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Research Article

Leaf Extract of *Carica papaya* alleviates Benign Prostate Hyperplasia in male Albino rats by Antioxidative Mechanisms

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ABSTRACT

The pathology of many disorders in humans such as inflammation has been linked with free radical activities. This study was done to evaluate the potential ameliorative action of *Carica papaya* leaf extract on benign prostatic hyperplasia which is highly prevalent today. The animals were grouped randomly into six with five (5) rats in each group. Group 1 served as normal control, while groups (2 – 6) were induced with prostate inflammation using 0.08 mg/kg of testosterone and 0.04mg/kg of oestradiol, parenterally for 28 days. Group 2 and 3 served as positive and negative control. The test groups (4 - 6) were treated with 200, 400 and 800 mg/kg body weight of *Carica papaya* extract for another 28 days. At the end of the treatment period, blood samples were collected for required biochemical analysis; PSA, testosterone, Dihydrotestosterone (DHT), malondialdehyde, (MDA), glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD). The results showed significant ($p<0.05$) increase in the serum PSA, testosterone, DHT and MDA levels of the animals exposed to the inducing agents, whereas there was a significant ($p>0.05$) decrease in the activities of GR, CAT and SOD as compared with the normal control. However, treatment with ethanol leaf extract of *C. papaya* caused significant ($p >0.05$) decrease in PSA, testosterone, DHT and MDA levels, and there was a significant ($p>0.05$) increase in the activities of the antioxidant enzymes in a dose dependent manner as comparable to the normal control. These findings are indication that the extract has potential remedial effects on benign prostatic hyperplasia through antioxidant mechanism.

Keywords: *Antioxidants, Benign Prostate Hyperplasia, Inflammation, Carica papaya*

INTRODUCTION

Every individual through body processes produces free radicals naturally, which is part of the body's intricate system (Teixeria *et al*., 2014). Oxidative stress occurs when the free radicals present in the biological system overwhelms the inherent antioxidants. This imbalance starts to silently cause damage to fatty tissues, DNA and proteins in a biological system (Chandra *et al*., 2015). Oxidative stress can lead to a vast number of diseases like diabetes, atherosclerosis, high blood pressure, heart diseases, aging, neurodegenerative diseases, inflammatory conditions and cancer (Joseph *et al*., 2015).

Benign prostate hyperplasia (BPH) is a malignant proliferation of stromal and epithelial cells of the prostate gland making the gland to enlarge. This often times may or may not be linked with lower urinary tract symptoms (Foo, 2017). BPH is seen mainly in older men between 51 and above and its percentage prevalence increases with age (Lim, 2017). The prostate undergoes two antagonistic processes to maintain a normal size: cell proliferation and

apoptosis. BPH is said to occur when an imbalance causes considerably increased cell proliferation rate more than the rate of apoptosis (Minutoli *et al*., 2016). Currently, drugs used for the treatment of BPH are grouped in six categories: herbal agents, inhibitors of the enzyme 5 α-reductase, selective α-adrenergic blockers, β 3-adrenergic agonists, antimuscarinic agents and inhibitors of the enzyme phosphodiesterase type 5 (Nunes *et al*., 2017).

Plants have been used since time immemorial to meet the primary health care needs of man in different parts of the world especially in developing countries. In Nigeria, majority of the rural dwellers still rely on herbal medicine for their health care needs due to one reasons or the other (Anitha *et al.,* 2018). Good enough, the phytochemicals in these medicinal plants are discovered every day and is been explored as a major source of novel drugs (Anitha *et al.,* 2018). *Carica papaya* commonly called pawpaw is an interesting tree in that some are either female (pistil late) or male (staminate), while others have both male and female flowers on the same plant (Anitha *et al*., 2018). Economically, *Carica papaya* is the most important of Caricaceae species (Singh *et al*., 2020). Men who consume glycopene rich fruits such as tomatoes, guava and paw-paw are less likely to suffer prostate cancer as compared to people who do not eat such fruits (Singh *et al.,* 2020). This study was done due to the prevalence of benign prostatic hyperplasia amongst African men and the promising potentials of *Carica papaya* plant.

