Evaluation of Oxidative Stress Status in Testosterone and Estradiol Induced Benign Prostatic Hyperplasia in Wistar Rats

Abstract

Background: The greatest risk factor for developing benign prostatic hyperplasia (BPH) is advanced age. As BPH and aberrant changes in reactive oxygen species become more common with aging, oxygen species signaling 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. **Method:** BPH was induced in male rats weighing 200-300g through exogenous administration of testosterone and estradiol by subcutaneous injection at a dose of 400µg T and 80µg 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 and group III received finasteride, 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 homogenized and used for the assays of oxidative activities.

Results: The activities of CAT and 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 GSH in the extract treated group significantly

(*P*<0.05) increased while 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 lipid peroxidation as a result of oxidative damage from BPH.

Keywords: Oxidative stress, Prostate, *Vernonia amygdalina,* finasteride, Wistar Rats

1. Introduction

Potential molecular and physiologic contributors to 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 loss of antioxidant defense mechanisms. Oxidative stress is associated with several pathological conditions including inflammation and infection [1]. Oxygen species are byproducts of normal cellular metabolism and play vital roles in stimulation of signaling pathways in response to changing intra and extracellular environmental conditions.

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 one [2, 3]. To date, these multifactorial and chronic conditions have been studied to prevent BPH progression. In the last few years, a relationship between prostatic inflammation and lower urinary tract symptoms related to BPH has been suggested [4]. Today, even though it is not yet known exactly when and why chronic inflammation occurs, it has been hypothesized that BPH is an immune-mediated inflammatory disease and inflammation may directly contribute to prostate growth [4].

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 ability to synthesize a wide variety of chemical compounds such as resins, alkaloids, glycoside, saponins, lactose and essential oils [5]. Many of these phytochemicals have beneficial effects on a long term human health and may be used to effectively treat human disease [6, 7].

Antioxidants are substance or molecules that are capable of neutralizing the harmful effects of the reactive oxygen species (ROS) through the endogenous enzymatic defense system. The antioxidant effect of plant is mainly due to phenolic components like flavonoids, phenolic acids and phenolic diterpenes [8]. The antioxidants capacity of phenolic compounds is mainly due to their redox properties, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen and decomposing peroxides [9].

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 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. Their dried leaves were milled using pestle and mortar to get a powder that was used for extraction.

2.1.1 Preparation of extract

The powered sample of *Vernonia amygdalina* 200g was soaked into 200ml 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 100mg/ml. The administration of extract was totally 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 400µg and 80µg E_2 [10]. 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. 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 42-day duration of the study. The animal room was well be ventilated with a temperature range of 27-29 ^{0}C. The Institutional 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 (three times a week respectively) for three weeks according to [10] with modification by [11].

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 100mg kg^{-1} body weight (bw) of *Vernonia amygdalina* extract; group III received finasteride (orthodox drug) at 0.1mg 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.

2.4. Determinations of Biochemical Parameters

After 42 days, the rats were anaesthetized 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 [12]. At low pH 3.5 and high temperature (100^0C) Malondialdehyde (MDA) binds with thiobarbituric acid (TBA) to produce a pink colour that can be measured at 532nm.

2.4.2. Assay for catalase activity

Calatase was assayed according to the method of [13]. Catalase can act on H_2O_2 to yield H_2O and O_2. The concentration of H_2O_2 in the in absorbance reading after 10min was determined as catalase activity expressed in terms of units/mg protein. The absorbance was measured at 230nm.

2.4.3. Determination of superoxide dismutase (SOD) activity

Superoxide Dismutase activity assay was carried out according to the method described by [14]. Exactly 920µL of assay buffer (Phosphate buffer pH 7.8) of 0.05M was added into clean test tube containing 40µL of sample; they were mixed and incubated for 2mins at 25^oC. 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 [14].

2.4.4. Estimation of glutathione concentration

The method of [15] 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 satble 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 412nm. Reduced GSH is proportional to the absorbance at 412nm.

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 $P < 0.05$.

3.0. Results

3.1. Liver and Kidney Superoxide Dismutase (SOD) Activity

There was a significant ($P< 0.05$) decrease in 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.

