EFFECT OF METHANOL EXTRACT OF *Duranta erecta* LEAVES ON THE OXIDATIVE STRESS STATUS IN TESTOSTERONE-INDUCED BENIGN PROSTATIC HYPERPLASIA IN ALBINO RATS

BY

UGWU, CHRISTOPHER CHIDIEBERE

(PG/M.Sc/17/02465)

DEPARTMENT OF BIOCHEMISTRY

UNIVERSITY OF NIGERIA

NSUKKA

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TITLE

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc) DEGREE IN PHARMACOLOGICAL BIOCHEMISTRY, UNIVERSITY OF NIGERIA, NSUKKA

BY

UGWU, CHRISTOPHER CHIDIEBERE

(PG/M.Sc/17/02465)

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SUPERVISORS: DR. (MRS) C. A. ANOSIKE

FEBRUARY, 2020

CERTIFICATION

Ugwu, Christopher Chidiebere, a postgraduate student of the Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, with Registration Number; PG/M.Sc/17/02465, has satisfactorily completed the requirements for the award of degree of Master of Science (M.Sc) in Biochemistry (Pharmacological Biochemistry). The work embodied in this dissertation is original and has not been submitted in part or full for any other diploma or degree of this or any other university.

Dr. (Mrs) C. A. Anosike	**Prof. H. A. Onwubiko**
(Supervisor)	(Head of Department)

External Examiner

DEDICATION

This work is dedicated to Almighty God for His divine provision throughout this programme.

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ABSTRACT

Benign prostatic hyperplasia (BPH) is a common urinary tract disorder reported among ageing men of which inflammation, oxidative stress, proliferative, and apoptotic changes play important roles. The present study was carried out to evaluate the antioxidant potential of methanol extract of *Duranta erecta* leaves in *in vitro* models and its effects on some biochemical parameters in testosterone-induced BPH in male albino rats. The extract was subjected to phytochemical screening, DPPH assay, ferric reducing antioxidant power assay, nitric oxide assay, and antioxidant vitamins and minerals analysis. Acute toxicity of the plant extract was determined using Lorke's method. Benign prostatic hyperplasia was induced in adult male albino rats (203-305 g) by subcutaneous injection of testosterone propionate for 28 days. All the rats injected with testosterone propionate had a serum prostate specific antigen (PSA) level ≥ 1.70 ng/ml. At the end of treatment, rats were anesthetised and blood was collected via cardiac puncture to determine serum levels of PSA, testosterone, dihydrotestosterone and acid phosphatase. The prostate was weighed, assayed and histologically examined. The phytochemical screening of the extract indicated the presence of flavonoid (24.20 ± 0.14 mg QE/g), alkaloid (15.87 ± 1.71 mg/g), total phenol (12.73 ± 0.61 mg GAE/g), tannin (9.24 ± 0.03 mg TAE/g), terpenoid (8.90 ± 0.96 mg/g), steroid (2.65 ± 0.55 mg/g) and saponin (5.55 ± 0.76 mg/g). The results of all the *in vitro* antioxidant assays proved that the extract had an antioxidant potential in a concentration dependent manner. The antioxidant activity of the sample was compared to the ascorbic acid and gallic acid as standards. The antioxidant mineral composition of the extract revealed the presence of zinc (1.82 ± 0.03 mg/100g) and selenium (0.59 ± 0.04 mg/100g). The antioxidant vitamins composition of the extract showed lower concentrations of vitamin C (0.35 ± 0.01 mg/100g) and vitamin E (0.68 ± 0.07 mg/100g). The extract had an LD_{50} greater than 5000 mg/kg b.w and therefore was not acutely toxic for oral use. *Duranta erecta* extract significantly ($p < 0.05$) reduced testosterone-induced increase in prostate weight, prostatic index, serum testosterone, dihydrotestosterone, PSA and acid phosphatase relative to the untreated rats. Testosterone caused significant ($p < 0.05$) increase in malondialdehyde as well as reduction in glutathione, superoxide dismutase, catalase and glutathione peroxidase activities which were attenuated by the extract. The disruption of the prostate histoarchitecture by testosterone was also ameliorated by *Duranta erecta* extract. Methanol extract of *Duranta erecta* leaves showed antioxidant properties and reduced the effect of testosterone-induced BPH possibly through enhancement of antioxidant defense mechanisms and inhibition of inflammatory mediators. Thus, *Duranta erecta* could be a potential phytotherapeutic agent in the management of BPH in men.

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CHAPTER ONE

INTRODUCTION

Benign prostatic hyperplasia (BPH) is among the most frequent diseases in ageing men. In the 4th decade of life, BPH is demonstrable in 30–40 % of men, and its prevalence increases almost linearly to 70–80 % in those older than 80 years (Madersbachera *et al*., 2019). Benign prostate hyperplasia (BPH) disease is characterized by uncontrolled proliferation of the prostate epithelial cells and stromal cells, which results in increased prostate size (Pais, 2010). As prostate enlarges, it constricts the urethra and reduces urine outflow, thus creating lower urinary tract symptoms (LUTS) (Manuj and Luk, 2014). Lower urinary tract symptoms (LUTS) includes symptoms such as increased urinary frequency, urgency, nocturia leads to an increased risk of obstructions of the urethra, urinary retention and urinary infections (Miller and Tarter, 2009).

A shift in prostatic androgen metabolism is the underlying factor in BPH (Dvorkin and Song, 2002). During that time, the conversion rate of testosterone to dihydrotestosterone by 5α-reductase increases within the prostate. This increase results in prostatic accumulation of dihydrotestosterone, an active form of testosterone that causes cell proliferation (Dvorkin and Song, 2002). The pathophysiology of this disease remains poorly understood. However, numerous studies reported that oxidative stress, suppression of apoptosis and proliferation surge play crucial roles in its development and progression (Shoieb *et al*., 2018). A compensatory cellular proliferation might be precipitated by oxidative stress resulting in hyperplastic growth in prostatic tissue (Minciullo *et al*., 2015). Moreover, oxidative stress can activate transcription factor NF-κB which in turn regulates inflammatory and cellular proliferation pathways (Hamid *et al*., 2011). Furthermore, the suppression of cell apoptosis, which effects a rise in the total number of both stromal and epithelial cells, has been highly associated with the development of BPH. Subsequently, enhancing cell apoptosis has been proposed as a promising strategy for the evolution of anti-BPH agents (Hong *et al*., 2013; Mosli *et al*., 2015).

1.5 ANATOMY AND HISTOLOGY OF THE HUMAN PROSTATE

1.1.1 Embryology

The prostate gland develops from the pelvic portion of the urogenital sinus, which is 10-12 weeks of gestation (Praveen, 2013). The prostate arises after the development of numerous endodermal buds, which initially proliferate throughout the entire length of the primitive urethra. The endodermal buds next invade the surrounding urogenital sinus mesenchyme which is responsible for the development of connective tissue and muscular constituent of the definitive prostate. The gland is well differentiated by the end of fourth month. Conversion to dehydrotestosterone is essential for the growth and development of the prostate (Praveen, 2013).

1.1.2 Gross Anatomy

The human prostate gland is a pyramid-shaped organ located beneath the bladder with the apex (corresponding to apex of the pyramid) contacting the penile urethra and the base contacting to the bladder (Paner, 2010). The prostate lies below the urinary bladder and is located in front of the rectum. The prostate surrounds the prostatic urethra that is the conduit for urine flow from the bladder. The normal prostate weighs about 15–20 g. The seminal vesicles are located bilaterally at the base of the prostate. The human prostate is a single gland with different histological zones (peripheral, transition, and central zones). As shown in Figure below, the peripheral zone is wrapped around the outer portion of the prostate distally and is the site of origin of the majority of prostate cancers. It constitutes about 70 % of tissue in the normal prostate (Paner, 2010). The transition zone is located near the prostatic urethra and is inconspicuous in most young men, constituting about 5 % of the prostate. In the majority of older men, the transition zone is enlarged considerably by benign prostatic hyperplasia, an extremely common benign proliferation in transition zone tissue. Most radical prostatectomy specimens show evidence of variable degrees of benign prostatic hyperplasia (Ittmann, 2017).

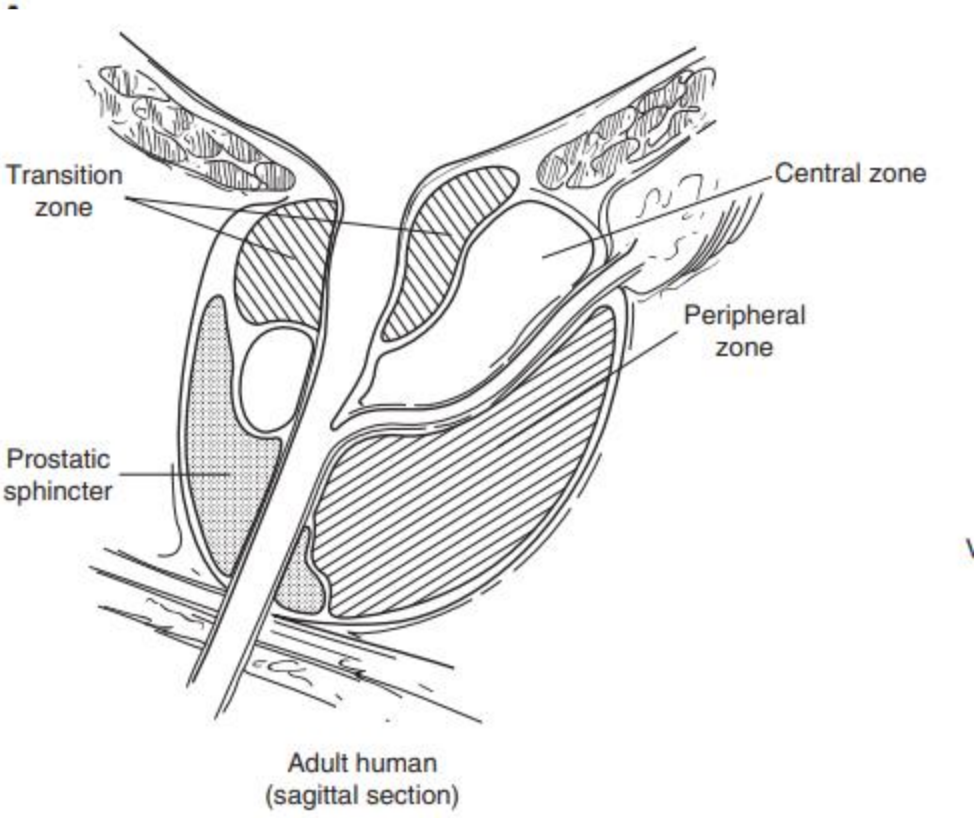

Figure 1: Schematic illustration of human prostate showing the location of the three zones of the prostate.

