EVALUATION OF LIPID PARAMETERS IN TESTOSTERONE-INDUCED BENIGN PROSTATE HYPERPLASIA IN ALBINO RATS

Abstract

Benign prostate hyperplasia (BPH) refers to the enlargement of the prostate gland, a condition that is becoming prevalent in men of 40 years and older. This study investigated lipid parameters in testosterone-induced benign prostate hyperplasia in albino rats. Thirty-six (36) albino rats weighing 180-240g were acclimatized for two weeks and divided into 6 groups of 6 rats each. The BPH was induced using 4mg/kg testosterone propionate in groups 2-6, and treatment was as follows; Group 1(Negative control), Group 2 (Positive control; induced with BPH and not treated), Group 3 (induced with BPH and treated with 1000mg/kg of plantain root extract), group 4 (induced with BPH and treated with 0.45mg/kg of Avodart), group 5 (induced with BPH and treated with combination of herbal extract and Avodart) and group 6 (prophylactic group; treated with herbal extract then induced with BPH). At the end of the treatment, the rats were sacrificed via cardiac puncture. Blood samples were collected into plain bottles for the assay of prostate specific antigen (PSA), malondialdehyde (MDA), and lipid profile. The results indicate that the PSA and MDA levels in the treated groups were significantly lower than the levels in the positive control group. However, there were no significant differences in the levels of the lipid parameters. The results from this study indicate that lipid abnormalities are not prevalent in the onset or progression of BPH. It is recommended that lipid parameters be assayed in the management of BPH in order to confirm or rule out changes in lipid parameters.

Keywords: Benign prostate hyperplasia (BPH), prostate, lipid parameters, Malondialdehyde, Prostate Specific Antigen

Introduction

Benign prostate hyperplasia (BPH) is defined medically as a condition in which there is proliferation of the stromal cells of the prostate gland, which results in the enlargement of the prostate[1]. In other words, BPH refers to a medical condition in which there is an increase in the size of the prostate, and commonly seen in men who are 40 years and older.

BPH is characterized by a number of symptoms known as lower urinary tract symptoms (LUTS). These symptoms are generally classified into two groups, namely voiding symptoms and storage symptoms. The voiding symptoms include hesitancy, intermittency, straining, dribbling and reduced calibre of urine stream, while storage symptoms include the frequency, urgency and nocturia associated with BPH [1].

A number of modifiable factors have been associated with BPH, including age, which is the principal factor. Other factors include diet, hypertension, obesity, smoking, alcohol consumption, hormonal imbalance and dyslipidaemia, and these contribute to the development of BPH [2]. Although dyslipidaemia is reported as one of the modifiable factors that may contribute to the development of BPH [2], there is a paucity of data to prove this. Several studies have reported different results on the levels of lipids in BPH while others have reported no association of lipids with BPH [3].

Materials and Methods

(a) Experimental Animals

Thirty-six (36) male albino rats weighing between 180 - 240g were used for this study. They were obtained from the animal farm of University of Port Harcourt, Nigeria and allowed to acclimatize for a period of fourteen (14) days before the commencement of study. They were allowed free access to standard rat feed and clean water *ad libitum*. Handling of animals in line with relevant guidelines for animal studies.

(b) Extract Preparation

Absolute ethanol was used for the extraction. The plantain roots were collected, properly washed and cut into piece then dried in the oven at 60^0C for 48 hours. The dried roots were milled into coarse powder form, and weighed. Absolute ethanol to root powder was 1:7 dilution, the mixture was placed on an orbital shaker for 24hours. After which, the extract was filtered using Whattman filter paper to separate the coarse root from the mixture. The filtrate was placed in the oven at 60^0C for evaporation of the solvent and proper concentration of the solute. Final extract was left in the oven at 40^0C for 48 hours following which total extract, concentration of extract and moisture content was determined.

(c) Phytochemical Analyses

The extract was then prepared in accordance with ISO:17025. Phytochemicals of interest were determined using the UV visible via scan analysis with the wavelength range of 200 – 1100nm. At each wavelength, its absorption was compared with the UV developed standard for

phytochemicals to determine the phytochemicals present and its quantification was done with the help of the dilution factor which gives the actual concentration which were expressed in mg/ml.

(d) Induction of Benign Prostatic Hyperplasia

This was done using 4mg/kg of testosterone propionate (TP) as adopted from the work of another researcher [4]. Each rat in the appropriate groups was given 4mg/kg of TP dissolved in 1ml of vehicle oil.