MATERIALS AND METHODS

Sample collections

leaves of *Carica papaya* were obtained during the raining season and identified by a taxonomist in the Department of Applied Biology, Ebonyi State University, Ebonyi State, Southeast, Nigeria. The leaves were thoroughly washed and dried under room temperature. The dried leaves were grounded into fine powder and stored.

Extraction of the ethanol leaf extracts

The homogenized *Carica papaya* sample (500g) each was soaked in 500 ml of ethanol for 48 hours. The mixture was shaken at intervals, filtered using a muslin cloth and evaporated to dryness using a rotary evaporator. The filtrate was stored in an airtight container.

Animal Treatment and Experimental design

The 12 weeks old healthy male wistar albino rats weighing approximately 120 - 160g were purchased from University of Nsukka Enugu State, Nigeria. The rats were housed in conventional cages under standard laboratory conditions and acclimatized for two weeks, before the commencement of the treatment. The study was conducted in accordance to the criteria outlined in Principles of Laboratory Animal Care, 1985. The albino rats were weighed and placed randomly into six different groups containing five rats per cage and oestradiol/testosterone were administered to induce benign prostatic hyperplasia. Group 1, not induced served as normal control while benign prostatic hyperplasia was induced in groups (2 – 6) using oestradiol (0.04 mg) and testosterone (0.08 mg)/kg body weight diluted with goya oil (0.01 ml) parenterally at the inguinal region every other day for 28 days. PSA analysis was used to confirm prostate hyperplasia. Group 2 which served as the positive control was given the standard drug (Finasteride 200 mg/kg) while group 3, the negative control was treated with normal saline. The test groups (4 – 6) were treated daily with 200, 400 and 800 mg/kg body weight of the reconstituted ethanol leaf extracts of *Carica papaya* orally for another 28 days. At the end of the treatment period, all rats were fasted overnight, sacrificed under anaesthesia and blood samples were collected through cardiac puncture into heparin and non-heparin bottles for biochemical analysis.

Biochemical Analysis

The serum testosterone and PSA levels were measured using testosterone/PSA ELISA kits (Monobind Inc., Lake Forest, CA92630, USA) while DHT was determined using DHT ELISA kit (ALPCO, 26-G Keewaydin Drive Salem NH03079, USA) respectively, following the manufacturer's instructions.

The oxidative stress parameters were determined according to the Randox kits manufacturer's protocols. Malondialdehyde (MDA) was assayed spectrophotometrically by Wallin *et al.,* (1993) method. Glutathione reductase (GSH) was determined by the method of Goldberg and Spooner (1983). Catalase (CAT) was assayed by Aebi (1983) method and Superoxide dismutase (SOD) was analysed following the method of Fridovich (1989).

Statistical analysis

Data generated were expressed as mean and standard deviations. Statistical significance of difference was determined by performing one- way Analysis of variance (ANOVA) with post-hoc comparisons between the control group and each of the treated groups by Duncan's multiple comparison tests. $P < 0.05$ was considered statistically significant

RESULTS AND DISCUSSION

Effect of Ethanolic Leaf Extract of *C. papaya* on Serum PSA Level

The result in Table 1, shows a significant increase in serum PSA in the rats induced with BPH. The positive control group (Finasteride group) showed a decrease in PSA level as compared to the negative control whereas the *C. papaya* extract treated groups (200, 400 and 800mg/kg) displayed a dose-dependent decrease as compared with negative group.

Table 1. Effect of Ethanolic Leaf Extract of *C. papaya* on Serum PSA of Testosterone/Oestradiol-induced BPH in Rats

Groups	PSA in ng/ml (before treatment)	PSA in ng/ml (after treatment)
Normal	2.78 ± 0.16^{a}	2.89 ± 0.10^{a}
Negative control	8.50 ± 0.20^{b}	8.32 ± 0.21^{b}
Positive control	$8.49 \pm 0.25^{b*}$	$3.32 \pm 0.06^{a*}$
Extracts 200mg/kg	$8.50 \pm 0.30^{b*}$	$4.95 \pm 0.10^{d*}$
Extracts 400mg/kg	$8.51 \pm 0.21^{b*}$	$3.84 \pm 0.15^{c*}$
Extracts 800mg/kg	$8.49 \pm 0.26^{b*}$	$3.58 \pm 0.16^{ab*}$

Results are presented as mean ± SD of 5 rats. Values in the same column having different superscripts differ significantly ($p<0.05$); values in the same row having asterisk (*) as superscripts differ significantly ($p<0.05$).