Source: (Ittmann, 2017)

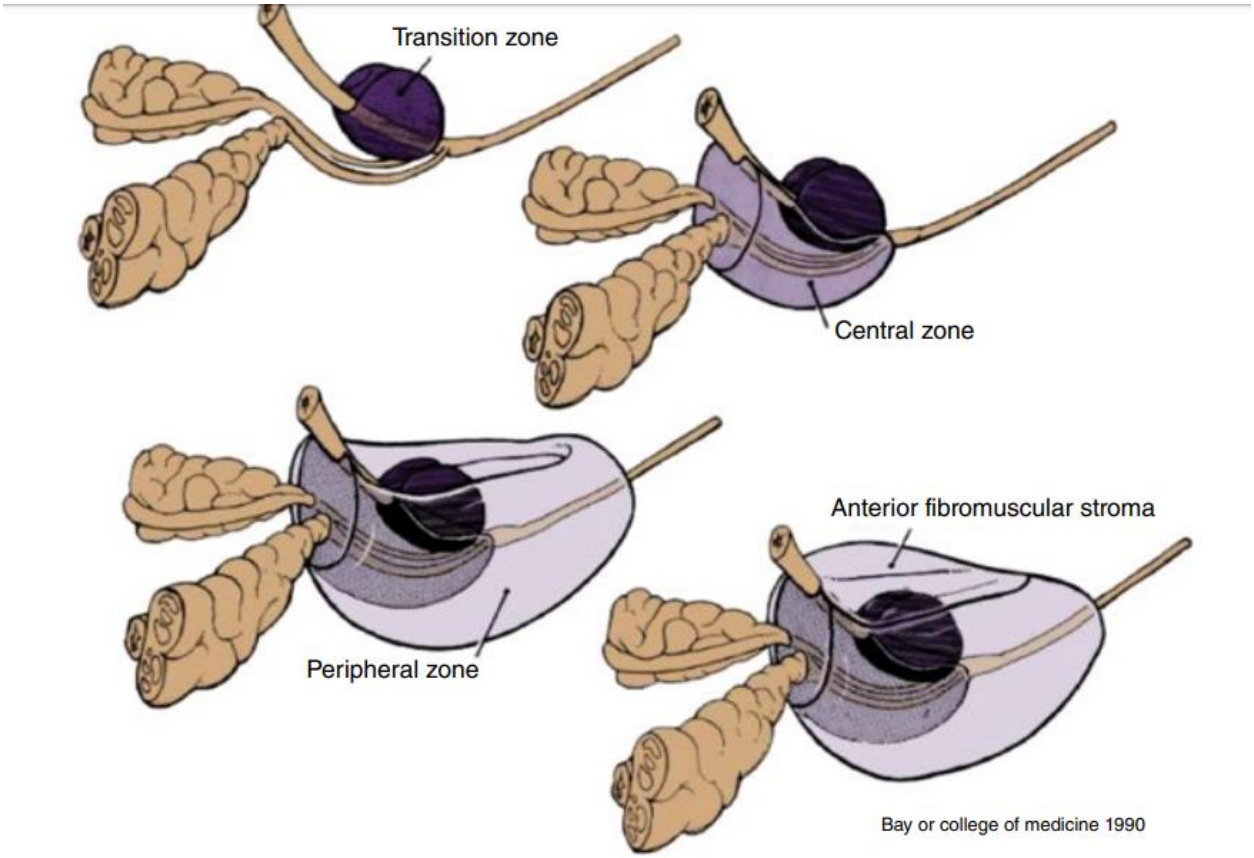

Figure 2: McNeal's zonal anatomy of prostate
Source: (McNeal, 1990)

Cancers also arise in the transition zone and there is considerable evidence that cancers arising in the transition zone are clinically and biologically different than peripheral zone cancers, although there is considerable overlap. The central zone is a cone-shaped region, with the wider portion at the base of the prostate and the apex at the verumontanum surrounding the ejaculatory ducts. It is not the site of origin of any disease process but of course can be secondarily involved by cancer (Ittmann, 2017).

1.2 RISK FACTORS FOR BENIGN PROSTATE HYPERPLASIA (BPH)

The risk factors for BPH include age, metabolic syndrome, diabetes, obesity, hypertension, diet and sex hormone levels. Typically, these factors do not occur in combination, but in certain men they can overlap (Chughtai *et al*., 2016).

1.2.1 Age

BPH increases with age, which has been confirmed by numerous studies. For example, in a study of 278 men (mean age at the start of the study: 58 years) enrolled in the Baltimore Longitudinal Study of Aging who had undergone at least two MRI scans to determine prostate volume, the median prostate volume was 28.1ml (range: 4.4–135.0ml) on the first MRI and 31.1ml (range: 8.7– 237.3ml) at the end of the study period with a median follow-up of 4.3 years. It was reported that prostate volume increased at a median rate of 0.6ml per year (range: 9.9 to 62.1 ml), which represented a median annual change of 2.5 % (Loeb, 2009). Although symptom severity cannot be correlated directly with prostate volume, having a large prostate volume is a risk factor for the development of LUTS. That is, larger prostates are associated with increased risks of urinary retention, increased future need for surgery and clinical progression of BPH (Roehrborn, 2011).

1.2.2 Metabolic Syndrome

Metabolic syndrome includes hypertension, dyslipidaemia, glucose intolerance, central obesity and insulin resistance with compensatory hyperinsulinaemia (De Nunzio *et al*., 2012). New findings on the development of BPH support the notion that metabolic syndrome can influence the natural course of these conditions. For example, in a study of 158 patients with LUTS secondary to BPH, significantly larger prostate glands were observed in men with individual components of metabolic syndrome (Hammarsten *et al*., 1998); these men also had faster annual prostatic growth than those without components of metabolic syndrome. Specifically, the annual prostatic growth rate was increased by 47 % in men with type 2 diabetes mellitus, 17 % in men with hypertension, 36 % in obese men, 31 % in men with low levels of HDL cholesterol and 28 % in patients with high levels of fasting insulin (Hammarsten *et al*., 1998). A recent meta-analysis on the relationship between metabolic syndrome and BPH reported that patients with metabolic syndrome had significantly higher total prostate volumes than those without (Gacci, 2015). Further meta-regression analysis showed that the differences in prostate volume were significantly higher in obese patients, older patients and in those with low serum concentrations of HDL cholesterol. However, no difference was noted between patients with and those without metabolic syndrome for International Prostrate symptom score (IPSS) (Gacci, 2015).

Men with LUTS also have significantly higher levels of glycosylated haemoglobin (indicating poorer control of blood glucose) than men without LUTS (Rohrmann *et al*., 2005). Having three or more components of metabolic syndrome is associated with an increased risk of having LUTS (Rohrmann *et al*., 2005). However, the effect of diabetes on LUTS and, in particular, bladder dysfunction is thought to be multifactorial (Golbidi and Laher, 2010). Urothelial dysfunction, detrusor physiology and neuronal impairment are involved in bladder dysfunction and have been reported to occur subsequently in LUTS (Yoshimura *et al*., 2005), complicating the diagnosis of LUTS that is purely related to BPH. Bladder dysfunction related to diabetes can manifest as either detrusor over activity or poor detrusor function (Golbidi and Laher, 2010). A similar effect on bladder function can be seen with atherosclerosis and hypoperfusion (Michel *et al*., 2015). Various experimental animal studies have shown that a chronic reduction of bladder perfusion can initially cause detrusor overactivity, but if severe or chronic, bladder underactivity can also occur (Michel *et al*., 2015). These recent studies outline that LUTS related to the different components of metabolic syndrome are multifactorial and cannot be purely attributed to BPH.

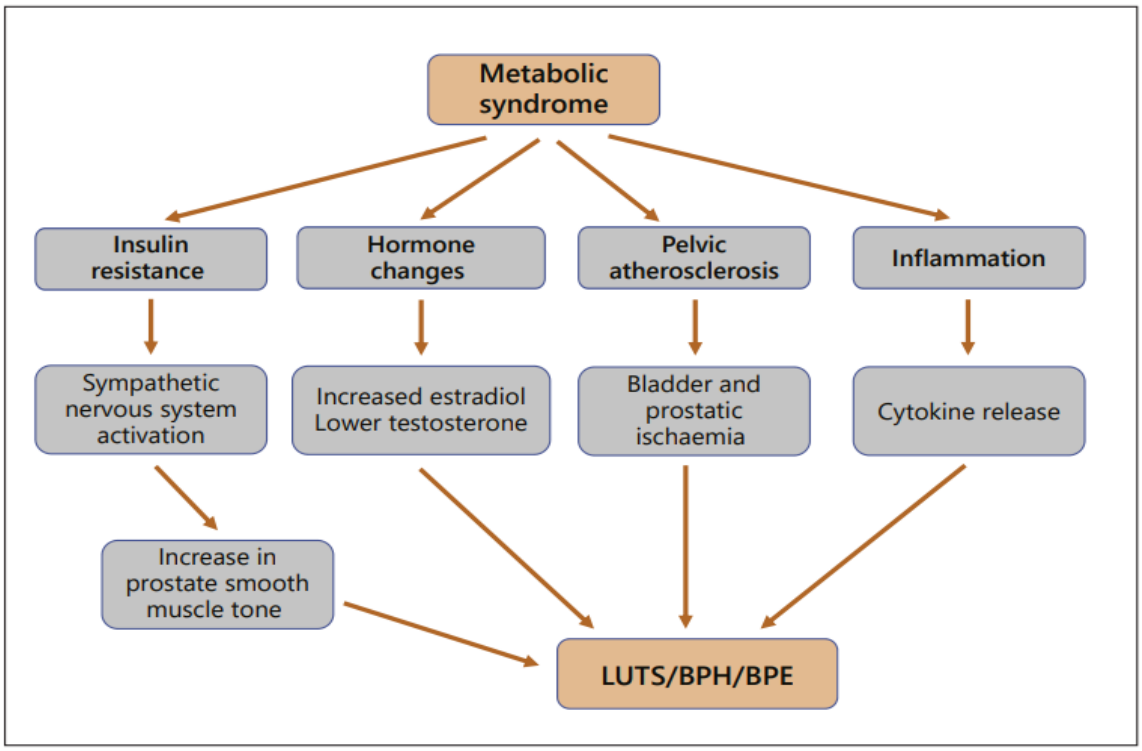

Figure 3: The potential role of the metabolic syndrome in the pathophysiology of BPE, BPH and LUTS.
Source: (Madersbacher *et al*., 2019).

1.2.3 Obesity

Increased levels of adipose tissue have been shown to be associated with greater prostate volume (Kellogg *et al*., 2013; Patel and Parsons, 2014). The effect of obesity on the risk of symptomatic BPH was assessed in 5,667 men enrolled in the placebo arm of the Prostate Cancer Prevention Trial (Kristal *et al*., 2007). Over a period of 7 years, participants were assessed annually with their weight and body circumferences measured and also had their LUTS evaluated using the IPSS questionnaire. In the study, 'total BPH' was defined as receipt of treatment or report of two IPSS values of > 14 and 'severe BPH' was defined as receipt of treatment or report of two IPSS values of ≥ 20. Each 0.05 increase in the waist-to hip ratio (a measure of abdominal obesity) was associated with a 10 % increased risk of total BPH ($P < 0.003$) and severe BPH ($P < 0.02$) (Kristal *et al*., 2007).

Obesity is one component of the metabolic syndrome, and both are associated with systemic inflammation and oxidative stress (Furukawa *et al*., 2004). Indeed, several lines of evidence connect BPH to inflammation. For example, the NHANES III study by Rohrmann *et al*. (2005) showed that higher levels of serum C-reactive protein are associated with an increased risk of LUTS. Furthermore, the presence of inflammation has been implicated as the stimulus that leads to the development of prostate cancer and it is, therefore, assumed that BPH might represent a non-malignant pathway that is promoted by oxidative stress and inflammatory mediators (De Nunzio *et al*., 2012).

1.2.4 Diet

Like many other risk factors, a clear correlation between diet and the development of BPH and LUTS has not been shown. However, some evidence suggests that various macronutrients and micronutrients might influence the risk of developing BPH and LUTS (Chughtai *et al*., 2016). The case–control study that was conducted in Italy by Bravi *et al*. (2006) evaluated a wide range of foods on the risk of developing BPH in 1,369 men < 75 years of age who were previously surgically treated for BPH and 1,451 age-matched controls who had been admitted for various acute, non-neoplastic conditions. A significantly increased risk for BPH was shown with more-frequent consumption of cereals (odds ratio: 1.55), bread (odds ratio: 1.69), eggs (odds ratio: 1.43) and poultry (odds ratio: 1.39). By contrast, inverse associations were observed for the

consumption of soup (including minestrone) (odds ratio: 0.74), legumes (odds ratio: 0.74), cooked vegetables (odds ratio: 0.66) and citrus fruits (odds ratio: 0.82) (Bravi *et al*., 2006). In addition, fruit might have a protective role in preventing BPH (Lagiou *et al*., 1999). Furthermore, one review suggested that the low occurrence of BPH in Asian men, as well as in vegetarian men, can be attributed to low-fat and high-fibre diets (Denis *et al*., 1999).

1.2.5 Genetics

A study from China reported that variants in 2q31 and 5p15 are associated with aggressive (defined by multiple criteria) BPH (Qi, 2013). Another study reported that rs103294, a single--nucleotide polymorphism of LILRA3 (encoding leukocyte immunoglobulin-like receptor A3), which has been shown to be associated with prostate cancer risk in a Chinese population, was associated with risk of developing BPH (Jiao *et al*., 2013). It was also noted that individuals with risk allele 'C' of rs103294 had increased risk for BPH (Jiao *et al*., 2013).

