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PHYTOSTEROL-ENRICHED FRACTION OF *Morinda lucida* EXTRACT INHIBITS TESTOSTERONE-INDUCED BENIGN PROSTATIC HYPERPLASIA IN RATS.

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ABSTRACT

Benign prostatic hyperplasia (BPH) is a prevalent disorder among ageing men and could lead to prostatic carcinoma if not managed early and properly. Although orthodox drugs have been used for managing BPH, they have various side effects which necessitated the search for alternatives, especially from plant sources. Plants such as *Morinda lucida* have been folklorically used to manage the condition. This study was designed to investigate the effect of the phytosterol-rich extract of *Morinda lucida* leaf on testosterone-induced BPH in rats. Thirty rats were administered treatment for twenty-eight days as follows: Normal control (NC), BPH control, 200 mg/kg *Morinda lucida* Phytosterol-enriched Extract (MLPE), 400 mg/kg MLPE extract, and 1 mg/kg Finasteride group. After twenty-eight days, BPH control, 200mg/kg, 400mg/kg extract, and finasteride groups received 5mg/kg testosterone subcutaneously for another twenty-eight days along their various treatments. The rats were fasted overnight, sacrificed, and the testes were excised. Sperm parameters were carried out under light microscopy. Antioxidant assays were done using spectrophotometry. Inflammatory biomarkers were assayed via the Enzyme Linked Immunosorbent Assay method. The results showed a significant reduction in the sperm motility, count and viability status of BPH control relative to NC. However, BPH rats treated with MLPE significantly increased the parameters. Malondialdehyde concentration was high, while GSH level, GPx, and catalase activities were low in BPH rats relative to NC. Conversely, antioxidant status was normalized as a result of MLPE administration. Elevation in TNF- α and interleukin-1 β was observed in BPH control compared to NC. Nevertheless, there were significant decreases in the inflammatory biomarkers in groups administered MLPE relative to BPH rats. The above results suggest that MLPE exerts its effects via free radical scavenging, antioxidant and anti-inflammatory activities. It could therefore be concluded that MLPE could serve as a promising therapeutic agent for the prevention and management of BPH.

1.0 Introduction

Benign prostatic hyperplasia (BPH) is one of the most common urological conditions affecting ageing men. It is characterized by a non-malignant enlargement of the prostate gland,

resulting from the proliferation of stromal and epithelial cells within the transition zone of the prostate. This histological growth frequently manifests as nodular hyperplasia, which can lead to significant anatomical and functional changes in the lower urinary tract [1].

BPH is a progressive condition that can cause lower urinary tract symptoms (LUTS), including increased urinary frequency, urgency, nocturia, weak urinary stream, and incomplete bladder emptying. While BPH itself is not life-threatening, the associated symptoms can significantly reduce quality of life. They may lead to complications such as acute urinary retention, recurrent urinary tract infections, and, in severe cases, kidney damage [1].

The pathogenesis of BPH is multifactorial, involving a complex interplay of hormonal, genetic, and age-related factors. Androgens, particularly dihydrotestosterone (DHT), play a pivotal role in driving prostate growth. Additionally, chronic inflammation, changes in the stromal-epithelial microenvironment, and alterations in growth factor signaling have been implicated in the development and progression of the disease [1].

Currently, the most widely used drugs for the treatment of BPH, 5 α -reductase inhibitors and alpha-blockers, are helpful because of their ability to relax the smooth muscles of the prostate and bladder neck, thus, aiding urine flow. However, they produce some side effects such as erectile dysfunction, decreased libido, reduced sperm count, fatigue, dizziness, and a higher risk of developing prostate cancer [2,3]. Thus, it is inevitable to search for alternative therapies with little or no side effects, especially from plant sources.

Morinda lucida Benth (Brimstone tree in English Language and Oruwo in Yoruba Language) is a plant commonly used in Nigeria for the treatment of a variety of diseases which include inflammations and cancers [4,5,6,7]. The *Rubiaceae* family of flowering plants includes the genus *Morinda*. It is widespread throughout the tropics and consists of roughly 80 species. These species could be shrubs, vines, or trees. All *Morinda* species produce clusters of fruits [8]. Five species, including *Morinda lucida* Benth, are known in Africa. The Brimstone tree is a common name for the characteristic West African

rainforest *Morinda lucida* [8]. The *Morinda lucida* is a medium-sized tree that grows to a height of 15 to 25m. It has crooked or twisted branches, smooth or roughly scaly grey or brown bark, and shiny foliage. Alkaloids of different kinds, including anthraquinones make up most of the *Morinda lucida* extract's ingredients. One of the four most popular traditional medicines used against various maladies in Nigeria is *Morinda lucida* which is a versatile species that produces colours, wood, fuel, and other products [8].

Studies have reported the antiproliferative potential of compounds isolated from this plant, including phytosterols [9,10,11,12]. There is, however, no pharmacologically relevant *in vivo* experimental study on the anti-BPH potential of phytosterols from this plant. This study therefore aimed to investigate the anti-BPH activity of *Morinda lucida* leaf phytosterol-enriched extract in testosterone-induced BPH rats.

2.0 Materials and Methods

2.1 Materials

2.1.1 Drug and Chemical: Testosterone, finasteride, dihydrotestosterone enzyme-linked immunosorbent assay (ELISA) kit, Silica gel 60, ethyl acetate, hexane, methanol, pentobarbital, formalin, and Bradford reagent were obtained from Bristol Scientific, Liverpool Road Apapa Lagos, an accredited supplier of Sigma Aldrich, St. Louis, MO, USA.

2.1.2 Plant Material: *Morinda lucida* leaves were collected from Ondo City, in Ondo State, Nigeria (7°05'35.38" N 4°50'7.01" E) and the plant was identified by a Taxonomist in the Herbarium Unit in the University.

2.1.3 Ethical approval

The protocols and experimental design of this research were approved by the Animal Care and Use Ethical Research Committee, University of Medical Sciences, Ondo, Ondo State, Nigeria, with approval number, UNIMED-AREC/Apv/2021/015, in compliance with international standard for the care and use of laboratory animals.