Effect of Ethanolic Leaf Extract of *C. papaya* on Serum Testosterone and Dihydrotestosterone (DHT) Levels

The induction of BPH using oestradiol/testosterone injection significantly increased serum testosterone and DHT as compared to the normal control (Table 2). The Finasteride was shown to have reduced the testosterone level significantly. The *C. papaya* extract treated groups also reflected a dose-dependent reduction after the treatment period as compared with the negative control.

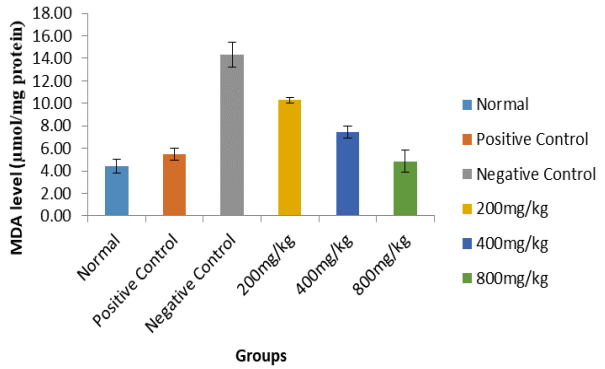

Figure 1. Effect of Ethanolic Leaf Extract of *C. papaya* on Serum Malondialdehyde Level of Rats induced with BPH. The results are mean ± SD of 5 rats.

Table 2. Effect of Ethanolic Leaf Extract of *C. papaya* on Serum Testosterone and DHT on Rats Induced with BPH

Groups	Testosterone (ng/ml)	Dihydro-testosterone (ng/ml)
Normal	6.58 ± 0.15^{a}	2.44 ± 0.04^{c}
Negative control	20.16 ± 0.11^{c}	9.68 ± 0.30^{d}
Positive control	8.95 ± 0.26^{a}	3.61 ± 0.20^{abc}
Extracts 200mg/kg	18.54 ± 0.33^{c}	7.86 ± 0.16^{a}
Extracts 400mg/kg	13.29 ± 0.21^{c}	5.89 ± 0.22^{bc}
Extracts 800mg/kg	9.11 ± 0.19^{b}	4.45 ± 0.28^{ab}

Results are presented as mean ± SD of 5 rats. Values in the same column having different superscripts differ significantly ($p<0.05$).

Effect of Ethanolic Leaf Extract of *C. papaya* on Oxidative Stress Parameters

The results showed that oestradiol/testoterone induced BPH caused an elevation of serum malondialdehyde (MDA) and a decreased level of serum catalase (CAT), glutathione reductase and superoxide dismutase (SOD). Figure 1 showed a decrease in the level of MDA on administration of *C. papaya* leaf extract as compared to the negative control.

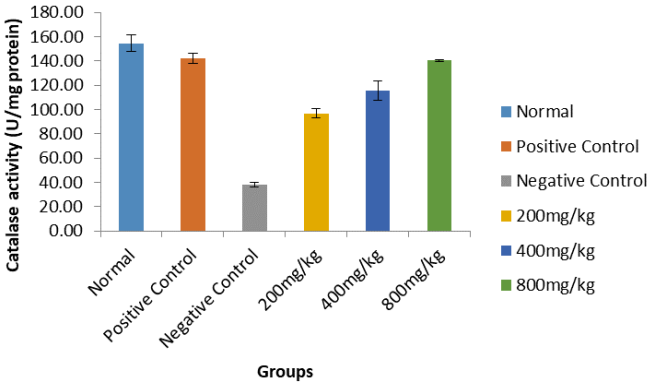

Figure 2: Effect of Ethanolic Leaf Extract of *C. papaya* on Serum Catalase Level of Rats induced with BPH. The results are mean ± SD of 5 rats.