The mode of inheritance for BPH has been suggested to be autosomal dominant for certain men (Pearson *et al*., 2003). Furthermore, twin studies have suggested that heritability is an important determinant of disease severity in BPH, including the development of LUTS. In a study of monozygotic and dizygotic twins, heritability accounted for 82.6 % of the variability in IPSS in men > 50 years of age (Meikle *et al*., 1999). In a similar study, the concordance rates for LUTS in 1,723 twin pairs were higher in monozygotic than in dizygotic twins36. The study by Rohrmann *et al*. (2006) suggested that 72 % of the risk of high-to-moderate and severe LUTS was attributable to genetic factors.

1.3 PATHOGENISIS OF BENIGN PROSTATE HYPERPLASIA

1.3.1 Sex Hormones

Testicular androgens are required in the prostate for the development of BPH (Chughtai *et al*., 2016). The enzyme steroid 5-α-reductase 2 (also known as 3-oxo-5-α-steroid 4-dehydrogenase 2), which is bound to the nuclear membrane, converts testosterone into dihydrotestosterone (DHT), the principal androgen in the prostate accounting for 90 % of total prostatic androgen. The levels of intraprostatic DHT continue to remain high with ageing (Roehrborn, 2008). The

stroma and epithelium of the prostate interact via cellular signalling mechanisms mediated by DHT and DHT-dependent growth factors (Foster, 2000).

Development of BPH involves the disruption of the DHT-supported homeostasis between cell proliferation and cell death allowing proliferative processes to predominate (Carson, 2003). It has previously been hypothesized that DHT acts on prostate cells via endocrine, paracrine or autocrine mechanisms (Griffiths *et al*., 1997) and plays a key part in regulating the homeostasis between apoptosis and cell proliferation (Chughtai *et al*., 2016). Growth factors stimulated by DHT, including epidermal growth factor (EGF), keratinocyte growth factor (KGF) and insulin-like growth factors (IGFs), modulate cellular proliferation in the prostate in humans (Carson, 2003). The expression of transforming growth factor β (TGFβ), which modulates apoptosis, is also influenced by DHT (Niu *et al*., 2001). Overall, rather than increased levels or activities of these growth factors that contribute to BPH, the interactions between growth factors and steroid hormones might alter the balance of cell proliferation versus cell death to result in BPH.

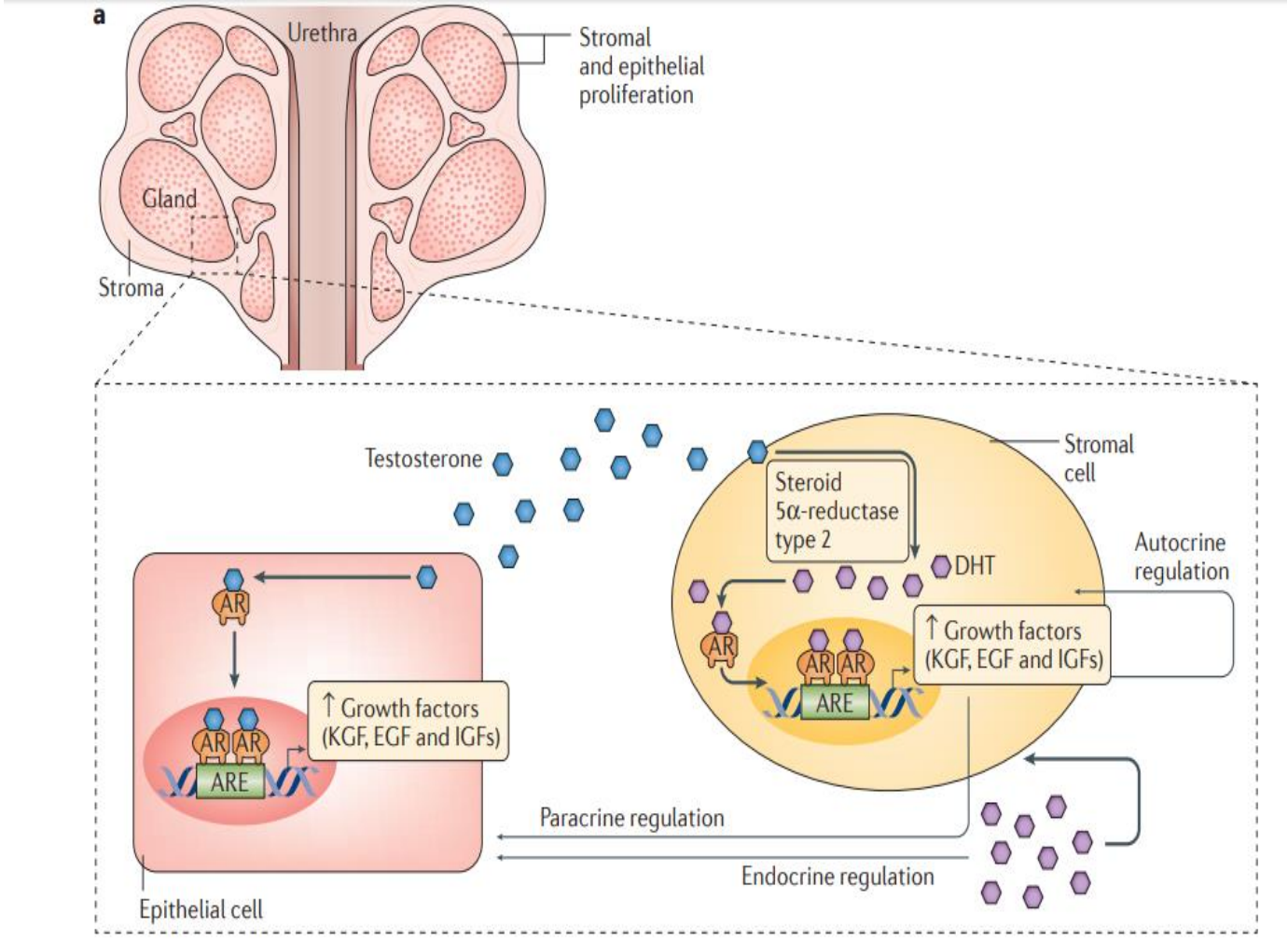

Figure 4: Role of testosterone in Benign Prostate Hyperplasia.

Source: (Chughtai *et al*., 2016).

From the above figure, Testosterone (produced in the testes) diffuses into stromal and epithelial cells of the prostate. Testosterone and its derivatives interact with the androgen receptor (AR), which upon binding translocates to the nucleus and binds to the androgen response element (ARE), promoting the expression of genes encoding various growth factors; including keratinocyte growth factor (KGF), epidermal growth factor (EGF) and insulin-like growth factors (IGFs). In stromal cells, most of the testosterone is converted to dihydrotestosterone (DHT), which then acts in an autocrine manner to promote stromal proliferation. DHT can also diffuse into the adjacent epithelial cell to act in a paracrine manner. DHT produced peripherally

in the liver and the skin can also diffuse from the circulation into the prostate to act in an endocrine manner.

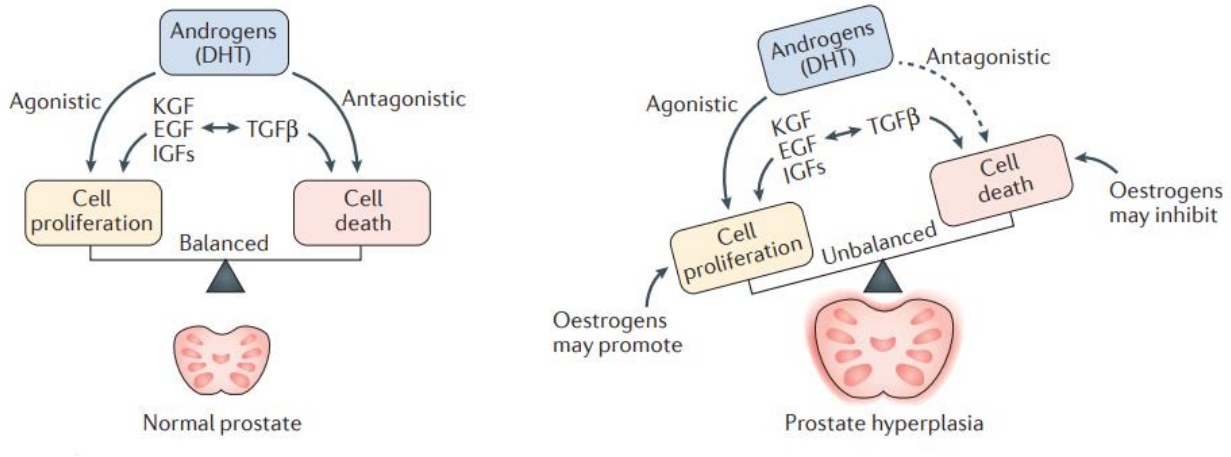

Figure 5: Molecular control of prostate growth.

Source: (Chughtai *et al*., 2016).

From the figure above, Benign prostatic hyperplasia (BPH) probably develops as a result of an imbalance between mechanisms that regulate cell death and cell proliferation. Growth factors such as KGF, EGF and IGFs, which are AR target genes, are probably involved, as is transforming growth factor-β (TGFβ), which is negatively regulated by androgens. The dashed arrow indicates a less antagonistic effect (than in the left panel) and the double-headed arrows indicate the bidirectional relationship of the growth factors.

1.3.2: Inflammation

Inflammation plays a role in the development of BPH (Madersbacher *et al*., 2019). Local inflammation may be triggered by viral or bacterial infection, which would lead to the secretion of cytokines, chemokines and growth factors involved in the inflammatory response with consequent growth of epithelial and stromal prostatic cells (Soler *et al*., 2013). T cells are known to secrete various growth factors that promote stromal and glandular hyperplasia of the prostate.

The pathways of specific inflammatory mediators have been studied to assess their potential role in the pathogenesis of BPH. In particular, increased levels of IL-2, IL-4, IL-7, IL-17, IFNγ and

their relevant receptors have been identified in BPH tissue (Kramer *et al*., 2002; Kramer *et al*., 2007). Robert *et al*. (2009) have been able to show that IL-2, IL-7 and IFNγ stimulate the proliferation of prostatic stromal cells *in vitro*. Chronic inflammation in BPH has also been associated with focal upregulation of cyclooxygenase 2 in the glandular epithelium, generating pro-inflammatory prostaglandins, which cause prostate cell proliferation (Wang *et al*., 2004; Chughtai *et al*., 2011).

1.4 DIAGNOSIS OF BENIGN PROSTATE HYPERPLASIA

Several key diagnostic tools and investigations are used to diagnose BPH in men who present with LUTS. The medical history is a critical component in the accurate diagnosis of BPH. Type and severity of symptoms must be assessed, but more importantly, LUTS resulting from BPH must be distinguished from LUTS resulting from another pathologic process (Kim *et al*., 2016). A thorough review of the patient's medications may also help identify contributing factors. Whilst there is little to no correlation between symptoms or urodynamics and prostate size (Girman, 1998).The American Urological Association (AUA) symptom score is a validated questionnaire that is used to objectively measure LUTS. Seven symptoms; incomplete emptying, frequency, intermittency, urgency, weak stream, straining, and nocturia are scored from 0 to 5. Total scores from 0 to 7 are considered mild, scores from 7 to 19 are considered moderate, and scores of 20 to 35 are considered severe. The reduction in quality of life due to LUTS is also qualitatively assessed and included (Barry *et al*., 1992). Dysuria may be a secondary indicator of BPH, as bladder outlet obstruction may lead to urinary stasis and resultant urinary tract infection (UTI) (Kim *et al*., 2016).

1.4.1 Physical Examination

Digital rectal examination (DRE) and a focused neurologic examination are used to detect an enlarged prostate (Kim *et al*., 2016). An enlarged, smooth, and symmetric prostate are DRE findings consistent with BPH. A gloved and lubricated finger is inserted in to the patient's rectum and feels the prostate to estimate its size and detect nodules or tenderness. The examination is quick and painful. Some find this method embarrassing (Praveen, 2013). This test helps to rule out prostate cancer but it underestimates the size of the prostate. It is not the primary diagnostic tool for benign prostate hyperplasia because its result is not accurate

(Praveen, 2013). A focused neurologic examination should be performed in order to evaluate for neurogenic causes of LUTS. This should include assessment of lower-extremity strength, sensation, and reflexes as well as perineal sensation (Kim *et al*., 2016).