2.1.4 Animals: Thirty male Sprague-Dawley rats (125 \pm 20 g, about 42 days old) were obtained from the Animal House of the University of Medical Sciences Ondo City, Ondo State.

2. 2 Methods

Table 1: Experimental design

Groups	Treatments –Duration	
	Day 1-28	Day 29-56
Control	Olive oil	Olive oil
BPH	Olive oil	Testosterone (5 mg/kg b.w)
MLPE	MLPE (200 mg/kg b.w)	MLPE (200 mg/kg b.w) + testosterone (5 mg/kg b.w)
MLPE	MLPE (400 mg/kg b.w)	MLPE (400 mg/kg b.w) + testosterone (5 mg/kg b.w)
Finasteride	Olive oil	Finasteride (1 mg/kg b.w) + testosterone (5 mg/kg b.w)

BPH = benign prostatic hyperplasia; MLPE = *Morinda lucida* leaf phytosterol-enriched extract

2.2.1 Preparation of phytosterols-enriched fraction of *Morinda lucida* leaf

The leaves were dried under shade and pulverized using a blender at the Biochemistry Department, University of Medical Sciences Ondo City Ondo State Nigeria. Extraction and preparation of phytosterols-enriched fraction was done as previously described [10]. Pulverized leaves were soaked in a solution of 50% methanol and 50% distilled water. The supernatant was collected and concentrated by evaporation using a rotary evaporator. The concentrate was dried in a lyophilizer, and the dried powder was subjected to column chromatography which was set up using column-grade silica gel (silica gel 60, Merck) in a glass column and eluted gradient-wise with 10% ethyl acetate in 90% hexane. Fractions 33-45, which has been identified as a combination of phytosterols, was used for the study [10].

2.2.2 Study design

The study utilised biological samples (serum/testis) collected from testosterone-treated experimental rats which were pre-treated with *Morinda lucida* leaf phytosterol-enriched extract for 28 consecutive days. Subcutaneous injection of rats with testosterone (5 mg/kg) for another 28 consecutive days is an accepted standard method of inducing benign prostatic hyperplasia in (BPH) experimental animals [13, 14]. Animals were housed in rooms maintained at 22 ± 2 °C and 30–70% humidity, with water and a standard pellet diet given *ad libitum*.

2.2.3 Assessment of sperm motility

This was determined following Zemjanis' method [15]. The sperm sample was collected from the epididymis and diluted with 2.9% sodium citrate dehydrate solution at 37°C. The suspension was moderately stirred and shrouded with a coverslip (24 by 24 mm). The motility was estimated by counting about fifteen microscopic area at X200 magnification power. The sperm was classified into two, namely motile and immotile.

2.2.4 Evaluation of epididymal sperm count

It was assessed according to the method of Rotruct, [16]. The epididymis was excised and homogenized in 0.9% normal saline and sifted via a nylon filter membrane. Fifty microlitre (50µl) suspension was collected by admixing 2.5 µl sperm with 47.5 µl diluent (0.35% formalin, 5% NaHCO₃ and 0.25 trypan blue). Ten microlitres of suspension was added to the Neubauer chamber and observed under the X400 magnification lens power of a light microscope.

2.2.5 Measurement of sperm morphology and viability

These were carried out in reference to the method of Habig [17]. A coat of sperm suspension was made on a neat glass slide and viability was evaluated via staining with eosin (1%) and 5% nigrosine, 3% C₆H₅Na₃O₇.2H₂O. Morphological damage was assessed by staining the coated dye that has 0.1 g eosin and 0.3 g fast green dissolved in ddH₂O and ethanol (2:1). Four hundred sperm

cells were estimated from the rats each in order to ascertain abnormalities

2.2.6 Sperm plasma membrane integrity

The HOST (hypo-osmotic swelling test) was carried out according to Turner and Lysiak's technique [18]. The hypo-osmotic solution was prepared by dissolving 1.838 g sodium citrate and 3.378 g fructose in 250 ml final volume water. An aliquot of 50 μ l of sperm suspension obtained from cauda epididymis was made in 500 μ l hypo-osmotic solution and incubated at physiological temperature for 1 hour. Not less than 200 cells were counted for each sample from various fields at X400 magnification using a light microscope. The HOST-positive sperms with twisted tails were also estimated.

2.2.7 Estimation of Hormonal status

The assay was performed in accordance with the manufacturer's instructions. 25 μ l of standards, control, and test samples were pipetted into ELISA wells. After that, 100 μ l of working testosterone-enzyme conjugate reagent was added to the wells. The microplate was slightly tilted for about thirty minutes for a thorough mixture. It was then covered and incubated for sixty minutes at room temperature. The liquid was removed and the wells were washed thrice with 300 μ l 1X wash buffer. The remaining water was removed using a clean absorbent. Afterwards, 100 μ l TMB substrate reagent was introduced into all the wells. The plate was covered, and the samples were incubated for fifteen minutes at room temperature. Stop solution (50 μ l) was added to the wells and mixed for fifteen to twenty minutes. The absorbance was read on an ELISA microplate reader at 450 nm within fifteen minutes after stopping the reaction.

2.2.8 Evaluation of antioxidant status

A post-mitochondrial fraction was obtained from the centrifugation (10,000 rpm, 15 minutes) of tissue samples homogenized in buffer containing 50 mM Tris-HCl and 1.15% KCl. In catalase (CAT) activity, H₂O₂ was used as the substrate by

the Claiborne method [19] and measured spectrophotometrically at 240 nm. GSH status was evaluated at wavelength 412 nm in tandem with Jollow *et al.* [20] method. Percentage malondialdehyde (MDA) was assayed at 532 nm spectrophotometrically by following the protocol of Farombi *et al.*, [21].

2.2.9 Histopathological investigation

Fixation of testis samples in Bouin's solution was done for one day [22]. The dehydration process (via tissue processor Leica TP 1020) was then followed by using ascending gradient alcohol concentrations, and cleaned with xylene. After that, it was immersed in paraffin wax [23]. About 4-5 μ m fractions were cut using a microtome, fixed on slides, stained with hematoxylin and eosin (H&E) then observed at X400 magnification under a light microscope. The nuclei were stained blue, and the cytoplasm, pink.

