Antioxidant Action of Aqueous Extract of *Ocimum gratissimum* on Testosterone and Estradiol induced Benign Prostatic Hyperplasia in Adult Male Rats

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ABSTRACT

Benign prostatic hyperplasia (BPH) is a common urological disorder reported among aging men. The pathophysiology of BPH is not clearly understood; however, its etiology is attributable to inflammation and/or oxidative stress. We investigated the effect of aqueous leaf extract of *Ocimum gratissimum* (OG) on BPH induced animal model. BPH was induced in male rats weighing (250-350 g) through exogenous administration of testosterone and estradiol. A total of 30 rats were divided into five groups. One group served as a normal control, and the other groups received subcutaneous injections of the hormones for 3 weeks to induce BPH. Groups 1 to 2 were treated orally with 50 and 100 mg kg⁻¹ body weight (bw) respectively. While group 3 received finasteride at 0.1mg kg⁻¹, group 4 was left untreated, and group 5 served as normal control. After forty-five days of treatment with the extract, the animals were sacrificed. Blood was collected by cardiac puncture and the sera centrifuged and used for the determination of different biochemical indices. The liver and kidney were collected and homogenised and used for the assays of oxidative stress parameters. The activities of tissue superoxide dismutase (SOD) and catalase (CAT) in the extract treated rats were significantly increased when compared with the BPH control which had a significant reduction in the activities of these enzymes. Thiobarbituric acid reactive substance (TBARS) concentration decreased compared to BPH control group, while the concentration of reduced glutathione (GSH) in the extract treated group significantly (P<0.05) increased. Prostate tissue is prone to attack by reactive oxygen species (ROS) resulting in tissue damage. However, the outcome of this research revealed that *Ocimum gratissimum* possesses the potential to maintain tissue integrity and reverse oxidative stress caused by BPH.

Keywords: Free Radicals, Prostate disease, lipid peroxidation, finasteride and dihydrotestosterone

INTRODUCTION

Benign prostatic hyperplasia (BPH) is a common aging disease in men affecting well over 42% of men in their 50s and more than 80% of octogenarians [1, 2]. At birth, the weight of prostate is only few grams; however, it continues to increase to be 20±6 g in young adults [3]. This increase in size is via androgen-dependent process [4]. After age of 60, more than 50% of males will have classical symptoms of BPH as a result of considerable both stromal and epithelial proliferation in the transitional zone compressing the urethra [5], [6].

One of the risk factors to the development of BPH is hormone changes, majorly the increased production of dihydrotestosterone (DHT) from testosterone through the action of 5α-reductase in the prostate, which eventually leads to prostatic growth and development of the disease [7], [8]. Also,
increased oxidative stress as well as prolonged inflammation is associated with BPH development [9]. Reactive oxygen species (ROS) are common by-products of normal aerobic cellular metabolism and play important physiological roles in intracellular cell signaling and homeostasis [10]. The human body is equipped with antioxidant systems to regulate the levels of these free radicals and maintain proper physiological function. Oxidative stress (OS) is defined as the interruption of the balance between oxidant and reductant molecules due to the excessive production of reactive oxygen species (ROS). This imbalance leads to oxidative DNA damage and performs a nidus for the etiopathogenesis of several diseases [11]. There is plenty of data regarding the relationship between ROS and age-related pathologies such as cancer, diabetes or several degenerative disorders [12].

It has also been reported that OS has a pivotal role in the aging process and disorders in aging males which include BPH and prostate cancer PCa [13]. Malondialdehyde (MDA) is the principal end product of the lipid peroxidation pathway and is used as a marker to reflect oxidative status in normal subjects, BPH and PCa patients [14], [15]. Superoxide dismutase (SOD) is the main endogenous antioxidant enzyme that counteracts the deleterious effects of ROS. Previous studies have reported that MDA levels are elevated and SOD activity is reduced in BPH and PCa patients compared to control groups [16], [17].

The oxidative damage can be exacerbated by a decreased efficiency of antioxidant defence mechanisms [18], [19]. Like many different cancer types, OS has been linked with benign prostatic hyperplasia (BPH) and prostate cancer (PCa) development, progression and the response to therapy [20], [21]. Several mechanisms for prostate hyperplasia development have been suggested and these include; oxidative stress (OS) [22], [23], [24], inflammatory mediators [25], [26], hormones (especially androgens whose increase in physiologic level can cause increase in oxidative stress and alterations in intracellular glutathione levels and the activity of other detoxification enzymes required for the maintenance of the cellular prooxidant-antioxidant balance such as gamma-glutamyl transpeptidase) [27], enzymatic factors, dietary factors [28], [29], [30], inflammatory genes [31], [32]. Reactive nitrogen species (RNS) and ROS are by products of normal cellular metabolism which impact on cell signaling. Increase in the levels of ROS and RNS induces oxidative stress, causing the cells to activate a variety of mechanisms that allow them to cope with these changes [33].