1.4.2 Urine Analysis

A urine analysis may be performed to determine if there is sign of bleeding or infection. Urinalysis involves a physical and chemical examination of urine. A urinalysis should be performed to detect blood, glucose, leucocytes, and nitrites. A positive urinalysis for infection should be treated and may well account for the patients LUTS. Urinalysis should be performed in men with LUTS to evaluate for glucosuria, pyuria, and hematuria (Kim *et al*., 2016).

1.4.3 Serum Creatinine

Serum creatinine measurement may be considered during the initial evaluation of patients with LUTS to ensure that renal insufficiency has not occurred as a result of obstructive uropathy. Although obstructive uropathy is a potentially serious complication of longstanding and untreated BPH, elevations in serum creatinine are much more commonly a result of medical renal disease. Patients with obstructive uropathy require early urinary decompression with urethral or suprapubic catheterization, stabilization during postobstructive diuresis, monitoring of kidney function recovery, and consideration of surgical BPH intervention (Kim *et al*., 2016).

1.4.4 Prostate Specific Antigen (PSA)

A PSA test measures the level of prostate specific antigen in the patient's blood sample. This is the standard test for prostate (Lam *et al*., 2004). Prostate specific antigen test is recommended annually for men over 50 years old. The decrease in prostate size corresponds to decrease in PSA levels. Every 19 % decrease in PSA levels, there is a decrease of prostate volume by 10 % (Praveen, 2013).

1.4.5 Post-Void Residual Urine

A post-void residual measurement checks how much urine is left in the bladder of a patient after urination (Birch *et al*., 1988). After urination, a thin tube called a catheter is placed into the patient's urethra. The tube will be threaded into the bladder to remove any urine that's left inside.

It can also be checked with an office ultrasound or bladder scanner. Cold jelly is placed over the bladder and the ultrasound measures the left over urine (Kim *et al*., 2016).

1.4.6 Urine Flow Test

To determine whether the bladder is obstructed, the speed of urine flow is measured electronically using a test called uroflowmetry (Praveen, 2013). The patient is instructed not to urinate for several hours before the test and to drink plenty of fluid so that he will develop a strong urge to urinate. . It is important that the patient remains still while urinating to ensure accuracy of test result. The test should be repeated at least twice since other factors may influence urine flow (such as straining or holding back because of self-consciousness). The values more than 15 ml per second and voiding volume of 150 ml or more is considered to be the normal range (Joseph, 2007).

1.5 Management of Benign Prostate Hyperplasia

Management approaches for benign prostate hyperplasia range from observation only, to medical therapy, to minimally invasive, endoscopic or open surgery (Woo *et al*., 2011). Men with bothersome lower urinary tract symptoms without complications from benign prostatic hyperplasia, such as urinary retention, hydronephrosis or impaired kidney function, are often good candidates for medical therapy (Nickel *et al*., 2010).

1.5.1 Watchful Waiting

Patients with mild symptoms (IPSS of ≤ 7) and no complicating factors or those with moderate symptoms and minimal bother can be managed with watchful waiting. Watchful waiting includes advice about lifestyle changes that can help to ameliorate or circumvent symptoms. These changes include advice about volume, type and timing of liquids consumed, avoidance of caffeine (a diuretic), and abstinence of alcohol consumption in the evening and regulation of bowel movements with avoidance of constipation (Gratzke *et al*., 2015). Over-the-counter ‘decongestants’ commonly used for cold and flu symptoms should also be avoided as they can exacerbate symptoms owing to their α-adrenergic agonist effects at the bladder neck causing urinary retention (Verhamme *et al*., 2008). Although watchful waiting avoids the risks and costs associated with medication and surgical procedures, patients should be warned that symptoms

can worsen overtime. A longitudinal study over a period of 4 years, which involved almost 400 men, demonstrated that the cumulative clinical progression rate was 6 %, 13 %, 15 %, 24 %, 28 % and 31 % at 6, 12, 18, 24, 36 and 48 months, respectively; 4.9 % of men developed acute urinary retention within the 48month follow-up period (Djavan *et al*., 2004).

1.5.2 Medical Management of Benign Prostate Hyperplasia

The aim of medical therapy is to improve symptoms, lower the risk of progression and improve quality of life. There are many pharmaceutical options, with guidelines and algorithms readily available to help guide selection (EAU, 2016).

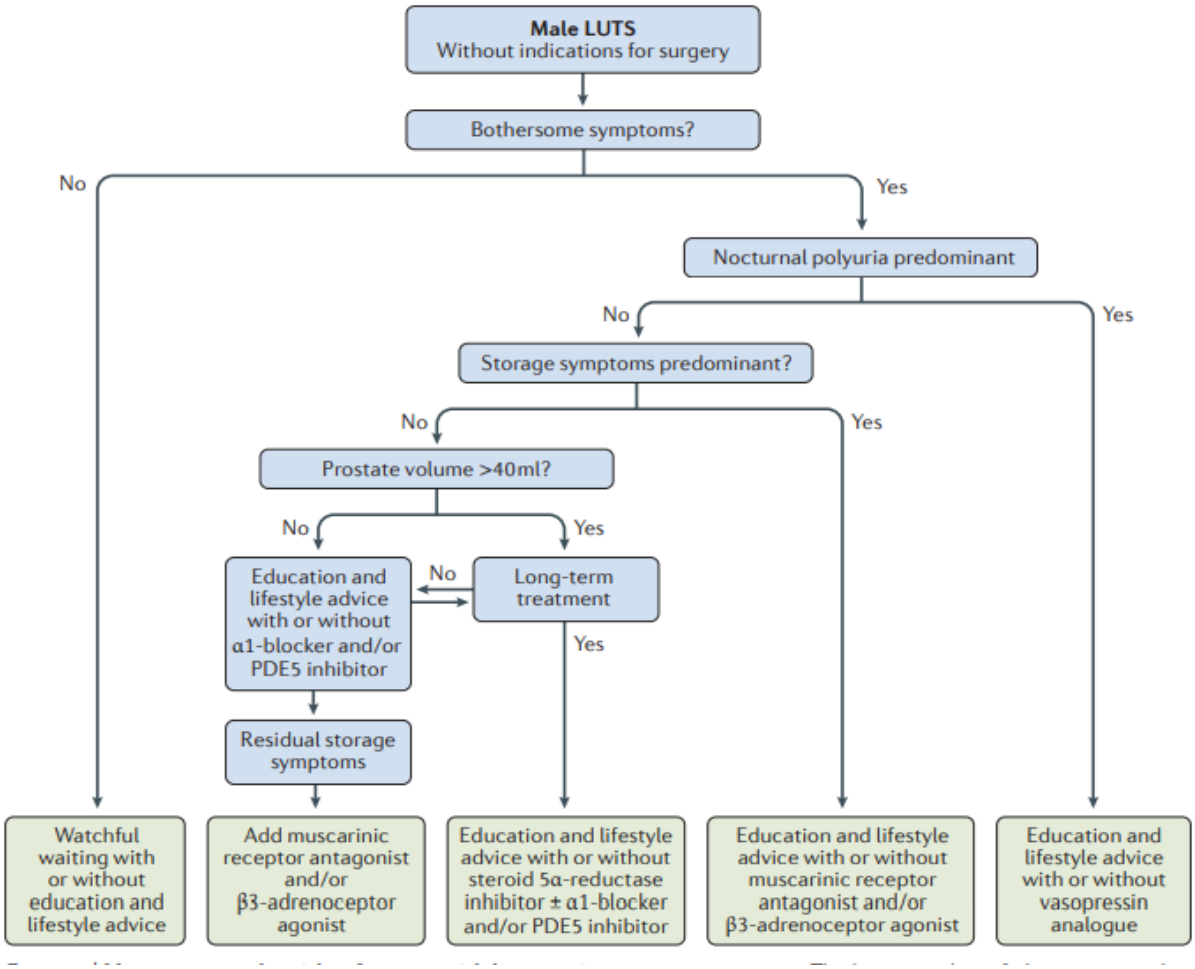

Figure 6: Management algorithm for men with lower urinary tract symptoms.

Source: (EAU, 2016)

1.5.2.1 Herbals

Herbal medications have been used for the management of benign prostate hyperplasia for many years in Europe (Daniel *et al*., 2019). *Prunus africana* and *Serenoa repens* are the two main plants extracts that have been used in the management of BPH but there are also other herbal preparations in use (Nyamai *et al*., 2016).

The exact mechanism of action of *Serenoa repens* has not been clearly understood but several theories exist. The mechanisms most likely involve inhibition of 5α-reductase and/or inhibitory effects on androgen and estrogen receptors. Inhibition of 5α-reductase results in a decrease in the conversion of testosterone to dihydrotestosterone (Dvorkin and Song, 2002). Some studies have shown that the antiestrogenic effect may be due to competitive blocking of the translocation of cytosolic estrogen receptors to the nucleus. A similar mechanism has been proposed for the effect on androgen receptors in regard to *S. repens'* antiestrogenic effects (Dvorkin and Song, 2002). Studies have shown that *S. repens* extract inhibits testosterone-stimulated prostate enlargement in rats.

Prunus bark contains a number of different ingredients, some of which have demonstrated benefit in the treatment of BPH. These constituents include long-chain fatty alcohols (n-docosanol, n-tetracosanol, and their trans-ferulic acid esters) (Dvorkin and Song, 2002), β-sitosterol, and its 3-O-glucoside, β-sitosterone, oleanolic, ursolic, crataegolic, and fatty acids (Dvorkin and Song, 2002). Different studies with the extract of *Prunus africana* postulates that it has several mechanisms of action. Research has shown that inflammatory cell infiltration, as well as fibroblast proliferation, may play a key role in development of BPH. *P. africana* exhibited anti-inflammatory activity and inhibited fibroblast proliferation, specifically fibroblasts induced by prostatic growth factors, basic fibroblast growth factor, and epidermal growth factor (Dvorkin and Song, 2002). It has also been shown that *P. Africana* improves prostate histologic architecture, restores normal secretory patterns, inhibits pathologic fibroblast proliferation, decreases hypersensitivity of the detrusor muscle, and has anti-inflammatory effects and even some estrogenic action (Mathe *et al*., 1995).

1.5.2.2 Alpha-Blockers (α-Blocker)

The mode of action of Alpha-adrenergic antagonists, or alpha blockers is to block the α-receptors located in the prostatic smooth muscle and bladder neck that mediate contraction and thereby reduced flow and the development of LUTS (Daniel *et al*., 2019). Blocking these receptors mediates relaxation of the tissues, thereby easing the flow of urine through the lower urinary tract (Roehrborn *et al*., 2005). Some of the α-blockers have also been shown to cause apoptosis of the prostatic epithelium, which may also contribute to their effect (Kyprianou *et al*., 1998).

There are three main types of α-receptors (Alpha-1A-adrenergic receptors predominate in the lower urinary tract, alpha-1B and alpha-1D receptors found in blood vessels, the central nervous system, and nasal passages.). (Daniel *et al*., 2019). The different types depend on their uroselectivity; uroselective α1A-blockers include Tamsulosin and alfuzosin and nonselective α1A-blockers are doxazosin and terazosin (Daniel *et al*., 2019). These drugs treat the dynamic component of benign prostatic hyperplasia by relaxing smooth muscle in the prostate and bladder neck. This causes the urethral lumen to widen so improving urinary flow (Elbakyan, 2018). Alpha blockers can improve symptoms and increase the maximal urinary flow rate. There are many α1-blockers now available, and each have similar efficacy for treatment of LUTS but have differing side effect profiles.

Prazosin was previously the most commonly used alpha blocker, but it requires multiple daily dosing. The efficacy of prazosin is limited; therefore international guidelines no longer recommend prazosin for lower urinary tract symptoms (EAU, 2018). Tamsulosin is a selective blocker for the alpha 1-receptor subtype. It is available in a slow-release formulation, which reduces the systemic adverse effects such as postural hypotension and the need for dose titration (Lepor, 2007). Silodosin is a newer drug that is highly selective for alpha 1-receptors. It has demonstrated a similar efficacy to tamsulosin (Wilt, 2002).

Adverse Effects

The main side effects of α-blockers are mild, occur in about 15 % of patients, and include (more common with non-neuroselective blockers): orthostatic hypotension, headache, dizziness, asthenia, drowsiness, and ejaculatory problems (i.e. retrograde ejaculation, more common with

the uroselective blockers) (McConnell *et al*., 2003). Alpha blockers, particularly tamsulosin, have been associated with intra-operative floppy iris syndrome (Elbakyan, 2018). This increases the technical difficulty of cataract surgery and increases the incidence of complications such as posterior capsule rupture, iris trauma and vitreous loss (Fung and McCluskey, 2010).