2.2.10 Statistical Analysis

Data were analyzed by employing One-way ANOVA and post hoc Dunnett's t-test through Graph pad Prism 8 (La Jolla, California, USA). P < 0.05 was deemed significant.

3.0 RESULTS

3.1 Phytosterol-enriched fraction of *Morinda lucida* ameliorates hormonal status in testosterone-induced BPH in rats

The results show that there were significant increases (p<0.05) in the testosterone, luteinizing hormone and oestrogen status in the BPH control compared to normal control. Meanwhile, there was no difference (p>0.05) in the concentrations of these hormones in the groups treated with doses of MLPE (figures 1, 2, and 3 respectively) relative to normal control.

In Figure 4, a similar increase in FSH was observed in the BPH control group, whereas a dose-dependent decrease was noticed in rats treated with MLPE.

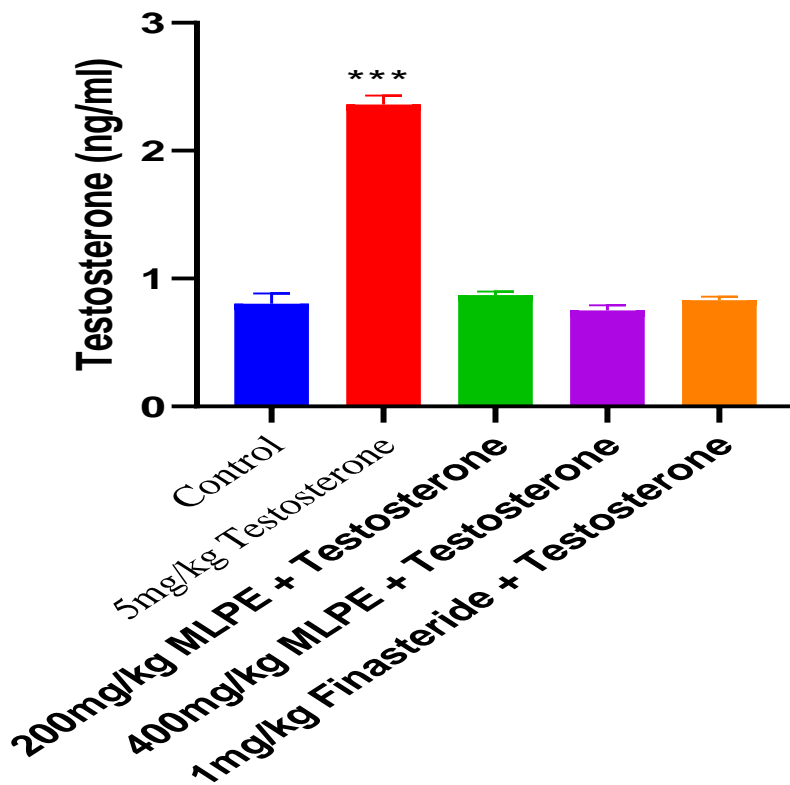


Figure 1: Effect of a phytosterol-rich fraction of *Morinda lucida* on testosterone level in testosterone-induced BPH in rats

MLPE: *Morinda lucida* phytosterol-enriched Extract

***P<0.001: Test groups vs Control

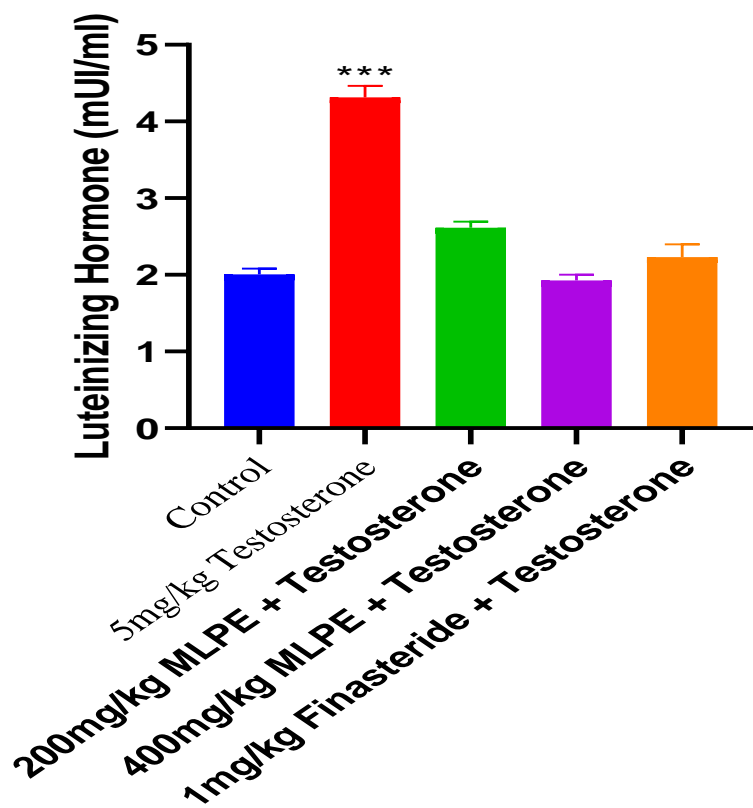


Figure 2: Effect of a phytosterol-rich fraction of *Morinda lucida* on luteinizing hormone status in testosterone-induced BPH in rats

