels of GSH, SOD, and catalase activities of BPH untreated rats and which parameters were significantly improved following treatment with finasteride or kolaviron [54,55].

Humans are naturally protected against free radical damage by oxidative enzymes and proteins such as superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) as well as phytochemicals. GSH serves as a redox buffer by removing toxic peroxides *via* reactions catalysed by GSH peroxidase. The ratio between the reduced and oxidized glutathione disulfide (GSSG) forms of glutathione is often used as an indicator of the cellular redox state, reflecting the balance between the capacity of the defence response for regeneration of GSH and the extent of neutralization by oxidants [47]. Superoxide dismutases (SODs) are a class of closely related enzymes present in almost all cells and in the extracellular fluids [56]. They catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide. Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in protecting the cell from oxidative damage by ROS [57]. These enzymes work synergistically in counteracting the deleterious effect of free radicals.

Many plants have been identified as good sources of natural antioxidants which protect against degenerative diseases and cancer [58,59]. In this study the administration of extract to the animals increased the activity of these endogenous antioxidant enzymes compared to the BPH control. The extract might have reactivated the activities of these enzymes through its active compounds that enhanced the scavenging effect of the enzymes against reactive oxygen species (ROS) thereby reducing its oxidative damages [12]. Some studies have justified the antioxidant mechanism of *Vernonia amygdalina* [31,60]. The antioxidant activity of *Vernonia amygdalina* has been attributed to the presence of flavonoids, as reported by Igile *et al.* [30].

Free radicals cause attack on polyunsaturated membrane lipid (lipid peroxidation) generating a product called malondialdehyde (MDA) [61]. MDA is an end-product derived from peroxidation of polyunsaturated fatty acids and related esters. In contrast to free radicals, aldehydes are relatively stable and therefore able to diffuse within or out of the cell and to attack targets distant from the site of original free-radical-initiated events. Increased lipid peroxidation can be destructive to various body tissues resulting in inflammation and other damages if not scavenged by antioxidant defense mechanism. The decreased level of plasma antioxidants indicates that BPH is a disease of increased oxidative stress [62].

Some studies have shown an increase MDA level in BPH patients which is an indicator of lipid peroxidation [63]. The activities of antioxidative enzymes, as well as the concentrations of the low molecular weight antioxidants decreases during ageing, which favours the increase of oxidative stress [32,64] and development and progression of BPH. The organism may not be able to counteract the intensified ROS synthesis due to this impaired antioxidant defense system in ageing organism, leading to the oxidative-induced damage of cellular structures and the pathological changes [65,66] that might result to BPH. In this study there was a significantly higher level of MDA in BPH control groups when compared with extract treated and normal control. Administration of the extract to the rats caused a significant reduction in the level of MDA compared to BPH control group. Some previous studies revealed similar trend [7,49,67].

The increase in the anti-oxidant activity seen as improved activities of SOD, CAT and rise in level of GSH and decrease in level of MDA of the rats treated with *Vernonia amygdalina* could provide a promising substitute revealing the protective effects of the plant against BPH [68,69,70]. The antioxidant compounds in *Vernonia amygdalina* might have caused this ameliorative effect.

5. CONCLUSION

The study showed that *Vernonia amygdalina* exhibited antioxidant properties and may have the capacity to prevent or delay the development and progression of BPH. It therefore means that consumption of *V amygdalina* leaves may have some therapeutic effect on benign prostatic hyperplasia. Hence a promising research for scientists to explore and find out its mechanism of action. This may also serve as a remedy for other prostate related diseases including prostate cancer.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The Institutional Animal Ethics Committee, Cross River University of Technology, Calabar, Nigeria, (IAEC/CRUTECH/17/101) approved the study before the experiment and certified all experimental protocols.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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