1.5.2.3 5-Alpha Reductase Inhibitors (5-ARIs)

The enzyme 5-alpha-reductase converts testosterone to dihydrotestosterone in the prostate (Thigpen *et al*., 1993). Inhibition of this enzyme reduces androgenic dihydrotestosterone and subsequently reduces prostatic tissue volume and the static contribution to symptoms (Hudak *et al*., 2006). There are two drugs available in this class, finasteride, which is a type II 5α-reductase inhibitor, and dutasteride, which is a type I & II 5α-reductase inhibitor (Elterman *et al*., 2012). These 5-ARIs block the action of the 5-AR enzyme and therefore the conversion of testosterone to DHT, the more potent ligand.

The 5-alpha-reductase inhibitors reduce the progression of benign prostatic hypertrophy, manifested as acute urinary retention or the need for surgery (Foley and Kirby, 2003). Compared to alpha blockers, dutasteride and finasteride are more effective in men with larger prostate volumes (> 40 mL) or prostate specific antigen (PSA) concentrations above 1.4 ng/mL (Foley and Kirby, 2003; Hudak *et al*., 2006).

Adverse Effects

The most common adverse effects of 5-alphareductase inhibitors are erectile dysfunction, decreased libido, decreased ejaculation and decreased semen count (Naslund and Miner, 2007).

1.5.2.4 Combination Therapy (α-blocker and 5-ARIs)

There have been two trials to study the efficacy of a combination therapy in the management of benign prostate hyperplasia. First was the Medical Therapy of Prostatic Symptoms (MTOPS) trial which studied a combination of doxazosin and finasteride (vs monotherapy with placebo, doxazosin or finasteride)(McConnell *et al*., 2003). Second was the CombAT trial which studied a dutasteride and tamsulosin combination (vs monotherapy with dutasteride or tamsulosin) (Roehrborn *et al*., 2010). They found that combination therapy with an alpha blocker and 5-

alpha-reductase inhibitor provided a greater improvement in lower urinary tract symptoms compared with single therapy (Elbakyan, 2018).

Adverse Effects

Combination therapy has similar adverse event profiles to other treatment procedures of benign prostate hyperplasia (Thorpe and Neal, 2003). Combination therapy has an increased risk of adverse effects such as sexual dysfunction, and this needs to be balanced against potential benefits for urinary symptoms (Füllhase and Schneider, 2016).

1.5.2.5 Phosphodiesterase-5 Inhibitors (PDE5-I)

Phosphodiesterase-5 inhibitors are more commonly used to treat erectile dysfunction. They can be effective in the treatment of lower urinary tract symptoms due to benign prostatic hyperplasia, however they are less effective than alpha blockade therapy according to measures such as I-PSS and maximum urinary flow rate (Wang *et al*., 2015). Phosphodiesterase-5 inhibitors reduce smooth muscle tone in the detrusor, prostate and urethra by increasing intracellular cyclic guanosine monophosphate (Lawrentschuk and Perera, 2018). As erectile dysfunction is a common adverse effect of 5-alpha-reductase inhibitors, they are sometimes used in combination to counteract it and also to reduce lower urinary tract symptoms (Favilla *et al*., 2016). The combination of phosphodiesterase-5 inhibitors with an alpha blocker results in greater reductions in I-PSS, post-void residual volumes and quality-of-life scores, and greater increases in maximum urinary flow rate than both drugs used as monotherapy (Wang *et al*., 2015).

Table 1: FDA-approved medications for the treatment of benign prostatic hyperplasia

Alpha blockers (nonselective)	Alpha blockers (alpha-1A selective)	5-Alpha reductase inhibitors	Phospho-diesterase type 5 inhibitors	Combination products
Alfuzosin extended-release 10 mg daily	Tamsulosin 0.4 mg daily	Dutasteride 0.5 mg daily	Tadalafil 5 mg daily	Dutasteride 0.5 mg/ tamsulosin 0.4 mg daily
Doxazosin immediate-release 1 mg daily (titrate up to 8 mg daily)	Silodosin 8 mg daily	Finasteride 5 mg daily		
Doxazosin extended-release 4 mg daily (titrate up to 8 mg daily)				
Prazosin 1 mg twice to three times daily (titrate up to 15 mg total daily)				
Terazosin 1 mg daily (titrate 2 mg, 5 mg, then 10 mg daily)				

Source: (Kim *et al*., 2016)

1.5.3 Surgical Management

Surgery is recommended for patients who have failed medical management or who have complications from bladder outlet obstruction due to BPH such as renal insufficiency due to obstructive uropathy, recurrent UTI, bladder stones, and refractory urinary retention (Kim *et al*., 2016). Many surgical options are available for men with BPH and they can be classified into three main groups: compressing the prostate tissue, debulking of the adenoma and removal of the entire adenoma (adenectomy) (Chughtai *et al*., 2016).

Compression: Compression procedures involve the insertion of a device that compresses the prostate laterally, widening the urethral channel. Urethral lift is a non-ablative technique and can be performed under local anaesthesia as a day patient procedure (Garcia *et al*., 2015). Owing to its tissue-sparing approach, this procedure seems to be promising in minimizing the co-morbidities of other BPH treatments and is able to considerably improve LUTS (Marra *et al*., 2015). Unlike surgical removal of a body part or tissue, prostatic urethral lift has the added benefit of preserving sexual function (Marra *et al*., 2015).

Adenoma debulking: Debulking surgery involves the endoscopic removal of some of the adenomatous component that obstructs the outlet (Chughtai *et al*., 2016).

Adenectomy: Adenectomy is a traditional approach and one of the oldest techniques to treat men with BPH and very large prostates (usually >100ml) (Chughtai *et al*., 2016). In this approach, the adenoma is enucleated off its capsule. Traditionally, adenectomy was an open surgical operation but can now be achieved with less morbidity with laser enucleation of the prostate transurethrally (Vincent and Gilling, 2000).

1.6 OXIDATIVE STRESS

Reactive oxygen species are produced by endogenous and exogenous processes and are balanced by the activities of antioxidants defences (Liguori *et al*., 2018). When there is an imbalance in reactive oxygen species and antioxidant defences, it results in oxidative stress. Imbalance between oxidants and antioxidants in favour of the oxidants, leading to a disruption of redox signalling and control and/or molecular damage is indicative of oxidative stress. Oxygen free radicals and other reactive oxygen species (ROS) are known to be formed in all aerobic organisms and play a plethora of useful roles (Halliwell, and Gutteridge, 2015). However, some of them have the potential to cause damage ("oxidative damage") to biomolecules, which is thought to contribute to the development and progression of certain diseases.

1.6.1 Pathophysiology of oxidative stress

Free radicals are highly reactive atoms or molecules with one or more unpaired electron(s) in their external shell and can be formed when oxygen interacts with certain molecules (Chandrasekaran *et al*., 2017). These radicals can be produced in cells by losing or accepting a single electron, therefore, behaving as oxidizing agent or reducing agent (Lobo *et al*., 2010). The terms reactive oxygen species (ROS) refers to reactive radical and non-radical derivatives of oxygen (Liguori *et al*., 2018). Reactive oxygen species (ROS) are produced by all aerobic cells and play an important role in aging as well as in age-related diseases (Venkataraman *et al*., 2013). ROS generation is not only limited to determine deleterious effects but also involved in the extraction of energy from organic molecules, in immune defences, and in the signaling process (Genestra, 2007).

1.6.2 Endogenous antioxidants enzymes

The antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase and glutathione peroxidase (GSH-Px) (Liguori *et al*., 2018).

1.6.2.1 Superoxide dismutase (SOD)

Superoxide dismutase are the first line of defense against oxygen free radicals, and the vast majority of organisms that live in the presence of oxygen express at least one SOD. Three classes of SOD have evolved in various organisms possessing different catalytic metal ions: Cu,Zn SODs, Mn SOD/Fe SODs, and Ni SODs SODs catalyze the conversion of superoxide into oxygen and hydrogen peroxide (Wang *et al*., 2018). Superoxide radicals are generated as by-products of several metabolic processes including mitochondrial respiration. They are negatively charged free radicals formed through a single electron donation to oxygen (Hayyan *et al*., 2016). Chemically, the dismutase activity of SODs accelerates the reaction of the superoxide anion ($O_2^{\bullet -}$) with itself to form hydrogen peroxide (H_2O_2) and oxygen ($2O_2^{\bullet -}+2H^+ \rightarrow H_2O_2+O_2$) (Wang *et al*., 2018).

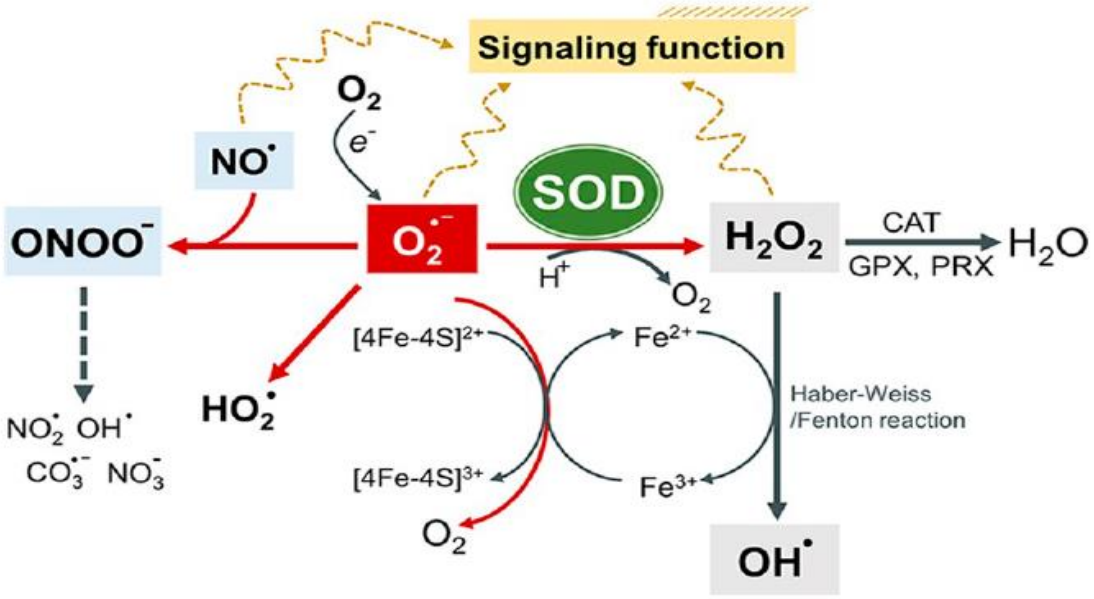

Figure 7: Reactions and transformations of the superoxide anion.

Source: (Wang *et al*., 2018)

1.6.2.2 Catalase (CAT)

Catalase is an antioxidant enzyme present in all living tissue that makes use of oxygen. It plays an important role in adaptive response to H_2O_2 (Hessam and Ali, 2018). It utilises iron or manganese as a cofactor in the reduction of H_2O_2 to H_2O and molecular oxygen thereby completing the detoxification process initiated by SOD.