MLPE: *Morinda lucida* phytosterol-enriched Extract

***P<0.001: Test groups vs Control

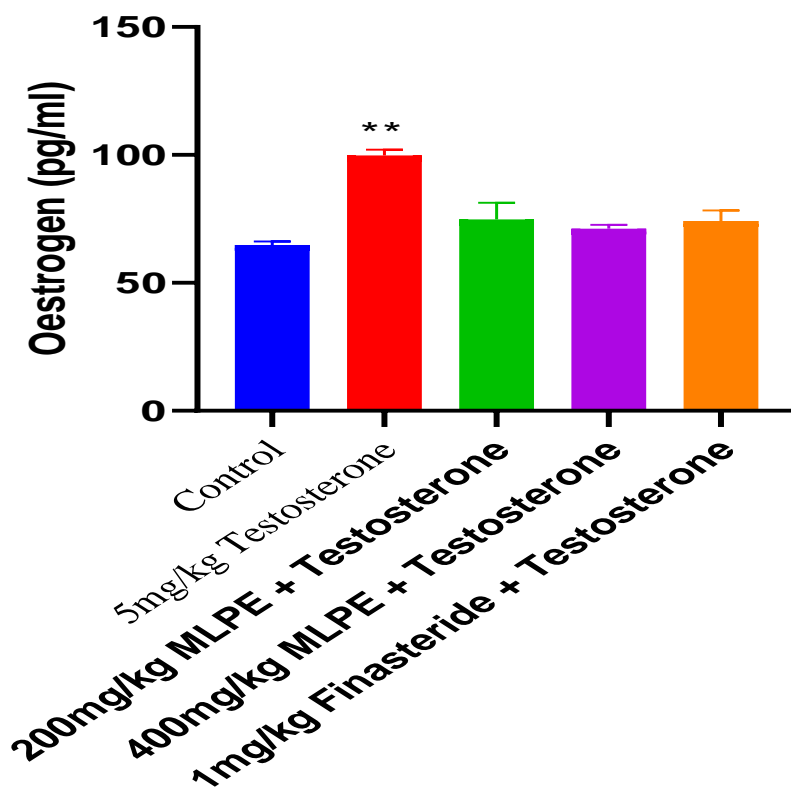


Figure 3: Effect of a phytosterol-rich fraction of *Morinda lucida* on Oestrogen level in testosterone-induced BPH in rats

MLPE: *Morinda lucida* phytosterol-enriched Extract

**P<0.01: Test groups vs Control

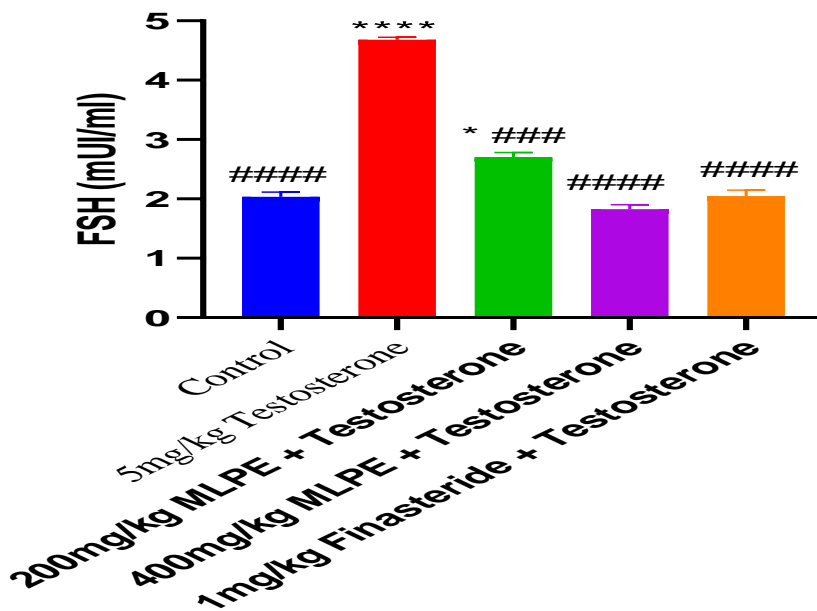


Figure 4: Effect of a phytosterol-rich fraction of *Morinda lucida* on follicle-stimulating hormone in testosterone-induced BPH in rats

MLPE: *Morinda lucida* phytosterol-enriched Extract

* $P < 0.05$: Test groups vs Control

**** $P < 0.0001$: Test groups vs Control

$P < 0.0001$: Other groups vs BPH control (Testosterone Group)

3.2 Enhancement of antioxidant status in testosterone-induced BPH rats by MLPE

There was a significant reduction ($p < 0.05$) in the catalase activity of the BPH control relative to normal control. However, no significant difference ($p > 0.05$) was observed in the activity of this enzyme in MLPE when compared to normal control (fig. 5). GSH level also significantly reduced in the BPH control group in comparison with the normal control rats. Whereas, there was a dose-independent increase

in the status of GSH in the groups treated with the doses of MLPE compared to normal control (fig. 6). GPx activity declined significantly in the BPH control compared to the normal control. Conversely, there was no significant difference ($p > 0.05$) in GPx activity in the treated groups when compared with the normal control (fig. 7). Malondialdehyde was found to significantly increase ($p < 0.05$) in BPH control in comparison to normal control. Meanwhile, no significant difference ($p > 0.05$) was observed between the treated groups and the normal control (fig. 8).

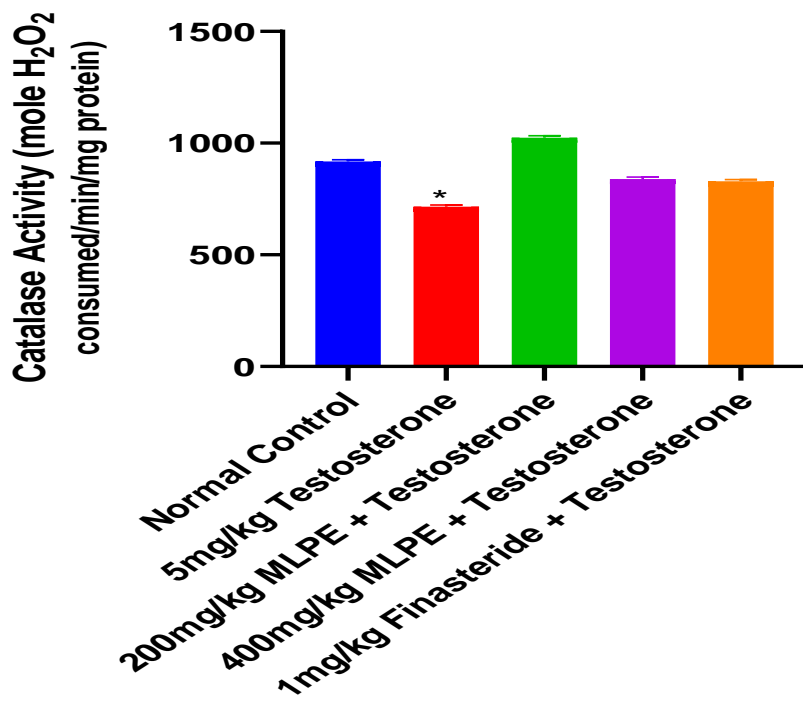


Figure 5: Effect of a phytosterol-rich fraction of *Morinda lucida* on catalase activity in testosterone-induced BPH in rats