The α1-adrenoreceptors antagonists, 5α-reductase inhibitors such as dutasteride and finasteride are pharmacological intervention in the management of BPH/LUTS [34], [35]. Furthermore, phytotherapeutic agents like saw palmetto fruits, *Pygeum africancum* bark extract and methyl jasmonate have been used in the management of BPH [36], [37]. Since Africa is endowed with enormous medicinal plants which are used to manage numerous diseases, therefore, the search for natural products with anti-BPH activity which can serve as better option to orthodox drugs is necessary. Previous study by Ugwu et al. [38] has established that *Ocimum gratissimum* was effective in reducing PSA, prolactin, testosterone, estradiol values and prostate weight caused as a result of BPH in a rat model.

**MATERIALS AND METHODS**

**Plant Material**

Fresh leaves of *Ocimum gratissimum* 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 extract**

The powered sample of *Ocimum gratissimum*, (100 g) was soaked into 100
mL of distilled water, and filtered after 48 h and filtrate was concentrated in hot air oven. The solutions were 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 E2 (puregynon depot) were used for the induction of prostate gland enlargement at a dose of 400 μg T and 80 μg E2 [39]. 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 gland 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 250-350 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 45-day duration of the study. The animal room was well ventilated with a temperature range of 27-29°C. The Cross River University of Technology, Calabar, Nigeria, 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, [40] with modification by Mbaka *et al.*, [41].

**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 100mg kg⁻¹ body weight (bw) of *Ocimum gratissimum* extract; group 3 received finasteride (orthodox drug) at 0.1mg kg⁻¹; all by gavaging 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.

**Determinations of Biochemical Parameters**

After 45 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°C 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.

**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.

**Assay for catalase activity**

Catalase was assayed according to the method of Machly and Chance [43]. Catalase can act on H₂O₂ to yield H₂O and O₂. The concentration of H₂O₂ 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.

**Determination of superoxide dismutase (SOD) activity**

Superoxide Dismutase activity assay was carried out according to the method described by Martin et al. [44]. Exactly 920 μL 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 2 mins at 25°C. 40 μL 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 [45].

**Estimation of glutathione concentration**

The method of Rukkumani et al. [46] 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.

**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.

**RESULTS**

**Effect of Extract of OG and Finasteride on Body Weight, Prostate Weight and Prostate/Body Weight Ratio**

The effect of oral administration of extract and finasteride on body weight is shown in Table 1. The BPH-control group exhibited a decline in body weight when compared with normal control. The extract and standard drug 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 1). 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 1).

**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 2 and 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.

**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 2 and 3).

**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 (Tables 2 and 3).
Table 1. Effect of extract of OG and finasteride body weight and prostate gland weight

<table>
<thead>
<tr>
<th>GROUP</th>
<th>BW (g)</th>
<th>PW (mg)</th>
<th>P/BW(mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPH + 50mg OG</td>
<td>304.40±8.23b</td>
<td>802.00±308.41ab</td>
<td>2.62±0.97b</td>
</tr>
<tr>
<td>BPH + 100mg OG</td>
<td>317.80±9.60c</td>
<td>1010.00±406.50b</td>
<td>3.18±1.28b</td>
</tr>
<tr>
<td>BPH + FINASTERIDE</td>
<td>320.40±8.99c</td>
<td>632.00±234.88ab</td>
<td>1.98±0.75ab</td>
</tr>
<tr>
<td>BPH CONTROL</td>
<td>270.40±8.93c</td>
<td>2214.00±275.37c</td>
<td>8.17±0.87c</td>
</tr>
<tr>
<td>NORMAL CONTROL</td>
<td>322.20±13.99c</td>
<td>418.00±70.50a</td>
<td>1.30±0.20a</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD. Benign prostate hyperplasia (BPH), Ocimum gratissimum (OG), body weight (BW), prostate weight (PW), prostate/body weight ratio P/BW) and protein content (PC). 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 and c) means there is significance between the comparing groups at P < 0.05.

