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Toxicological evaluation of Aqueous Extract of *Ocimum gratissimum* on Liver and Kidney of Testosterone and Estradiol induced Benign Prostatic Hyperplasia in Adult Male Rats

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ABSTRACT

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 *Ocimum gratissimum* extract (OG) on kidney and liver function indices in BPH was investigated. 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 gavaging for thirty-five days. While group IV was left untreated, group V served as normal control. After thirty-five days of treatment with OG 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 various different biochemical indices. The prostates were harvested and weighed. The levels of urea and creatinine in the treated groups were significantly (P<0.05) reduced when compared with to the BPH control. No significant (P>0.05) differences in serum concentrations of AST, ALT, ALP, and GGT were observed in all the treated groups compared to the BPH control. The extract of *Ocimum gratissimum* leaf demonstrated the potential to reverse the damage caused by BPH on the kidney. There were no noticeable changes recorded in the levels of liver enzymes indicating that *Ocimum gratissimum* had no toxicological effect on the liver. Likewise, BPH condition has no sequela effect on the liver.

Keywords: Toxicology, Evaluation, Aqueous, extract, liver, kidney, Testosterone.

INTRODUCTION

Benign Prostate hyperplasia (BPH) is a common disease in the aging men and causes substantial adverse health effects, leading to enlargement of the prostate and in turn resulting in lower urinary tract symptoms (LUTS). This results to high prevalence rate of the disease which causes serious socio-economic burden [1], [2], [3]. The natural history and evolution of benign prostatic enlargement ends up in urinary obstruction causing degradation of renal function over time [4]. In older men, chronic kidney disease (CKD) is an important medical problem that can even be life-threatening [5]. It has been reported that an average of 13.6% of patients presented to urological clinics for the treatment of BPH had renal failure [6].

Benign prostatic hyperplasia is characterized by the non-malignant overgrowth of prostatic tissue surrounding the urethra, ultimately constricting the urethral opening [7], [8]. Diagnosis of BPH is made based on histologic examination of a prostatic tissue (biopsy, surgery or autopsy), however surrogate measures, namely

lower urinary symptoms, bladder outlet obstruction and prostate enlargement are often used to define BPH as a clinical syndrome [9]. This fact gives us limited insight into the incidence and progression of the disease [10]. The prevalence of BPH thus can be calculated on the basis of histologic criteria (autopsy prevalence) or clinical criteria (clinical prevalence) [11].

Indiscriminate use of herbs in treating various diseases has attracted the interest of many researchers in science and medicine to undertake researches that will significantly unfold the medical importance, mechanism of action as well as toxic effect (if any) of these medicinal plants [12]. The organs mostly affected by toxins from medicinal plants include liver, heart and kidney [13]. Nephrotoxicity constitute a whole gamut of disorders reflecting damage to different nephron segments as a consequence of individual drug mechanisms [14]. Consequences of toxicity might include both glomerular and tubular injuries leading to acute or chronic functional changes [15]. Nephroprotective agents are material that has potential to minimize the effects of nephrotoxic agents. Medicinal plants have curative properties due to the presence of various complex chemical substances [16, 17].

Many people suffering from some diseases prefer herbal medicines instead of orthodox drugs and a significant number of such individuals attest to the effectiveness of such herbal remedies [18]. The use of complementary and alternative medicine (CAM) for the treatment of BPH is becoming popular. It is estimated that 30% of men diagnosed with prostate disease in North America use some CAM products [19], while such products constitute approximately 50% of all medicines prescribed for BPH in Italy [20] and almost 60% of such prescriptions in Germany and Austria [21].

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Ocimum gratissimum* were 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 specimen number 431 deposited in a herbarium in the Department of Botany. The fresh leaves were washed and dried under the shade for seven days. The dried leaves were pulverized using pestle and mortar to get a powder that was used for extraction [22].

Preparation of extract

The powered sample of *Ocimum gratissimum* 200 g was soaked into 200 mL of distilled water, this was filtered after 48 h and filtrate was concentrated in water Bath using rotary evaporator. The solutions were diluted with corn oil, to produce a solution 100 mg/ml [23]. The administration of extract was totally by gavage via oral intubation tube.

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 enlargement at doses of 400 μg T and 80 μg E2 [24] respectively. This was administered to the rats for three weeks subcutaneously in the groin region. 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 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 commenced. The rats were exposed to approximately 12 h 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, Cross River University of Technology, Calabar, Nigeria, (IAEC/CRUTECH/17/083) 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. 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 1mL of estradiol to make up a stock solution of 25 mL. From the stock solutions prepared, 200g rat was injected with 400 μg of testosterone and 80 μg of estradiol separately at the different thighs [25] with modification by Mbaka et al. [26].