MLPE: *Morinda lucida* phytosterol-enriched Extract

*P<0.05: Test groups vs Control

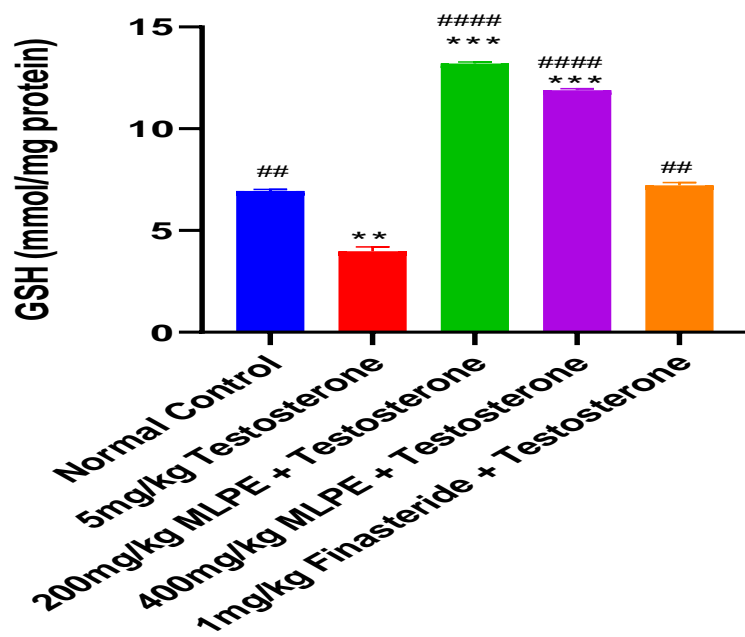


Figure 6: Effect of a phytosterol-rich fraction of *Morinda lucida* on reduced glutathione level in testosterone-induced BPH in rats

MLPE: *Morinda lucida* phytosterol-enriched Extract

**P<0.01: Test groups vs Control

***P<0.0001: Test groups vs Control

***P<0.0001: Test groups vs Control

####P<0.0001: Other groups vs BPH control (Testosterone Group)

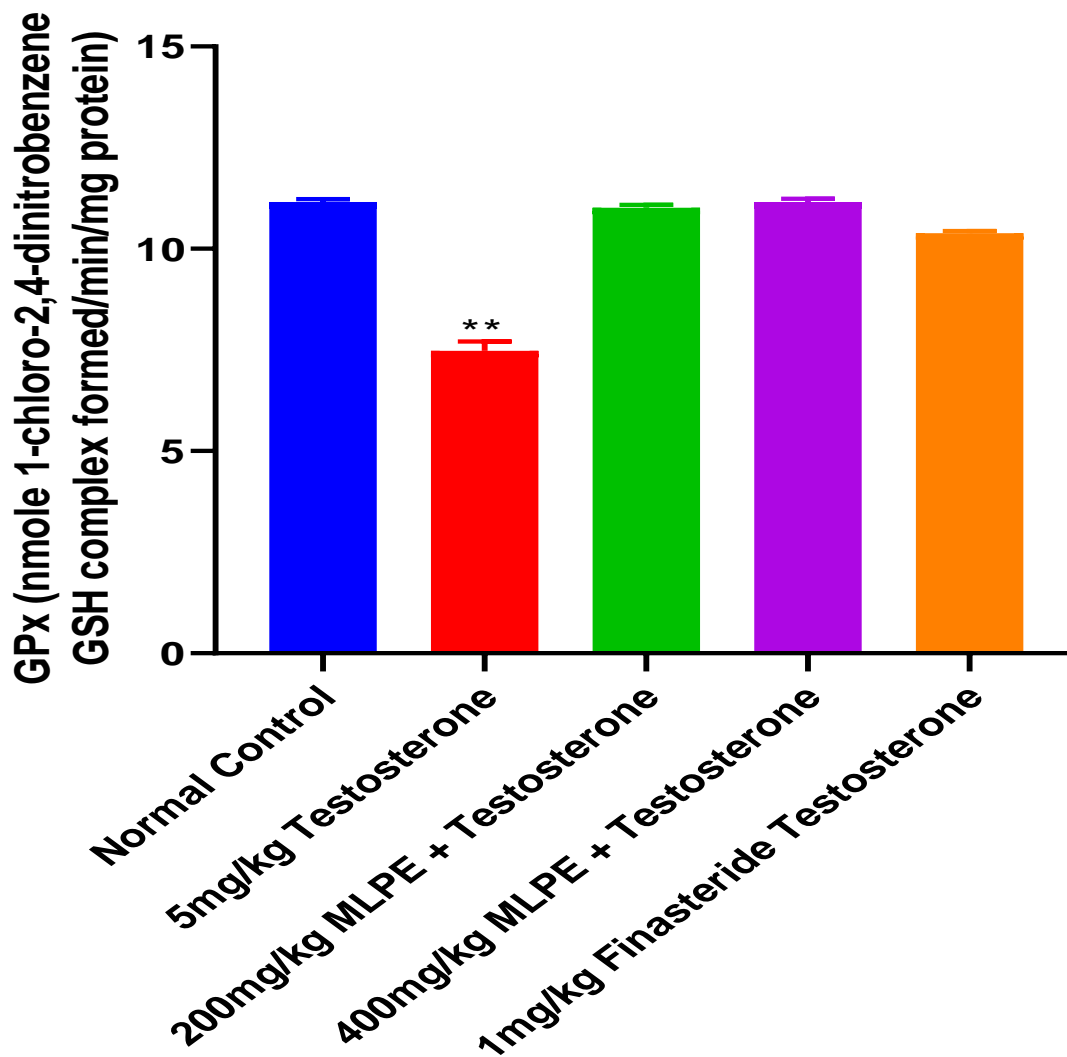


Figure 7: Effect of a phytosterol-rich aqueous fraction of *Morinda lucida* on glutathione peroxidase in testosterone-induced BPH in rats