(e) list 1 : Experimental Design

Groups	Treatment	Dosage/administration
Group 1	Negative control	Water + Normal meal
Group 2	Positive control Testosterone propionate	Testosterone propionate (TP) daily for 12 days
Group 3	TP Herbal extract	TP for 12 days Herbal extract for 28 days
Group 4	TP dutasteride (avodart)	TP 12 days Dutasteride for 28 days
Group 5	TP Dutasteride (Avodart) + Herbal extract	TP for 12 days Herbal extract & dutasteride for 28 days
Group 6	Herbal extract TP	Herbal extract for 28 days TP for 12 days

The dosage of Avodart (the standard drug) used was 0.045mg/kg, as extrapolated from human dose. The dosage of plantain root extract (*Musa paradisaica*) was 1000mg/kg as determined from our pilot study (not reported).

(f) Sample Collection and Analyses

Blood samples were collected via cardiac puncture in appropriate EDTA, heparinized and sterile bottles for standard laboratory analysis.

Lipid parameters (total cholesterol, tryglycerides and HDL) were determined using enzymatic colorimetric method. LDL was derived from Frielwald's equation. Malodialdehyde (MDA) levels were determined using ELISA technique.

Total white blood cell and platelet counts were determined using haematology autoanalyzer.

(g) Data of Analysis

The data obtained in this study were analyzed using Statistical Package for Social Sciences (SPSS), version 23. Results were expressed as mean ± SD, and p-values less than or equal to 0.05 were considered statistically significant.

Results

(a) Results of Phytochemical Analysis of Plantain Root Extract

The results of phytochemical analysis showed the presence of the phytochemicals indicated in table 1 below.

Table 1: Results of Phytochemical Analysis of Plantain Root Extract

Phytonutrient	Qualitative Results	Quantitative Results (mg/ml)
Steroids	++	42.94 ± 4.16
Terpenoids	+	1.88 ± 0.23
Triterpenoids	+	0.89 ± 0.11
Cardiac glycoside	+	1.25 ± 1.77
Flavonoids	+	0.16 ± 0.05
Alkaloids	+	3.53 ± 0.54
Saponins	+	0.44 ± 0.18
Phenols	-	-
Carbohydrates	+	23.94 ± 2.10
Resins	-	-
Tannins	+	2.18 ± 0.63

KEY: +++ Most absolutely detected

++ Absolutely detected

\+ Slightly detected

\- Not detected

(b) Comparison of Prostate Specific Antigen and Malondialdehyde Levels

The mean values of prostate specific antigen (PSA) and malondialdehye (MDA) of the rats are as shown in table 2 below. The mean PSA and MDA values of the treated rats were significantly lower than the levels of the respective parameters in the positive control group.

Table 2: Comparison of Prostate Specific Antigen and Malondialdehyde

	PSA (ng/ml)	**MDA (nmol/l)**
Group 1 (Negative Control)	0.57 ± 0.09^{a}	1.04 ± 0.35^{a}
Group 2 (Positive Control)	0.73 ± 0.12^{b}	1.15 ± 0.06^{b}
Group 3 (Extract only)	0.62 ± 0.08^{a}	1.05 ± 0.01^{a}
Group 4 (Avodart only)	0.52 ± 0.02^{a}	1.02 ± 0.01^{a}
Group 5 (Combination Therapy)	0.64 ± 0.05^{a}	1.04 ± 0.02^{a}
Group 6 (Prophylactic)	0.56 ± 0.05^{a}	1.04 ± 0.03^{a}
p-value	<0.001	<0.001
F-value	5.778	12.027
Remarks	S	S

Values with different superscripts are significantly different from each other ($p \leq 0.05$).

(c) Comparison of Lipid Parameters

The mean lipid parameters are as shown in table 3 below. There were no significant differences in the lipid parameters.

Table 3: Comparison of Lipid Parameters

	TC (mmol/l)	**HDL (mmol/l)**	**LDL (mmol/l)**	**TG (mmol/l)**
Group 1 (Negative Control)	2.22 ± 0.53	0.73 ± 0.34	1.20 ± 0.50	0.95 ± 0.26
Group 2 (Positive Control)	2.06 ± 0.61	0.53 ± 0.46	1.24 ± 0.70	0.64 ± 0.11
Group 3 (Extract only)	2.26 ± 0.21	0.86 ± 0.61	1.11 ± 0.76	0.67 ± 0.15
Group 4 (Avodart only)	2.47 ± 0.42	0.80 ± 0.15	1.25 ± 0.43	0.88 ± 0.18
Group 5 (Combination Therapy)	2.58 ± 0.25	0.83 ± 0.48	1.38 ± 0.46	0.82 ± 0.11
Group 6 (Prophylactic)	2.79 ± 0.52	0.89 ± 0.31	1.54 ± 0.80	0.82 ± 0.34
p-value	0.151	0.780	0.910	0.195
F-value	1.799	0.490	0.297	1.611
Remarks	NS	NS	NS	NS

Discussion

Benign Prostate hyperplasia (BPH) is one of the commonest urinary tract infections in men from age 40 years and older. This study investigated the immune and lipid parameters in testosterone-induced BPH in albino rats, and treated with plantain root extract and standard drug. The phytochemical analysis of the plantain root extract showed the presence of some important phytonutrients. It has been reported that the pharmacological and medicinal effects of herbal substances could be due to the presence of phytonutrients [[5].