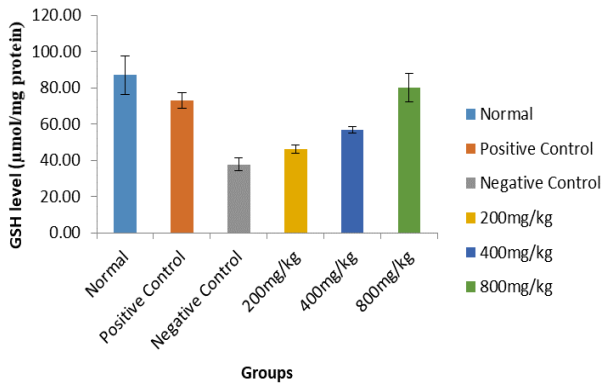

Figure 3: Effect of Ethanolic Leaf Extract of *C. papaya* on GSH level of Rats induced with BPH. The results are mean ± SD of 5 rats.

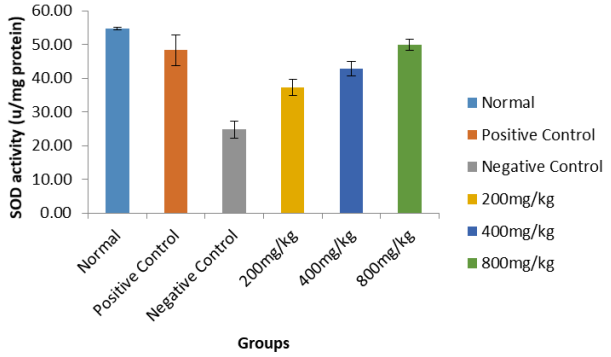

Figure 4: Effect of Ethanolic Leaf Extract of *C. papaya* on Serum Superoxide dismutase Level of Rats induced with BPH. The results are mean ± SD of 5 rats.

DISCUSSION

In the present study, Oestradiol and testosterone were used to induce BPH in albino rats. Since abnormal serum PSA level is a direct indicator of prostatic disorder, the serum elevation of PSA indicated that Oestradiol/testosterone injection successfully induced benign prostatic hyperplasia. The serum PSA level of positive control and extract treated groups were significantly ($P<0.05$) lowered after the treatment period as compared to the negative control (Table 1). Finasteride selectively inhibits type II 5α-reductase that catalyses dihydrotestosterone (DHT) formation from testosterone (Traish and Morgentaler, 2013). The administration of leaf extract of C. papaya significantly lowered the prostatic condition close to normal as observed by a decrease in serum PSA level. This reduction in serum PSA observed may be attributed to its inhibitory effect on the 5α-reductase activity which converts testosterone (Ekeyi *et al*., 2021). This work followed the same trend with Ekeyi *et al*., (2021) who reported that treatment of BPH induced rats with ethanol extract of *C. sieberiana* exerted potent BPH, hypolipidaemic and antioxidant effects. The result also agrees with Joshua *et al*., (2018) who reported that treatment of BPH-induced rats with *Z. portoricensis* stem ethanol extract has a positive outcome by reducing PSA level. The percentage reversal effect of the standard drug and the extracts did not return the serum PSA level to normal, which might be due to the short duration of treatment. Thus, an extension of the treatment period could reveal a maximum effect of the extract.

The significant decrease in serum level of testosterone and dihydrotestosterone (DHT) in rats treated with ethanol leaf extract of C. papaya could be attributed to improved clearing of unbound testosterone in the bloodstream, preventing the conversion of active DHT by 5 α-reductase (Liu *et al*., 2019, Roehrborn *et al*., 2007).

High level of malondialdehyde is an indication of tissue damage associated with lipid peroxidation which leads to the development of BPH (Aydin *et al*., 2006). The reductive action of ethanol leaf extract of C. papaya on the level of malondialdehyde is indicative of its antioxidant potential that can reduce the rate of lipid peroxidation while the increase in the activities of antioxidant enzymes (CAT, GSH and SOD) might be due to the extracts ability to reduce the accumulation of superoxide anion radicals and hydrogen peroxides which accentuates peroxidative activity. This work agrees with Mbaka *et al*., 2013 which reported the antioxidant ability of medicinal plant in the treatment of oestradiol/testosterone induced BPH.

AUTHORS' CONTRIBUTIONS

LNE conceived and designed the study and performed the experimental aspect. CBA performed the experimental aspect of the study. MCO wrote and edited the manuscript. OO analyzed and interpreted the statistical analysis and MEO contributed to the editing and proofreading of the manuscript. All the authors consent to be accountable to every aspect of the paper and gave approval for the publication of the revised version.

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None

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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