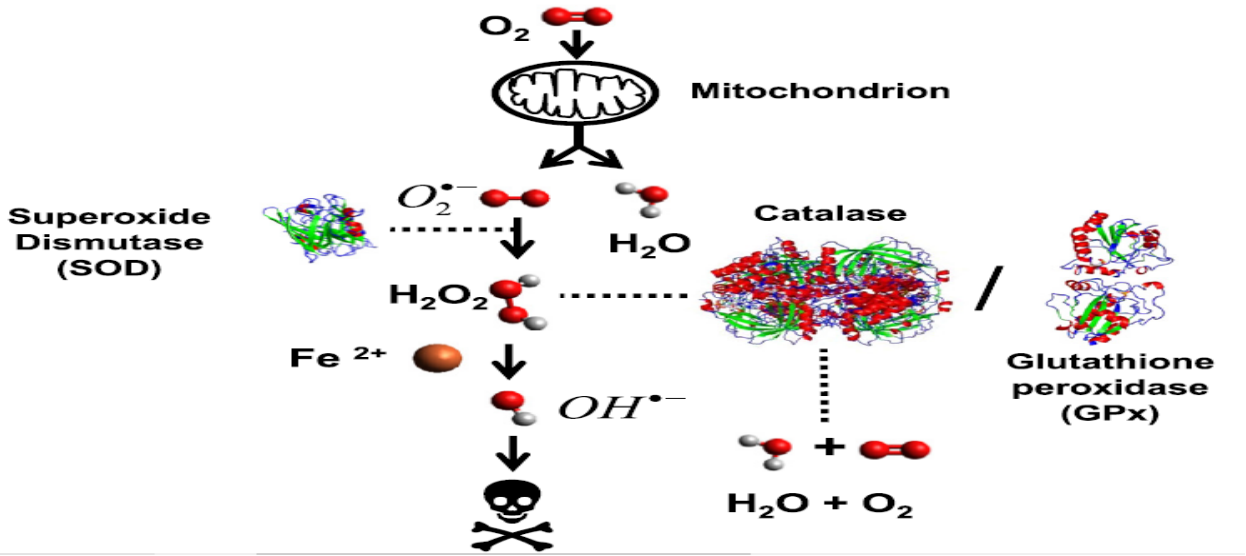

Figure 8: Reactive oxygen generation in organisms and the countermeasures taken by catalase. Source: (Hessam and Ali, 2018).

1.6.2.3 Glutathione peroxidase (GPx)

Glutathione peroxidase (GPx) converts peroxides and hydroxyl radicals into nontoxic forms by the oxidation of reduced glutathione (GSH) into glutathione disulfide and then reduced to GSH by glutathione reductase (Hessam and Ali, 2018).

1.6.3 Exogenous antioxidants

the exogenous antioxidants include; ascorbic acid (vitamin C), which scavenges hydroxyl and superoxide radical anion, α-tocopherol (vitamin E), which is involved against lipid peroxidation of cell membranes, and phenolic antioxidants, which include stilbene derivatives (resveratrol, phenolic acids, and flavonoids), oil lecitinas, selenium, zinc, and drugs such as acetylcysteine (Pisoschi and Pop, 2015).

1.6.3.1 Vitamin C

Vitamin C (ascorbic acid) is a cofactor for mixed function oxidase involved in the hydroxylation of lysine and proline, synthesis of carnitine, and required for protocollagen to cross-link properly into normal collagen fibrils (Chaney, 2011). It acts as a reducing agent and maintains normal connective tissue. It is necessary for bone formation, wound healing and aids in absorption of iron by reducing it to the ferrous state in the stomach (Bsoul and Terezhalmy, 2004). It enhances utilization of folic acid, either by aiding conversion of folate to tetrahydrofolate or formation of polyglutamate derivatives of terahydrofolate. Vitamin C spares vitamin A, E and some B vitamins by protecting them from oxidation and its deficiency causes capillary fragility (Chaney, 2011).

1.6.3.2 Vitamin E

Vitamin E occurs in diet as a mixture of several closely related compounds called tocopherol and tocotrienol all of which are naturally occurring antioxidants (Chaney, 2011). Due to their lipophilic character, they accumulate in circulating lipoproteins, cellular membranes and fat deposit where they act as scavengers of free radicals, protecting unsaturated fatty acids from peroxidation reaction (Zingg *et al.,* 2008). Alpha-Tocopherol is the most potent scavenger for Reactive Oxygen Species (ROS) while γ-Tocopherol is the most potent for Reactive Nitrogen Specie (RNS) (Chaney, 2011). Vitamin E has a role to play in cellular respiration and also prevents the oxidation of LDL. Tocopherol enhances haeme synthesis by increasing the levels of δ-aminolevulinic acid (ALA) synthase and ALA dehydratase (Chaney, 2011). Studies have shown that vitamin E is required for maintaining normal immune function and also may be important in preventing muscular degeneration and cognitive decline (Zingg *et al.,* 2008).

1.7 MEDICINAL PLANTS

Medicinal plants constitute an effective source of both traditional and modern medicine. These plants have been shown to have genuine utility and about 80 % of the rural population depends on them as primary health care (Akinyemi, 2000). Plants have been used as sources of remedies for the treatment of many diseases since ancient times and people of all continents especially Africa have this old tradition. Despite the remarkable progress in synthetic organic medicinal products of the twentieth century, over 25 % of prescribed medicines in industrialized countries

are derived directly or indirectly from plants (Newman *et al*., 2000). However, plants used in traditional medicine are still understudied (Kirby, 1996). In developing countries, notably in West Africa, new drugs are not often affordable. Thus, up to 80 % of the population uses medicinal plants as remedies (Kirby, 1996; Hostellmann and Marston, 2002).

1.7.1 Bioactive Constituents of Medicinal Plants

The medicinal values of plants lie in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Edeoga, 2005).

1.7.1.1 Alkaloid

Alkaloids are nitrogen-containing secondary metabolites that are considered as the largest group of bioactive natural compounds from plants (Barbosa-Filho *et al*., 2006). Plants are estimated to produce approximately 12,000 different alkaloids, which can be organized into groups according to their carbon skeletal structures (Ziegler and Peter, 2008). They have an extensive range of biological activities, such as antitumor, anticholinergic, antimicrobial, antiviral, antihypertensive, anti-inflammatory, antidepressant, emetic, diuretic, sympathomimetic, hypnoanalgesic, and miorelaxant (De Sousa Falcão *et al*., 2008).

1.7.1.2 Flavonoid

Flavonoids are the important groups of secondary metabolites in plants, and also the good sources of natural antioxidants in human diets (Kim *et al*., 2003). Hence, flavonoids are known to play an important role in the control of different human diseases. The flavonoids have polyphenolic structure, which makes it responsible for the variety of pharmacological activities (Mahomoodally *et al*., 2005). Functional hydroxyl groups in flavonoids show their antioxidant effects by scavenging free radicals or by chelating metal ions. This helps in the prevention of radical generation that damage the biomolecules leading to oxidative stress and many diseases (Leopoldini *et al*., 2006; Kumar and Pandey, 2013).

Besides possessing antioxidant property, flavonoids also posses diverse biological activities that owes to the health aspects for human. These activities are anti-inflammatory, anti-ulcer, anti-viral, anti-cancer, anti-diabetic and cytotoxic (Ghasemzadeh and Jaafar, 1986).

1.7.1.3 Saponin

The saponins are naturally occurring surface-active glycosides. They are mainly produced by plants, but also by lower marine animals and some bacteria (Riguera, 1997). Saponins consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin) which may be tri-terpenoid or steroid in nature (Francis *et al*., 2002).

1.7.1.4 Tannin

Tannins (commonly referred to as tannic acid) are water-soluble polyphenols that are present in many plant foods. Tannins are a heterogeneous group of polyphenolic polymers of varying molecular weight and complexity. Plant phenolics and tannins are synthesized to meet ordinary physiological demands but also as a response to biotic and abiotic stresses (Alonso *et al*., 2007).

1.7.1.5 Phytosterols

Sterols comprise several major groups of steroids characterized by having a hydroxyl group at C-3, normally in the beta configuration and branching side chains from eight to ten or more carbon atoms at C-17. They occur widely throughout the animal and particularly the plant kingdoms. They have both structural roles, as membrane constituents, and a key place in the biosynthetic sequences which lead to the steroidal species. The sterols are the starting material for biosynthesis of plants (Jain *et al*., 2019).

The phytosterols, known to be universally present in higher plants are the starting materials for the biosynthesis of plant steroids. They cannot be synthesized by human and are thus consumed from the diet. They have decreased serum cholesterol level as reported in several studies. Plant sterols are industrially important chemical sources for steroid compounds, insecticides, antioxidant and anticancer drugs. A summary of some of the sterols, isolated from various plants has been reported. The most often isolated sterols from higher plants is β-sitosterol, β-

stigmasterol, lanosterol and campesterol are also quite common. A similar finding was also reported by some other workers with β-sitosterol, campesterol and stigmasterol as most often isolated compounds from higher plants (Ju *et al*., 2004).

Evidence suggests that phytosterols possess antioxidant activity, anti-inflammatory activity, anti-cancer activity against cancer of lungs, stomach, ovary, and estrogen-dependent human breast cancer (Ju *et al*., 2004).

1.8 *Duranta erecta*

Duranta erecta belongs to the Verbenaceae family and it comprises 35 species. It is a native plant of Asia, Africa, and South and Central America (Aymard and Grand, 2012). It is used as an ornamental plant in tropical nations (Puri, 2018). The genus *Duranta* was described by Linnaeus (1753). This genus is named after Castor Durante (1529–1590), a French physician and botanist (Puri, 2018).

1.8.1 Taxonomical Classification of *D*uranta *erecta*

*D*uranta *erecta* can be classified as follows:

Domain: Eukaryota

Kingdom: Plantae

Subkingdom: Tracheobionta (Vascular plants)

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledonous

Subclass: Asteridae

Order: Lamiales

Family: *Verbenaceae* (the Vervain family)

Genus: *Duranta*

Specie: *erecta*

Common Name: Golden Dewdrop or sky flower (Puri, 2018)

Plate 1: Morphology of *Duranta erecta* leaves

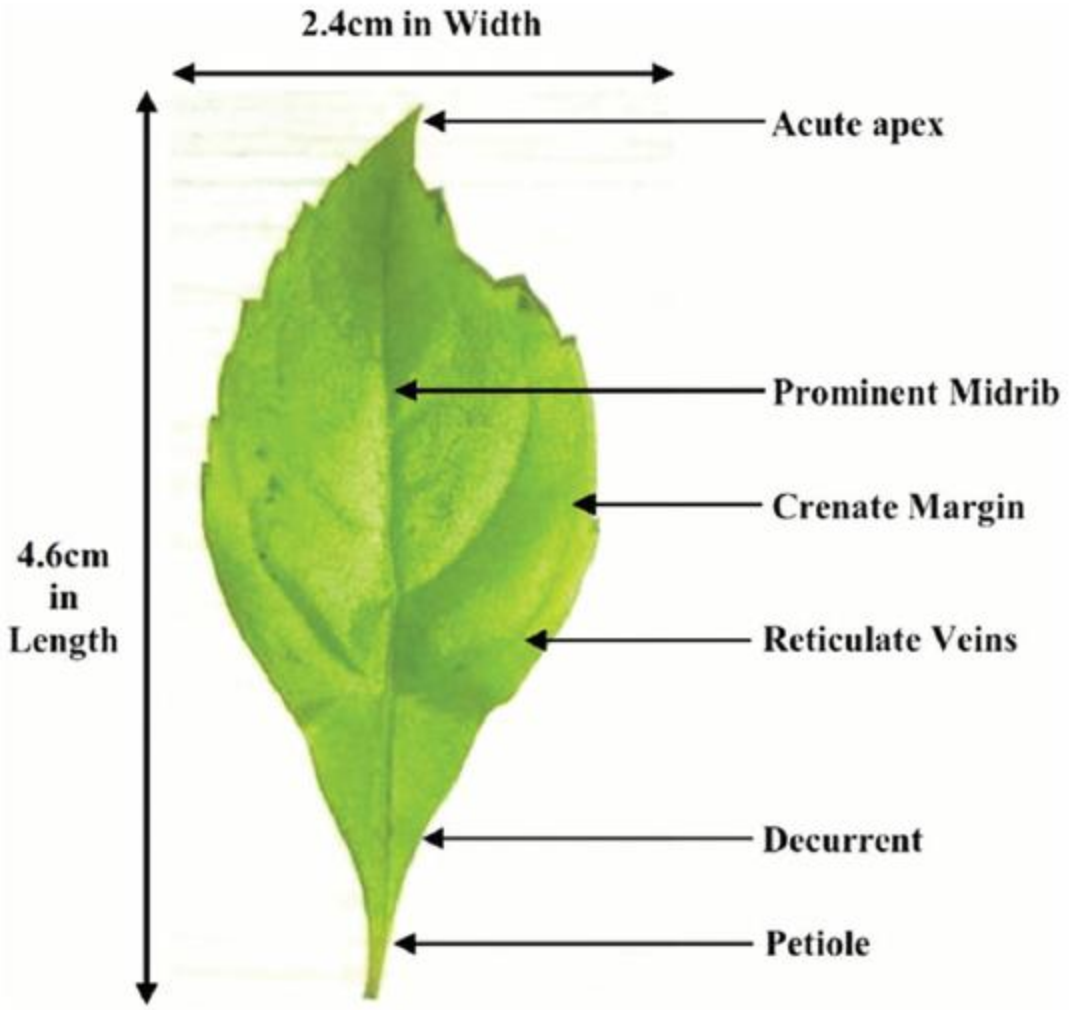

Figure 10: Macroscopical characteristics of *Duranta erecta* leaf

Source: (Puri, 2018)

1.8.2 Traditional uses of *Duranta erecta* Plant

Traditional plants are an important source of natural remedies and remain to be broadly used to treat many diseases (Badgujar *et al*., 2017). An infusion of the leaf and juice of the fruit is diuretic, and flower is said to have stimulant properties. Both leaf and fruit give a positive test for hydrocyanic acid. In the Chinese system of medicine, fruits are mentioned as poisonous berries. The plant is claimed to treat malaria which still stands as a major disease in the developing countries. Water-macerated fruits yield a juice which even in dilutions of 1:100 parts of water are lethal to mosquito larvae; the action is less marked on culicine larvae. The juice can

be used as a larvicidal in ponds and swamps. Fruit contains an alkaloid analogous to narcotine while leaf contains saponins. Some glycosides are also reported from the bark of the plant. In Chinese medicine, the fruits were used for the treatment of malaria, and the leaves are employed for the treatment of abscesses (Puri, 2018). The seeds yield oil with the following characteristics: Acid value (58.62 %), saponification value (210.9), iodine value (101.3), and unsaponifiable matter (0.4 %) (Puri, 2018).