The mean prostate specific antigen (PSA) and malondialdehyde (MDA) levels of the treated rats were significantly lower compared to the positive control. There is proliferation of the cells of the epithelium and stroma of the prostate, which leads to increased prostate [1]. This enlargement may be responsible for the raised level of PSA in the positive control group. The rats in the treatment groups had reduced PSA probably because the treatment administered arrested the further proliferation of the cells of the prostate thereby reducing the mean PSA levels.

The MDA levels in the positive control group were significantly higher than the levels in the treated groups and the negative control group. It has been reported that MDA levels are higher in BPH than in healthy subjects [6]. Inflammation in the prostate is one of the factors that promote the growth prostatic cells and enhance the symptoms of BPH [7]. The inflammation causes lipid peroxidation in the prostatic cells leading to raised MDA levels as the product of lipid peroxidation. The significant reduction in the MDA levels of the rats in the treatment groups could be due to the attenuation of inflammation, and subsequently lipid peroxidation, by the herbal formula and the orthodox drug. It is known that herbal extracts have the potential to attenuate lipid peroxidation [8].

The results from this study show that there were no significant differences in the mean levels of the lipid parameters (Total cholesterol, triglycerides, HDL and LDL). Though there is evidence of lipid peroxidation as shown the results of this study, the non-significant differences in the lipid parameters may be due to the fact that they may not be involved in the lipid peroxidation which is the results of inflammation. Lipid peroxidation involves only polyunsaturated fatty acids (PUFA), which are components of cellular membranes. Other biomolecules that can be involved in peroxidation include hemoproteins, nucleic acids, carbohydrates or steroids [9]. The observation of no significant differences in the lipid parameters could be because there was no dyslipidaemia in the rats. Dyslipidaemia results from insulin resistance. It has been reported that in insulin resistance, there is increased level of lipolysis which leads to increased fatty acid levels, which in turn are converted into triglycerides [10].

The results from this study have also shown that the group of rats treated with a combination of orthodox drug and herbal extract at the respective doses used in this study had results that are similar to the other treatment groups. This is an indication that combination therapy using the substances used in this study do not lead to negative outcomes or side effects.

Conclusion

The results from this study indicate that there are no alterations in the lipid parameters in testosterone-induced benign hyperplasia in albino rats. However, the rats treated with herbal extract and orthodox had significantly reduced levels of PSA and MDA compared to the positive control group, probably due to the attenuation of inflammation by the treatment.

COMPETING INTERESTS:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

References

1. Kapoor, A. (2012). Benign prostate hyperplasia (BPH) management in the primary care setting. The Canadian Journal of Urology, 19(Suppl 1), 10-17.
2. Kim, E. H., Larson, J. A., Andriole, G. L. (2016). Management of benign prostate hyperplasia. Annual Reviews Medicine, 67, 37-51.
3. Erbay, G., & Ceyhun, G. (2022). Association between hyperlipidemia and prostatic enlargement: A case-control study. Urologia Journal, 89(1),58-63.
4. Obisike, U. A., Nwachuku, E. O., Boisa, N. & Nduka, N. (2019). Determination of exogenous testosterone propionate dose for induction of benign prostatic hyperplasia in rat model. European Journal of Biomedical and Pharmaceutical Sciences, 6(13), 141 – 147
5. Afolabi, F. (2013). Phytochemical constituents of some medicinal plants in SouthWest Nigeria. IOSR Journal of Applied Chemistry, 4(1), 76-78
6. Arsova-Sarafinovska, Z., Eken, A., Matevska, N., Erdem, O., Sayal, A., & Savaser, A. (2009). Increased oxidative/nitrosative stress and decreased antioxidant enzyme activities in prostate cancer. Clinical Biochemistry, 42,1228-1235.
7. Chughtai, B., Lee, R., Te, A., & Kaplan, S. (2011). Role of inflammation in benign prostatic hyperplasia. Review Urology, 13(3),147-50
8. Félix, R., Valentão, P., Andrade, P.B., Félix, C., Novais, S.C., & Lemos, M.F.L. (2020). Evaluating the In Vitro Potential of Natural Extracts to Protect Lipids from Oxidative Damage. Antioxidants, 9(3),231.

9. Fritz, K.S., Petersen, & D.R. (2011). Exploring the biology of lipid peroxidation-derived protein carbonylation. Chemical Research in Toxicology, 24(9),1411-9.
10. Haile, K., Haile, A., & Timerga, A. (2021). Predictors of Lipid Profile Abnormalities Among Patients with Metabolic Syndrome in Southwest Ethiopia: A Cross-Sectional Study. Vascular Health Risk Management, 17,461-469