3.2. Concentration of Gluathione (GSH)

There was a significant reduction in the concentration of gluathione in the BPH control compared to normal control ($P< 0.05$). Administration of extract and standard drug showed a significant ($P< 0.05$) increase in the concentration of glutathione when compared to the BPH control group.

3.3. 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.

3.4. Concentration of Liver and Kidney Malondialdehyde (MDA)

There was a significant ($P<0.05$) increase in malondialdehyde (MDA) in the liver and kidney of BPH control group when compared with the normal control. Treatment with the extract and the standard drug significantly ($P< 0.05$) decreased the MDA concentration in the liver and kidney of treated groups.

Table 1: Effect of aqueous extracts of *Vernonia amygdalina* (VA) in BPH induced Wistar rats on Catalase and MDA of Liver.

GROUP	SOD LIVER (mg Protein)	CAT LIVER (mg Protein)	MDA LIVER (mg Protein)	GSH (mg/g)
BPH + 50mg VA	9.70 ± 0.90^{bc}	65.66 ± 1.87^{b}	30.12 ± 0.89^{ab}	65.87 ± 1.71^{bc}
BPH + 100mg 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). 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 $P < 0.05$.

177 **Table 2:** Effect of aqueous extracts of *Vernonia amygdalina* (VA) in BPH induced Wistar
178 rats on Catalase and MDA of Kidney.

Group	SOD Kidney (mg Protein)	CAT Kidney (mg Protein)	MDA Kidney (mg Protein)
BPH + 50mg VA	8.75±0.66^{bc}	72.99±4.67^{bc}	36.51±2.55^{bc}
BPH + 100mg 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}

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

184

185 **4. Discussion**

186 Lipid peroxidation is a well-established mechanism of cellular injury in both plants
187 and animals. This process, leading to the production of lipid peroxides and their byproducts,
188 and ultimately to the loss of membrane function and integrity, is widely accepted to be
189 involved in the pathogenesis of several human diseases [16, 17]. Several studies
190 demonstrated the presence of oxidative stress (OS) in BPH [3]. The cause of enhanced
191 oxidative stress could be the overproduction of free radicals or decrease in the activities of
192 free radical scavenging enzymes like SOD, GST, GR and glutathione levels in their
193 circulation, or both [3, 4, 18].

194 Humans are naturally protected against free radical damage by oxidative enzymes and
195 proteins such as Superoxide Dismutase (SOD), Catalase (CAT) and glutathione as well as

196 phytochemicals. Many plants have been identified as good sources of natural antioxidants
197 which protect against degenerative diseases and cancer [19, 20]. In this present study the
198 administration of extract to the animals increased the activity of these endogenous antioxidant
199 enzymes compared to the BPH control. The extract might have reactivated the activities of
200 these enzymes through its active compounds that enhanced the scavenging effect of the
201 enzymes against reactive oxygen species (ROS) thereby reducing its oxidative damages [7].
202 Some studies have justified the antioxidant mechanism of *Vernonia amygdalina* [21, 22]. The
203 antioxidant activity of *Vernonia amygdalina* has been attributed to the presence of
204 flavonoids, as reported by [23]

205 Free radicals cause attack on polyunsaturated membrane lipid (lipid peroxidation)
206 generating a product called malondialdehyde (MDA) [24]. MDA is an end-product derived
207 from peroxidation of polyunsaturated fatty acids and related esters. In contrast to free
208 radicals, aldehydes are relatively stable and therefore able to diffuse within or out of the cell
209 and to attack targets distant from the site of original free-radical-initiated events. In this study
210 there was a significantly higher level of MDA in BPH control groups when compared with
211 extract treated and normal control. Administration of the extract to the rats caused a
212 significant reduction in the level of MDA compared to BPH control group. Some previous
213 studies revealed similar trend [2, 25, 26].

214 The increase in the oxidative capability seen as improved activities of SOD, CAT and
215 rise in level of GSH and decrease in level of MDA of the rats treated with *Vernonia*
216 *amygdalina* could provide a promising substitute revealing the protective effects of the plant
217 against BPH [27, 28]. The antioxidant compounds in *Vernonia amygdalina* might have
218 caused this protective effect against BPH.

219 **5. Conclusion**

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

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