MLPE: *Morinda lucida* phytosterol-enriched Extract

**P<0.01: Test groups vs Control

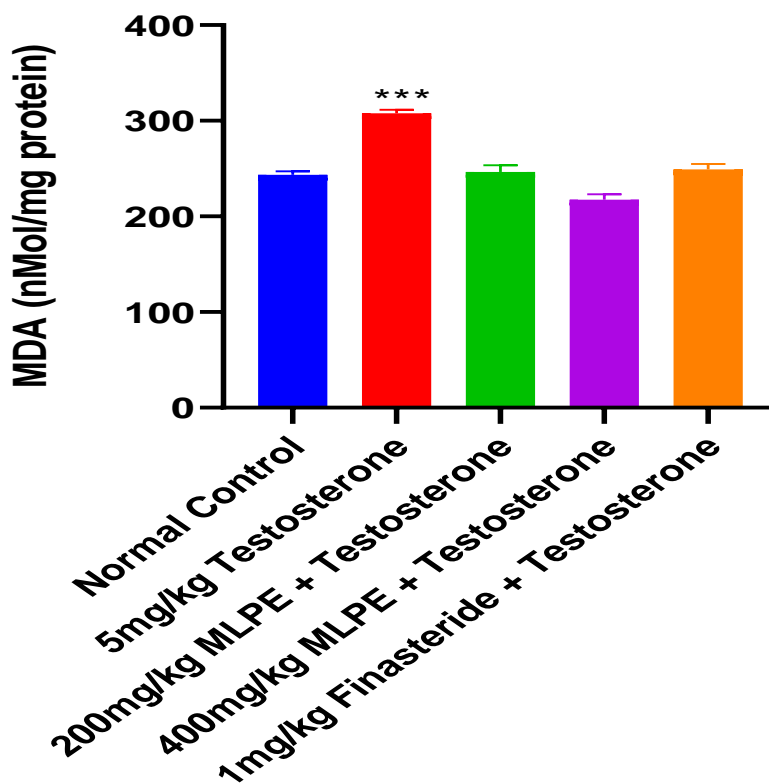


Figure 8: Effect of a phytosterol-rich fraction of *Morinda lucida* on malondialdehyde concentration in testosterone-induced BPH in rats

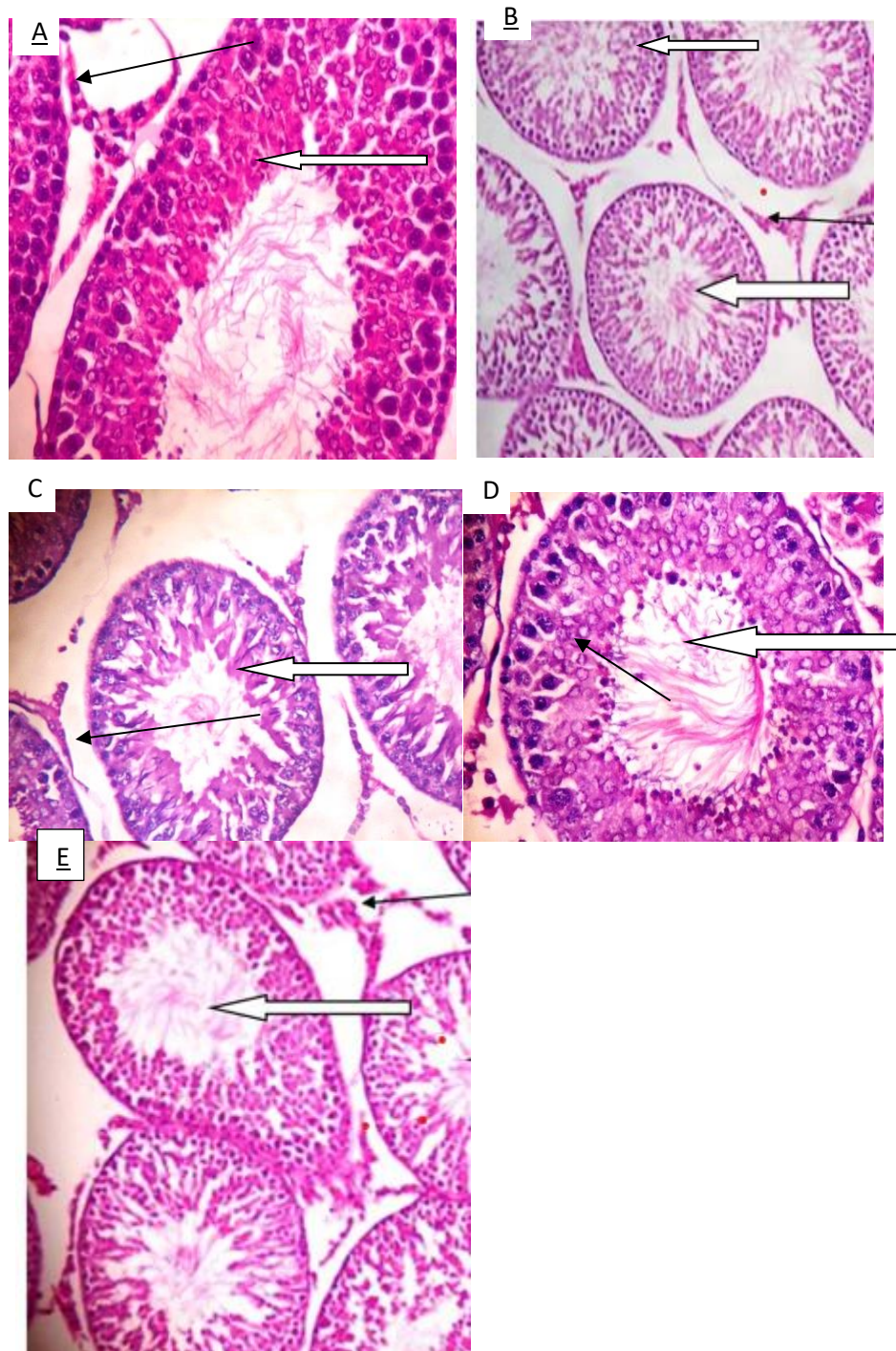
MLPE: *Morinda lucida* phytosterol-enriched Extract