Animal grouping and treatment

The animals were divided into five (5) groups comprising of six (6) male rats each. Four groups, I to IV were induced with BPH. Groups I and II received 50 and 100 mg kg–1 body weight (bw) of *Ocimum gratissimum* extract; group III received finasteride (orthodox drug) at 0.1 mg kg–1; all by gavaging for thirty-five days, group IV was left untreated for thirty-five days before sacrifice to assess possible reversal of the exogenous induction and group 5 served as normal control [27]. 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 35 days, the rats were anaesthetised by a brief exposure to trichloromethane vapour and bled by cardiac puncture, 5 ml of blood was collected from each animal and was dispensed into plain bottles, allowed to clot for 30 min and centrifuged at 3500 rpm for 10 min and the clear sera aspirated off for biochemical evaluation viz; alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (γ-GT), urea and creatinine.

The sera were carefully separated and used for the determination of various biochemical analyses.

Assay of Serum Aspartate Aminotransferase (AST) Activity

The assay of the blood serum aspartate aminotransferase (AST) activity was assayed by examining the level of oxaloacetate hydrazone produced with 2, 4-dinitrophenylhydrazine [28].

Assay of Serum Alanine Aminotransferase (ALT) Activity

Serum Alanine aminotransferase (ALT) activity was analyzed by checking the level of pyruvate hydrazone produced with 2, 4-dinitrophenylhydraine [29].

Assay of Serum Alkaline Phosphatase (Alp) Activity

The activity of ALP was assayed by using the kinetic colorimetric technique of optimized Deutsche Gesellschaft for Klinische Chemie (DGKC) by German Society of Clinical Chemists/Dewtsche Gessellschaft fu klinische chemie [30].

Assay of Gamma Glutamyl Transferase (γ-GT) Activity

The serum activity of this enzyme was assayed by using the kinetic colorimetric method as described by Persijin and Van der-Silk, [31].

Determination of Urea Concentration

Serum urea concentration was estimated using the Agape assay kit procedure as explained by Tobacco, [32].

Determination of Serum Creatinine Concentration

Serum creatinine concentration was estimated using the Agape diagnostic kit procedure as described by Allen, [33].

Statistical Analysis

The experimental data were analysed for statistical significance by one-way analysis of variance and post hoc comparison using the SPSS version. All data were reported as mean ± SD and the probability tested at 95 percent level of confidence.

RESULTS

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.

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.

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).

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 OG and finasteride body weight, prostate weight and prostate/body weight ratio

Group	BW (g)	PW (mg)	P/PW ratio (mg/g)
BPH + 50 mg OG	254.30±7.22^{b}	700±300.00^{ab}	2.75±0.04^{c}
BPH + 100 mg OG	267.70±8.60^{b}	1000±400.00^{b}	3.74±0.05^{c}
BPH + Finasteride	270.30±7.98^{c}	530±220.00^{ab}	1.96±0.03^{b}
BPH Control	220.30±7.92^{a}	2110±270.00^{c}	9.58±0.07^{d}
Normal control	272.10±7.90^{c}	310±60.00^{a}	1.14±0.1^{a}

Values are expressed as Mean ± SD. Benign prostate hyperplasia (BPH), *Ocimum gratissimum* (OG), Body weight (BW), Prostate weight (PW), Prostate/body weight (P/BW) ratio. Non- identical superscripts (i.e. a, b, c) means there is significance between the comparing groups at P < 0.05

Table 2: Effect of extract of OG and finasteride on serum enzyme activities

GROUP	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)
BPH + 50mg OG	26.06±1.23^{a}	36.06±0.99^{a}	244.29±2.78^{a}	21.20±2.28^{a}
BPH + 100mg OG	26.15±1.12^{a}	36.22±0.55^{a}	244.24±0.75^{a}	21.20±1.48^{a}
BPH + FINASTERIDE	26.07±1.14^{a}	35.55±3.18^{a}	244.14±2.62^{a}	21.17±1.71^{a}
BPH CONTROL	26.56±1.50^{a}	36.82±1.27^{a}	244.58±2.40^{a}	21.15±0.60^{a}
NORMAL CONTROL	24.20±5.18^{a}	36.01±0.99^{a}	244.12±2.97^{a}	21.15±0.97^{a}

Values are expressed as Mean ± SD. Benign prostate hyperplasia (BPH), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline

phosphatase (ALP), gamma-glutamyl transferase (γ-GT), *Ocimum gratissium* (OG). Identical superscript (i.e. a) means there is no significant difference between the comparing group $P>0.05$.