Table 2: Effect of aqueous extracts of Ocimum gratissimum.(OG) in BPH induced Wistar rats on SOD of the liver and kidney and GSH.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>SOD LIVER (µml)</th>
<th>SOD KIDNEY(µml)</th>
<th>GSH (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPH + 50mg OG</td>
<td>7.33±0.72b</td>
<td>6.18±0.45b</td>
<td>50.76±0.82b</td>
</tr>
<tr>
<td>BPH + 100mg OG</td>
<td>8.75±1.53c</td>
<td>7.07±0.74c</td>
<td>52.46±2.18c</td>
</tr>
<tr>
<td>BPH + FINASTERIDE</td>
<td>8.03±0.67c</td>
<td>6.75±1.24c</td>
<td>55.61±2.94d</td>
</tr>
<tr>
<td>BPH CONTROL</td>
<td>3.13±0.76a</td>
<td>2.47±0.63a</td>
<td>27.76±1.52a</td>
</tr>
<tr>
<td>NORMAL CONTROL</td>
<td>11.19±1.10e</td>
<td>9.11±0.63d</td>
<td>63.81±0.67e</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD. Benign prostate hyperplasia (BPH) Ocimum gratissimum (OG). 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.
Table 3: Effect of aqueous extracts of *Ocimum gratissium* (OG) in BPH induced Wistar rats on catalase and MDA of liver and kidney.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>CAT LIVER (mg Protein)</th>
<th>CAT KIDNEY (mg Protein)</th>
<th>MDA LIVER (nmol/ml)</th>
<th>MDA KIDNEY (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPH + 50mg OG</td>
<td>52.05±3.08^b</td>
<td>48.40±2.08^b</td>
<td>20.53±4.35^c</td>
<td>17.66±0.82^d</td>
</tr>
<tr>
<td>BPH + 100mg OG</td>
<td>56.10±1.22^c</td>
<td>52.16±4.03^b</td>
<td>19.25±1.03^b</td>
<td>17.13±0.56^c</td>
</tr>
<tr>
<td>BPH + FINASTERIDE</td>
<td>57.46±1.81^c</td>
<td>56.30±5.71^d</td>
<td>19.62±0.57^b</td>
<td>16.11±0.35^b</td>
</tr>
<tr>
<td>BPH CONTROL</td>
<td>20.53±4.88^a</td>
<td>18.17±1.60^a</td>
<td>31.32±1.40^d</td>
<td>24.29±2.45^e</td>
</tr>
<tr>
<td>NORMAL CONTROL</td>
<td>68.43±10.44^d</td>
<td>60.63±5.60^e</td>
<td>16.49±4.44^a</td>
<td>14.34±1.65^e</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD. Benign prostate hyperplasia (BPH) *Ocimum gratissium* (OG). 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**

Oxidative stress is implicated as one of the risk factors in the natural process of aging as well as in a variety of disease states such as benign prostate hyperplasia. A comprehensive understanding about the association of oxidative stress and etiology of some diseases can be used to find out disease status as well as to work out preventive and therapeutic measures that can be use to tackle such condition in humans [47]. The free radicals, generated during oxidative stress conditions, can consequently act as secondary messengers in intracellular signaling pathways involved in cell cycle progression and proliferation, cell survival and apoptosis, cell migration and angiogenesis, tissue invasion and metastasis, and tumor stemness, thereby resulting to different disease conditions [48], [49]. In addition to these oxidative stress-mediated signaling events, high levels of ROS can also lead to nonspecific damage of macromolecules such as nucleic acids, proteins, and lipids, often creating more free radicals, and triggering a chain of destruction, to promote transformation and alteration in tissue architecture [50].

Emerging research evidence has suggested that antioxidant can control the autoxidation by interrupting the propagation of free radicals or by inhibiting the formation of free radicals and subsequently reduce oxidative stress, improve immune function, and increase healthy longevity [51]. Indeed, oxidation damage is highly dependent on the inherited or acquired defects in enzymes involved in the redox-mediated signaling pathways. Therefore, the role of molecules with antioxidant activity that promote healthy aging and counteract oxidative stress is very crucial to human survival. In this study we investigated the antioxidant effect of aqueous leaf extract of *Ocimum gratissimum* on BPH induced animal model.