*****P<0.001: Test groups vs Control**

3.3 Histological study of testicular architecture of testosterone-induced BPH rats treated with MLPE

Plate A shows a photomicrograph of a testicular section stained by Haematoxylin and Eosin showing normal testicular architecture with normal seminiferous tubules and normal maturation stages with the presence of spermatozoa within their lumen (white arrow). The spermatogonia cells and the Sertoli cells are normal. The interstitial spaces show normal Leydig cells (slender arrow). Plate B shows some normal seminiferous tubules with normal maturation stages and the presence of spermatozoa within their lumen (white arrow). A few other seminiferous tubules with maturation

arrest are also seen (black arrow). Plate C shows several normal seminiferous tubules with lumen containing spermatozoa (white arrow), normal and completely developed germinal cells. The interstitial spaces show Leydig cells (slender arrow). Plate D shows normal seminiferous tubules with lumen containing spermatozoa (white arrow), normal and completely developed germinal cells. The interstitial spaces show Leydig cells (slender arrow). In plate E, several normal seminiferous tubules are observed with lumen containing spermatozoa (white arrow), and normal as well as completely developed germinal cells. Very few seminiferous tubules are seen with maturation arrest (black arrow), and the interstitial spaces show Leydig cells (slender arrow).



3.4 Inflammatory biomarkers status reduced in testosterone-induced BPH rats after administering MLPE.

In Figures 9 and 10 respectively, TNF- α and interleukin-1 β levels significantly increased in BPH rats when compared to the normal control. Upon treatment with phytosterol-rich MLPE, the inflammatory biomarkers were reduced to normal as compared to the normal control.

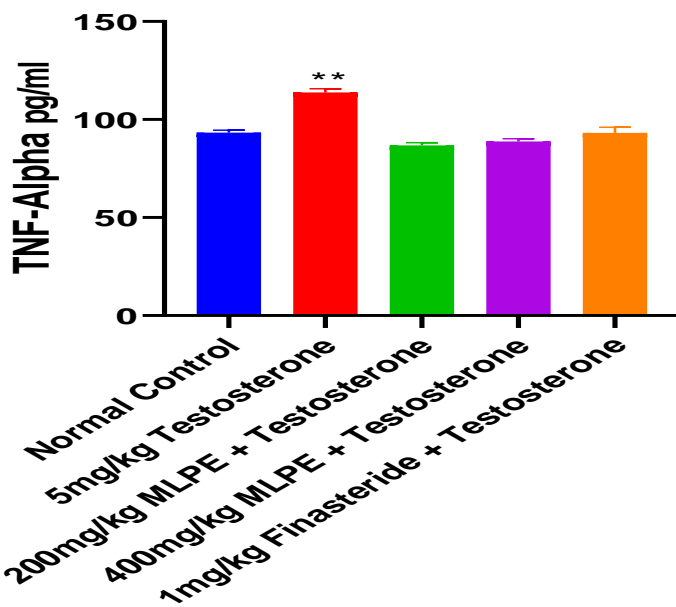


Figure 9: Effect of phytosterol-rich fraction of *Morinda lucida* on TNF- α in testosterone-induced BPH in rats.

MLPE: *Morinda lucida* phytosterol-enriched Extract

**P<0.01: Test groups vs Control

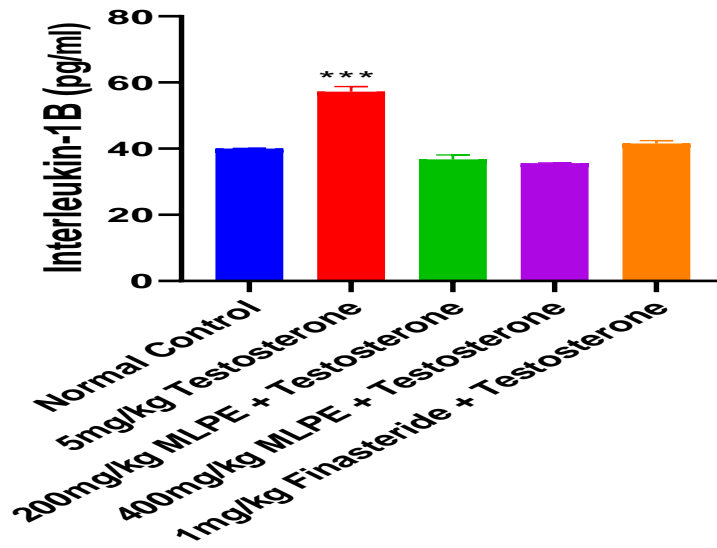


Figure 10: Effect of phytosterol-rich fraction of *Morinda lucida* on interleukin-1 β concentration in testosterone-induced BPH rats.