1.8.3 Biological and Pharmacological Activities of *Duranta erecta*

Natural products have been playing a substantial part throughout the world in treating and averting human diseases (Acharya *et al*., 2015). *D. erecta* has been accounted for a wide variety of medicinal activities. The whole plant is used in the treatment of infertility, pneumonia, and malaria, as diuretic, itches, anthelmintic, neuralgic disorders and also as an insect repellent (Puri, 2018). *D. erecta* also exhibits activities such as anti-shigellosis (Nikkon *et al*., 2008), cytotoxic potency (Ahmed *et al*., 2009), antiviral activity (Abou-Setta *et al*., 2007), antioxidant (Donkor *et al*., 2019), antibacterial, and antimicrobial (Ogbuagu *et al*., 2015) against human pathogens.

1.9 Justification of the Study

It is believed that *Duranta erecta* leaves have antioxidant properties. Plants with antioxidant potentials have been found to be effective in amelioration of benign prostate hyperplasia. This study was therefore designed to evaluate the effect of *Duranta erecta* leaves on the oxidative stress status in testosterone-induced BPH in rats.

1.10 Aim and Objectives of the Study

1.10.1 Aim of the Study

This research was aimed at determining the antioxidant potentials of *Duranta erecta* and its effects on some biochemical parameters in testosterone-induced benign prostate hyperplasia in rat models.

1.10.2 Specific Objectives of the Study

This research work is set out to achieve the following specific objectives. To:

- determine the phytochemical constituents of methanol extract of *Duranta erecta* leaves.
- determine the *in vitro* antioxidant potentials of methanol extract of *Duranta erecta* leaves.
- determine the median lethal dose (LD_{50}) of the methanol extract of *Duranta erecta* leaves.
- determine the effect of methanol extract of *Duranta erecta* leaves on markers of prostatic activities in rats induced with benign prostatic hyperplasia.
- determine the effect of methanol extract of *Duranta erecta* leaves on oxidative activities in testosterone-induced benign prostatic hyperplasia in male albino rats.
- carry out histological study of the prostate glands implicated in the research.

CHAPTER TWO

MATERIALS AND METHOD

2.1 Materials

2.1.1 Plant material

The leaves of *Duranta erecta* were used for this study. They were collected From Amagu-uwani village in Nsukka and were identified by Mr. Felix Nwafor, a botanist in the Department of Pharmacognocy, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka.

2.1.2 Animals

Thirty (30) adult albino rats were used for the studies and eighteen (18) albino mice were used for the acute toxicity (LD_{50}) study. All the animals used were purchased from the animal house of the Faculty of Veterinary Medicine, University of Nigerian, Nsukka. The rats were fed with standard finisher mash rat pellets (Vital Feeds) and water *ad libitum*. The guide for the care and use of Laboratory animals procedures were followed in this study.

2.1.3 Equipment

The equipment used were obtained from the Department of Biochemistry and other scientific shops in Nsukka. They include the following: Spectrophotometer (Spectronic 20D, England), refrigerator (Thermocool, England), centrifuge (ABMAN centrifuge), water bath (Gallenkamp, London), hot air oven (0-200 ^{0}C) (Gallenkamp, England), weighing Balance (Metler HAS) and microtiter plate reader (Teco Diagostic Laboratory, USA).

2.1.4: Chemicals and Reagents

The chemicals and reagents used were obtained from reputable companies and were of analytical grade, they include but not limited to the following: absolute Methanol (BDH, Poole England), aluminium chloride (BDH, England), ascorbic acid (May & Baker), ethyl acetate (BDH, England), Fehling's solutions A and B (BDH, England), glacial acetic acid (Sigma, London), hydrochloric acid (BDH, England), lead acetate (BDH, England), 1, 1- Diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich), picric acid (LabTech Chemicals, London), potassium

dihydrogen sulphate (BDH, England), potassium sulphate (May & Baker, England), potassium hydroxide (May & Baker, England), phosphoric acid (Sigma, London), sulphuric acid (BDH, England), testosterone propionate (TESTOST, Laborate Pharmaceuticals Ltd, India), testosterone ELISA kit (Cayman, Ann Harbor, USA), prostate specific antigen (PSA) ELISA kit (Enzo Life Sciences, Farmingdale, NY, USA) and dihydrotestosterone ELISA kit (Cayman, Ann Harbor, USA).

2.2. Methods

2.2.1 Preparation of Plant Material

The fresh leaves of *Duranta erecta* were collected from Amagu-uwani village in Nsukka. They were dried under shade for several days and then pulverised into powder.

2.2.2 Extraction of Plant Material

A quantity (837.11g) of the powdered leaves of *Duranta erecta* was macerated in 2.5litres of methanol for 48 hr. The solution was filtered with Whatman no.1 filter paper and the filtrate was concentrated to a semi-solid residue using rotary evaporator.

2.2.3 Preparation of Reagents for Phytochemical Analysis

5 % (w/v) Ferric Chloride Solution

A quantity, 5.0 g of ferric chloride was dissolved in 100 ml of distilled water.

Ammonium solution

A quantity, 187.5 ml of the stock concentrated ammonium solution was diluted in 31.25 ml of distilled water and made up to 500 ml with distilled water.

45 % (v/v) ethanol

Absolute ethanol (45 ml) was mixed with 55 ml of distilled water.

Aluminium chloride solution

A quantity, 0.5 g of aluminium chloride was dissolved in 100 ml of distilled water.

Dilute sulphuric acid

Concentrated sulphuric acid (10.9 ml) was mixed with 5.0 ml of distilled water and made up to 100 ml.

Lead sub-acetate solution

A quantity, 45 ml of 15 % lead acetate (i.e. 15.0 g of lead acetate in 100 ml of distilled water) was dissolved in 20 ml of absolute ethanol and made up to 100 ml with distilled water.

Wagner's reagent

A quantity, 2.0 g of iodine crystal and 3.0 g of potassium iodide were dissolved in 40 ml of distilled water and made up to 100 ml with distilled water.

Mayer's reagent

A quantity, 13.5 g of mercuric chloride was dissolved in 50 ml of distilled water. Also, 5.0 g of potassium iodide was dissolved in 20 ml of distilled water. The two solutions were mixed and the volume made up to 100 ml with distilled water.

Dragendorff's reagent

Bismuth carbonate (0.85 g) was dissolved in 100 ml of glacial acetic acid and 40 ml of distilled water to give solution A. Another solution called solution B was prepared by dissolving 8.0 g of potassium iodide in 20 ml of distilled water. Both solutions were mixed to give stock solution.

2 % (v/v) Hydrochloric acid

Concentrated hydrochloric acid (2.0 ml) was diluted with some distilled water and made up to 100ml.

2.2.4 Qualitative Phytochemical Analysis of Methanol Extract of *Duranta erecta*

The phytochemical analysis of the methanolic leaves extract of *Duranta erecta* was carried out according to the method of Harborne (1984) and Trease and Evans (1983) to identify its active constituents.

2.2.4.1 Test for Alkaloids

A quantity, 0.2 g of the sample was boiled with 5 ml of 2 % HCl on a steam bath for 5 minutes. The mixture was filtered and 1 ml of the filtrate was treated with 2 drops of the following reagents:

(i) Dragendorff's reagent: An orange precipitate indicated the presence of alkaloids.

(ii) Mayer's reagent: A creamy-white precipitate indicated the presence of alkaloids.

(iii) Wagner's reagent: A reddish-brown precipitate indicated the presence of alkaloids.

2.2.4.2 Test for Flavonoids (Ammonium test)

The sample (0.2 g) was heated with 10 ml ethylacetate in boiling water for 3 minutes. The mixture was filtered, and the filtrate was used. A quantity, 4 ml of the filtrate was shaken with 1ml of dilute ammonium solution to obtain two layers. The layers were allowed to separate. A yellow precipitate observed in the ammonium layer indicated the presences of flavonoids.

2.2.6.3 Test for Glycosides

A known weight, 2.0 g of the sample was mixed with 30 ml of distilled water and heated in a water bath for 5 minutes. The mixture was filtered and the filtrate was used for the following tests. A quantity, 0.3 ml of Fehling's solutions A and B were added to 5 ml of the filtrate until it turned alkaline (tested with litmus paper) and heated on a water bath for 2 minutes. A brick-red precipitate indicated the presence of glycosides.

2.2.4.4 Test for Saponin

A quantity, 0.1 g of the sample was boiled with 5 ml of distilled water for 5 minutes. The mixture was filtered while still hot. The filtrate was used for the following tests. A quantity, 1 ml of the filtrate was diluted with 4 ml of distilled water. The mixture was shaken vigorously and observed on standing for a stable froth.

2.2.4.5 Test for Tannins

A quantity, 2 g of the sample was boiled with 5 ml of 45 % ethanol for 5 minutes. The mixture was cooled and filtered. The filtrate was treated with the following solutions.

(i) Lead sub-acetate solution: To 1 ml of the filtrate was added 3 drops of lead sub-acetate solution. A gelatinous precipitate indicated the presence of tannins.

(ii) Ferric chloride solution: A quantity, 1 ml of the filtrate was diluted with distilled water and then 2 drops of ferric chloride solution was added. A transient greenish to black colour indicated the presence of tannins.

2.2.4.6 Test for Terpenoids and Steroids

Ethanol (9 ml) was added to 1 g of the sample and refluxed for 10 minutes and filtered. The filtrate was concentrated to 2.5 ml on a boiling water bath, and 5 ml of hot water was added. The mixture was allowed to stand for 1 hour, and the waxy matter filtered off. The filtrate was extracted with chloroform (2.5 ml) using a separating funnel. To 0.5 ml of the chloroform extract in a test tube was carefully added 1 ml of concentrated sulphuric acid to form a lower layer. A reddish-brown interface showed the presence of steroids.

Another 0.5 ml aliquot of the chloroform extract was evaporated to dryness on a water bath and heated with 3 ml of concentrated sulphuric acid for 10 minutes on water. A grey colour indicated the presence of terpenoids.

2.2.4.7 Ferric chloride Test for Phenolic Compounds

Two (2.0) ml of the extract was measured in a test tube and 0.01 $mldm^{-3}$ Ferric chloride solution was added drop by drop. Appearance of bluish black precipitate indicated the presence of phenolic compounds and tannins.

2.2.5 Quantitative Phytochemical Analysis of Methanol Extract of *Duranta erecta*

2.2.5.1 Estimation of Alkaloids Concentration

The method described by Harbone (1998) was used to determine the amount of alkaloids present in the extract. A quantity (5 g) of the dried powder of sample was weighed into a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added. The mixture was covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath until it reached one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried and weighed.

2.2.5.2 Estimation of Saponins Concentration

The method described in Soni and Sosa (2013) was used for saponin determination. A quantity (20 g) of sample was placed into a conical flask and 100 ml of 20 % aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20 % ethanol. The combined extracts were reduced to 40 ml over water bath at 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. N-butanol (60 ml) was added. The combined n-butanol extracts were washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and saponin content was calculated.