Table 3: Effect of extracts OG and finasteride on kidney function parameters

GROUP	UREA (mg/dl)	CREATININE (mg/dl)
BPH + 50mg OG	20.51±0.91^{bcd}	0.98±0.06^{b}
BPH + 100mg OG	19.74±1.14^{abcd}	0.98±0.20^{b}
BPH + FINASTERIDE	18.97±1.07^{ab}	0.83±0.15^{ab}
BPH CONTROL	26.41±2.81^{e}	1.96±0.33^{c}
NORMAL CONTROL	17.69±1.07^{a}	0.67±0.35^{a}

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

Benign prostatic hyperplasia (BPH) is one of the most common diseases in older men [34]. This disease is determined by benign prostatic enlargement, which may cause lower urinary tract symptoms that include both obstructive and voiding symptoms. The obstructive symptom causes pressure in the bladder from urinary retention which can directly damage the kidneys or allow bladder infections to reach the kidneys.

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 [35], [36]. Kumar et al. [37] showed in their studies that acute renal failures in patients with obstructive uropathy were due to BPH (38%), neurogenic bladder (19%), obstructive pyelonephritis (15%). This study is aimed at finding the effect of aqueous extract of *Ocimum gratissimum* on the kidney and liver integrity of rats induced with BPH.

Usually, a mixture of biochemical and physiological phenomena plays a role in kidney susceptibility and renal toxicity. The kidney is highly sensitive to chemical poisoning, compared to other organs, partly due to its unequally high blood flow, and because of its complexity both functionally and anatomically [38]. Toxic effects on kidneys are represented through the structure damage of kidneys and changes in the excretory function [39], [40], [41], [42], [43].

In this study, the renal profile parameter urea and creatinine levels significantly increased in BPH control rats compared to treated animals. Changes in urea and creatinine levels indicate that BPH condition may pose serious damaging effect on the kidney at long run. The administration of the extracts seems to reverse this alteration in the excretory function of the kidney which was impaired by the prostate disorder. Similar results of changes in the levels of urea and creatinine were observed in other studies [44], [45]. The plant seems to have nephroprotective effect on the kidney affected by the BPH. Nephroprotective agents are material that has potential to minimize the effects of nephrotoxic agents. Medicinal plants have curative properties due to the presence of various complex chemical substances [46]. This present study indicates that *Ocimum gratissimum* may play a significant role in the management of nephrotoxicity posed by the induction of benign prostatic hyperplasia in rats.

Serum enzymes such as aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) are useful biomarkers of liver injury. These are the enzymes found mainly in the liver, red blood cells, heart, pancreas, kidneys and biliary ducts of the liver. The levels of AST and ALT in serum are used to diagnose if body tissues especially the heart and the liver is injured or not. Research suggests that, when body tissues are damaged, additional AST and ALT are released into the bloodstream and raise the serum enzyme level [47]. As a result, the amount of AST and ALT in the blood is directly associated with the amount of tissue damage.

One of the major organs usually affected by xenobiotics is the liver. Hepatic injury is often associated with changes in the serum and liver levels of some enzymes notably ALT, AST, ALP and GGT [41], [42] and studies with medicinal plant extracts have shown the varying effects of photochemicals on serum and liver enzyme levels. While some phytochemicals are hepatotoxic, others are hepatoprotective. Akpanabiatu *et al.,* [43] in a study of the biochemical effect of *Eleophorbia drupifera* reported a decreased AST level in treated groups. Other studies have similarly reported changes in ALT, AST, ALP and GGT (gamma glutamyl transferase) activities in animals treated with plant extracts [44, 45, 46, 47]. The fact that the levels of ALT, AST, ALP and GGT in serum of both control and treated groups in this study were similar entails that *Ocimum gratissimum* may not have caused any toxic effect on the liver when used tradomedically in the treatment of BPH. The levels of these liver enzymes also indicate that BPH condition may not have damaging effect on the hepatic tissue.

Conclusively, this study shows that *Ocimum gratissimum* may play a crucial role in the management of nephrotoxicity posed by the induction of benign prostatic hyperplasia in rats and that the extract has no toxic effect on the liver and is safe for use depending on the dose.

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