MLPE: *Morinda lucida* phytosterol-enriched Extract

***P<0.001: Test groups vs Control

TABLE 1: HOSTS/SPERM PARAMETERS OF BPH RATS TREATED WITH PHYTOSTEROL-RICH *Morinda lucida* EXTRACT

Groups	HOST Positive (%)	HOST Negative (%)	Sperm Count (X10 ⁶ /ml)	Motility (%)	Viability (%)
Normal Control	91.3±2.52	8.67±2.52	146±14.11	93.3±2.89	95.67±1.15
5m/kg Testosterone	76±2.10**	22.2±1.0**	123±9.11**	85±2.2*	73±1.2**
200mg/kg MLPE + Testosterone	82.5±5.80*	17.5±5.80*	132.5±10.25	90±0	95.5±1.0
400mg/kg MLPE + Testosterone	81.75±7.46*	18.25±7.46*	142.75±22.35	90±4.08	94.75±3.30
1mg/kg Finasteride + Testosterone	82.1±1.23*	16±3.12*	144.31±2.5	89.2±4.6	90.6±23

3.5 Sperm analysis parameters of BPH rats treated with MLPE

In Table 1, the sperm count, motility and viability reduced significantly ($p < 0.05$) in BPH control when compared to normal rats. In contrast, upon treatment, there was no significant difference ($p > 0.05$) observed in the sperm parameters relative to normal control. HOST positive declined significantly ($p < 0.05$) in the BPH group in comparison with the normal control. Significant amelioration ($p < 0.05$) was evident upon treatment of BPH rats with doses of phytosterone-enriched MLPE relative to BPH control. HOST negative shows a noticeable elevation in BPH control compared to the normal control. However, after treatment of BPH rats with doses of the extract, there was significant improvement ($p < 0.05$).

MLPE: *Morinda lucida* phytosterol-enriched Extract

* $P < 0.05$: Test groups vs Control

** $P < 0.01$: Test groups vs Control

4.0 Discussion and Conclusion

4.1 Discussion

Benign prostatic hyperplasia is becoming alarmingly high in the world population, especially people in low-income and developing countries. It is characterized by the enlargement of the prostate which results in urine retention (acute or chronic). The major intervention for the management of this disorder is the use of 5 α -reductase inhibitor, a drug that helps prevent the conversion of testosterone to dihydrotestosterone (DHT) implicated in the onset of BPH. However, side effects such as impotence or decreased sexual desire, anxiety, depression, and ejaculatory disorder among others, have been associated with the use of this drug. Therefore, bioactive principles in plants with less or non-toxic effects are being employed to manage BPH. Plants such as *Garcinia kola*, *Coco nucifera*, *Heliotropium indicum* and *Morinda lucida* among others, are traditionally used for managing BPH [24].

Elevated testosterone level has been discovered to be involved in the onset of BPH as it is converted into dihydrotestosterone, which precipitates BPH. From the study, increased testosterone observed in BPH control was significantly reduced upon administration of MLPE doses. This result was corroborated by a previous study which reported that male quails which were fed with plant sterols experienced declined testosterone levels [25]. It was also observed from the results that other reproductive hormones were significantly high in the BPH group. Studies have shown that a high level of oestrogen in males stimulates prolactin which in turn triggers prostate enlargement and reduces apoptosis, thus, causing BPH [26]. Different oestrogen receptor isoforms have been discovered to be implicated in cell proliferation, growth factor secretion and differentiation which could lead to the onset of BPH. The follicle-stimulating hormone usually sends signals to the testes to synthesize sperm and its level remains stable after puberty. Elevated level FSH, however, could promote excessive secretion of sperm, thus leading to enlargement of the prostate; this was observed in the BPH control group in our results. However, the administration of doses of phytosterol-enriched extract was able to circumvent this condition by reducing the hormonal level to normal. It has been reported that an increased level of LH is associated with the onset of benign prostatic hyperplasia or prostate carcinoma [27].

In the sperm parameters, the observed significant reduction in the sperm count, motility, and viability in the BPH rats was suggestive of the fact that spermatogenesis occurs in the animals. This assertion was corroborated by the reduced HOST positive and increased HOST negative parameters. HOST explains if the membrane of the sperm cells have been compromised or not. The results suggest a compromise in the integrity of the sperm membrane. The histological investigation which reveals that there was maturation arrest noticed in BPH control rats, is also in concord with previously reported sperm parameter results. However, treatment with doses of phytosterol-rich extract increased motility, viability and sperm count to normal. An increase in HOST

positive and a reduction in HOST negative are suggestive of sperm cell membrane integrity compromise.

Excessive free radical generation which leads to oxidative stress was discovered to be the third cause of BPH. During the onset of the disease, prostatic stromal cells promote the induction of inflammatory cytokines which results in free radical generation. Our results reveal that malondialdehyde was significantly high in the BPH control rats, thereby depicting lipid peroxidation of the membrane as a result of free radical production. Malondialdehyde was significantly reduced to normal upon administration of a phytosterol-rich extract of *Morinda lucida*. This report was in agreement with our results on the antioxidant status of BPH rats treated with doses of phytosterol-rich extract. Elevated catalase, GPx activities, and GSH status in the extract-treated rats suggest that the free radical generated during the disease was scavenged by the antioxidant capacity of the extract [28].

In BPH rats, levels of the pro-inflammatory cytokines TNF- α (Figure 9) and interleukin-1 β (Figure 10) were markedly elevated compared to the normal control group. This increase underscores the involvement of chronic inflammation in the progression of BPH, as both TNF- α and interleukin-1 β are key mediators in inflammatory signaling and are known to contribute to tissue remodeling, cellular proliferation, and stromal-epithelial interactions within the prostate. Although under physiological conditions TNF- α contributes positively to spermatogenesis, elevated level of this cytokine results to enlargement of prostate [29].

Treatment with phytosterol-rich MLPE led to a significant reduction in these inflammatory biomarkers, effectively restoring their levels to near-normal values as observed in the control group. These results suggest that MLPE exerts a potent anti-inflammatory effect, potentially through the inhibition of pro-inflammatory cytokine production or by modulating signaling pathways associated with inflammation.

The ability of MLPE to normalize TNF- α and interleukin-1 β levels indicates its therapeutic promise in mitigating inflammation-driven mechanisms in BPH. This finding not only supports the anti-inflammatory properties of phytosterols but also positions MLPE as a candidate for further research and potential use in the management of BPH.

4.2 Conclusion

From the above data, it could be inferred that the phytosterol-rich fraction of *Morinda lucida* leaf extract exhibited a preventive effect on testosterone-induced BPH rats via its antioxidant, anti-inflammatory, cytoprotective, and sperm-boosting activities; and it may be a good phytotherapeutic agent for the prevention of BPH.

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Conflict of interest

We declare no conflict of interest on this research work.

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