In previous study, treatment of rats with testosterone propionate and estradiol valerate for three weeks increased prostate weight as well as levels of serum testosterone and PSA, indicating BPH [52], [53]. Increased level of MDA and reduced levels of GSH, SOD, and catalase activities were observed in this study which is suggestive of oxidative stress. Superoxide dismutase (SOD) is a family of antioxidant enzymes that regulate ROS levels by catalyzing the conversion of superoxide to hydrogen peroxide and molecular oxygen [54], [55]. Catalase is a ubiquitously expressed antioxidant enzyme that is responsible for the degradation of hydrogen peroxide generated from oxidative stress into water and oxygen [56], [57]. Inside the cell, free
Glutathione (GSH) can exist as the reduced GSH and oxidized GSSG forms, although it is primarily maintained in the former state by glutathione reductase \[58\]. GSH is the most abundant intracellular low-molecular-weight thiol and plays a critical role in metabolic protective functions, including hydroperoxide reduction, xenobiotic detoxification, and free radical scavenging \[59\], \[60\].

Glutathione is a pivotal antioxidant present in the microorganisms, plants, and animals. Glutathione prevents the cell damage induced by ROS including lipid peroxides, peroxides, free radicals, and heavy metals \[61\]. Glutathione can scavenge ROS via non-enzymatic and enzymatic reactions. The non-enzymatic antioxidant activity is contributed by the free thiol group of glutathione \[62\]. Additionally, glutathione also detoxifies oxidants and electrophiles via enzymatic reactions which involve glutathione reductase, glutathione peroxidase, and glutathione-S transferase \[63\]. Glutathione plays a crucial role in regulating redox state of the cell, specifically via modulation of the proper tertiary structure of proteins through thiol-disulfide exchange concomitantly with glutaredoxin and protein disulfide isomerases \[64\]. Besides antioxidant properties, glutathione also involves hormones metabolisms such as estrogens, leukotrienes, and prostaglandins and signal transduction for transcription \[65\]. Alteration of glutathione concentration has been linked to adverse health impacts such as dysregulation of cell proliferation, transcription of detoxification enzymes, and apoptosis \[58\].

Malondialdehyde (MDA) is a physiological keto-aldehyde produced as a by-product of peroxidative decomposition of unsaturated lipid. MDA is also a secondary product of lipid peroxidation and is used as an indicator of free radical tissue damage \[59\]. Increasing evidence has indicated that oxidative stress is associated with aging and several age related degenerative diseases \[27\]. A wide variety of reactive oxygen species (ROS) and reactive nitrogen species (RNS) attack DNA directly and form mutagenic lesion. ROS may cause formation of adducts indirectly by inhibiting autocatalytic lipid peroxidation which generates a large variety of genotoxic breakdown products including alkoxy radicals, peroxy radicals and aldehyde such as malondialdehyde \[60\].

With advancing ages, the oxidative stress increases and may aggravate many pathological conditions including BPH in elderly man. The present study showed increased MDA level, indicator of lipid peroxidation. Increased MDA level is indicative of excessive lipid peroxidation in BPH signifying oxidative stress. The decrease of plasma antioxidants level indicates the imbalance between pro-oxidants and antioxidants status in favour of pro-oxidants. There are some studies in this regard, which support this finding of elevated MDA level in BPH suggesting oxidative stress in BPH \[37\], \[61\], \[62\].

Superoxide dismutase, catalase and glutathione which were decreased significantly in BPH control than other groups treated. With this decrease of GSH, SOD and CAT the body were probably unable to detoxify \(\text{H}_2\text{O}_2\) completely. An accumulation of \(\text{H}_2\text{O}_2\) might occur resulting in higher production of OH-radical. The circulating antioxidant enzymes may be used up in an attempt to counteract the enhanced lipid peroxidation in the affected tissue \[63\]. With the decrease of antioxidants in BPH, an accumulation of free radicals such as OH· might occur. These highly reactive oxidant molecules oxidize DNA, lipid and proteins and it reacts with various structures in the vicinity. Any oxidative lesion that is not repaired may lead to mutations, increasing the risk of damaging tissues \[64\]. The enhanced lipid peroxidation occurs as consequence of the insufficient power of depleted antioxidant defense system for a prolonged duration. In addition, it has been suggested that antioxidants have protective role against BPH as well as progressive prostate cancer \[65\].

Conclusively, treatment of the BPH-induced rats with Ocimum gratissimum reversed the effect caused by oxidative stress from BPH as shown by the increase of the endogenous antioxidants.
Interestingly, *Ocimum gratissimum* might have enhanced the antioxidant defence system of the rats, indicating that the plant might be helpful in the management of benign prostatic hyperplasia.

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