2.2.5.3 Determination of Total Flavonoid Content

The total flavonoid content was determined using the method by Riaz *et al*. (2012).

Preparation of standard solution

Quercetin (10mg) was weighed and made up to 10ml with methanol in a 10 ml volumetric flask. From the above solution (1mg/ml), 1 ml was pippeted out and made up to 10ml with methanol to get 100 mcg/ml Quercetin standard solution (stock solution). From the stock solution, solutions of concentration 25, 50, 75, 100, 125 and 150 mcg/ml were prepared. To each of these 4ml water

was added followed by 0.3 ml of 5 % sodium nitrite. After 5 min 0.3 ml of 10 % Aluminium chloride solution and at the 6th minute 2 ml of 1 M Sodium hydroxide was added. The total volume was made up to 10 ml with distilled water. A blank was prepared without addition of aluminium chloride solution. The solutions were mixed well and the absorbance was measured against the blank at 510 nm using UV-Visible spectrophotometer. A standard graph was plotted using various concentrations of Quercetin and their corresponding absorbance.

Preparation of sample solution

The total flavanoids content of each plant extract was estimated by method described by Riaz *et al*. (2012). Based on this method, each sample (1.0 ml) was mixed with 4 ml of distilled water and subsequently with 0.3ml of $NaNO_2$ solution (10 %). After 5 min, 0.3ml $AlCl_3$ solution (10 %) was added followed by 2 ml of NaOH solution (1 %) to the mixture. Immediately the mixture was thoroughly mixed and absorbance was then determined at 510 nm versus blank. The standard curve of quercetin was prepared (0-12 mg/ml) and the results were expressed as quercetin equivalents (mg quercetin/g dried extract).The estimation of flavonoid content was carried out in triplicate.

2.2.5.4 Concentration of Terpenoids

The method reported by Ladan *et al*. (2014) was used for the quantitative determination of terpenoids. Extract (2g) was weighed and soaked in 50 ml of 95 % ethanol for 24 hrs.The mixture was filtered and the filtrate extracted with petroleum ether and concentrated to dryness. The dried ether extract was treated as total terpenoids.

2.2.5.5 Estimation of Total Phenolic Content

The total phenolic content was determined using the method by Ainsworth and Gillepie (2007). The total phenolic content of dry extracts was performed with Folin-Ciocaltaeu assay. One (1) ml of sample (1 mg/ml) was mixed with 1 ml of Folin Ciocalteu's phenol reagent. After 5 minutes,10 ml of 7 % sodium carbonate solution was added to the mixture followed by the addition of 13ml of deionized distilled water and mixed thoroughly. The mixture was kept in the dark for 90 minutes at 23 ^{0}C, after which the absorbance was read at 760 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing

Gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of Gallic acid equivalents (GAE)/g of dried sample.

2.2.5.6 Estimation of Total Tannin Content

The total tannin content was determined using the method by Saeed *et al*. (2012). The tannins were determined by Folin-Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 mi of 35 % sodium carbonate solution and diluted to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. a set of reference standard solutions of tannic acid (20, 40, 60, 80, 100 µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 700 nm with an UV/Visible spectrophotometer. The estimation of the tannin content was carried out in triplicate. The tannin content was expressed in terms of mg of tannic acid equivalents/ g of dried sample.

2.2.6 1, 1-diphenyl-2-picrylhydrazyl (DPPH·) radical-scavenging assay

Scavenging activity on DPPH free radicals by the extract was assessed according to the method by Gyamfi *et al*. (1999). A 1.0ml solution of the extract at different concentrations (20-100 µg/ml) in 80 % methanol was mixed with 1.0ml of 0.3mM DPPH in methanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 25 min. The negative control was 1.0ml of 0.3mM DPPH solution plus 2ml of methanol. L-Ascorbic acid was used as positive control. Thereafter, the absorbance of the assay mixture was measured at 518 nm against each blank with UV/Visible spectrophotometer. The lower absorbance of the reaction mixture indicated higher radical scavenging activity. The DPPH radical scavenging activity was calculated using the formula: $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the samples. IC_{50} values were calculated for plant and standard samples.

2.2.7 Nitric Oxide Scavenging Activity

Sodium nitroprusside (5 mM) in standard phosphate buffer solution was incubated with different concentrations (200-1000 µg/ml) of the methanolic plant extract dissolved in phosphate buffer (0.025 M, pH 7.4) and tubes were incubated at 25^0C for 5 hours. Control tube without the plant

extract, but with equivalent amount of buffer was maintained in an identical manner. After 5 hours, 0.5ml of the incubated solution was removed and diluted with 0.5ml of Griess reagent (1 % sulfanilic acid, 5% phosphoric acid, and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite ions with suphanilic acid and its subsequent coupling with Napthylethylenediamine was read at 546nm. The experiment was repeated in triplicate (Vijayakumar *et al*., 2015).

Calculation

$$\text{NO2 Scavenging activity } (\%) = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

The antioxidant activity of the extract was expressed as IC_{50}.

2.2.8 Ferric Reducing Antioxidant Power Assay

The reducing power was determined according to the method of Vijayakumar *et al*. (2015)

Procedure

A volume (1 ml) of varying concentrations (20-100 µg/ml) of plant extract was mixed with 2.5 ml phosphate buffer and 2.5 ml of potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Aliquots of 2.5 ml of trichloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10min. The upper layer of the solution (2.5 ml) was mixed with equal volume of distilled water, to this 0.5ml of freshly prepared 1 % ferric chloride solution was added and the absorbance was measured at 700 nm against a blank. Gallic acid was used as a standard for comparison of the activity. An increased in absorbance of the reaction mixture indicated an increase of reduction capability.

Calculation

$$(\%) \text{ increase in reducing power} = \frac{\text{Absorbance of test}}{\text{Absorbance of blank}} \times 100$$

The antioxidant activity of the extract was expressed as IC_{50}.

2.2.9 Determination of Antioxidant Vitamins Concentrations

The vitamins estimation was performed using the method of Pearson (1976).

2.2.9.1 Determination of Vitamin C Concentration

The sample (1 g) was macerated with 20 ml of 0.4 % oxalic acid. The mixture was filtered.

Indophenol reagent (9 ml) was added to 1ml of the filtrate. The standard solution of vitamin C (ascorbic acid) was prepared similarly and the absorbance of the standard solution and the sample were read at 520 nm. The absorbance of different concentrations of the standard ascorbic acid was plotted against their corresponding concentrations. The amount of vitamin C in the extract was determined using the following formula:

$$\text{Amount of vitamin C} = \frac{\text{Absorbance of sample}}{\text{Slope (standard curve)}} \times \text{dilution factor}$$

$$\text{Dilution factor} = \frac{\text{Total volume}}{\text{weight of sample}}$$

2.2.9.2 Determination of Vitamin E Concentration

The extract (1.0 g) was weighed, macerated with 20 ml of ethanol and then filtered. To 1 ml of the filtrate was added 1 ml of 0.2 % ferric chloride in ethanol and 1 ml of 0.5 % α-dipyridine. This was diluted to 5ml with distilled water. The absorbance of the solution was then read at 520nm. The absorbance of different concentrations of the standard solutions (α-tocopherol) was taken and plotted against their respective concentrations. The slope was calculated and the amount of vitamin E was determined using the following formula:

$$\text{Amount of vitamin E} = \frac{\text{Absorbance of sample}}{\text{Slope (standard curve)}} \times \text{dilution factor}$$

$$\text{Dilution factor} = \frac{\text{Total volume}}{\text{weight of sample}}$$

2.2.10 Determination of Antioxidant Mineral Contents

The minerals were determined using Atomic Absorption Spectrophotometer as described by APHA (1998) after the digestion of the sample.

Wet digestion: The extract (1.0 g) was weighed into a conical flask. Conc. Nitric acid (HNO_3) (20 ml) and 20 ml of Hydrogen peroxide (H_2O_2) were added to the conical flask. The solution was digested on a hot plate using a watch glass. The digestion continued until the solution became clear. The solution was cooled and made up to 50 ml using distilled water. It was then transferred to a dried container and stored for mineral determination.

2.2.10.1 Determination of Zinc Content

The sample (5 ml) was measured into a tube, 2 ml of citric acid solution and two drops of phenolphthalein were added. The solution was neutralized with ammonia. A volume, 5 ml of dithizone was added and the solution shaken. To the supernatant, 2 ml of carbon tetrachloride was added and the solution was shaken, the carbon tetrachloride layer was discarded and 5 ml of dilute dithizone was added and the absorbance taken at 532 nm against the blank.

2.2.10.2 Determination of Selenium Content

The sample (100ul) was pipette into 25ml flask and 5ml of concentrated HCL and 2ml of 2,4 DNPH-NEDA was added into the reagent mixture. The mixture was allowed to stand for 10 minutes with occasional shaking. It was then diluted to the mark with distilled water. The absorbance was measured at 520nm.

2.2.11 Acute Toxicity Test

Acute toxicity study of the plant extract was carried out using the method of Lorke (1983). Eighteen (18) albino mice were utilized in this study. The test involved two phases. In the first phase, nine mice randomly divided into three groups were given 10, 100, 1000 mg/kg body weight (via a cannula), respectively. Based on the result of the phase one study, the procedure was repeated using another set of mice randomly divided into three groups of three mice each, given 1600, 2900 and 5000 mg/kg body weight respectively. The mice were observed for signs of toxicity and death during 24 hours of constant observation.

2.2.12 Experimental Design

2.2.12.1 BPH Induction

Thirty (30) male albino rats weighing 203-305g were used for the study. They were acclimatized for seven (7) days with free access to feed and water. After 7 days of acclimatization, rats were randomly divided into two groups; the first group made up of five male albino rats which served as normal control group throughout the experiment and the other group made up of twenty five male albino rats. Briefly, BPH was induced in the latter group by daily subcutaneous injection of testosterone propionate (3 mg/kg) for 28 days according to the method of Jang *et al*. (2010). On day 29, ocular puncture was carried out by a trained technician to collect blood for baseline serum PSA level determination. All the rats injected with testosterone propionate had a serum PSA level ≥ 1.70 ng/ml. The BPH induced rats were randomly divided into five experimental groups of five animals each.

2.2.12.2 Experimental Groups

Group 1: Normal control

Group 2: Positive control (BPH induced and untreated)

Group 3: BPH induced rats treated with a standard drug (finasteride 3 mg/ kg body weight)

Group 4: BPH induced rats treated with 200 mg/kg body weight of the extract.

Group 5: BPH induced rats treated with 400 mg/kg body weight of the extract.

Group 6: BPH induced rats treated with 600 mg/kg body weight of the extract.

The treatment lasted for 21 days. At the end of the 21 days treatment, all the rats were anesthetised using chloroform inhalation following an overnight fasting, and cardiac puncture was performed to collect blood samples. The prostates were immediately dissected out and weighed.

2.2.13 Determination of Prostate Weight and Prostatic Index

At the end of the experiment, the prostate glands of all the experimental animals were harvested and weighed. The prostatic index was calculated as the ratio of the prostate weight to the total body weight (Ishola *et al*., 2017).

2.2.14 Determination of Serum Testosterone Concentration

Enzyme immunoassay technique was used for the quantitative determination of testosterone concentration (Marcus and Durnford, 1985; Ekins, 1990).

Principle

The principle of the enzyme immunoassay test follows the typical competitive binding schematic. Competition occurs between an unlabeled antigen (present in standards, control and patient samples) and an enzyme-labeled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stop solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of testosterone in the sample. A set of standards is used to plot a standard curve from which the amount of testosterone in the samples and controls can be directly read.

Procedure

Working solutions of the testosterone-HRP conjugate and wash buffer were prepared. The required numbers of microwell strips were removed, then the bag was reseal and unused strips were returned to the refrigerator. Each of the calibrator, control and specimen sample (50 µl) were pipette into correspondingly labelled wells in duplicate. The conjugate working solution (100 µl) was pipetted into each well. The plate was incubated on a plate shaker (approximately 200 rpm) for 1 hour at room temperature. The wells were washed 3 times with 300 µl of diluted wash buffer per well and the plate taped firmly against absorbent paper to ensure that it is dry. TMB(3,3’,5,5’-Tetramethylbenzidine) substrate (150 µl) was Pipette into each well. The plate was incubated on a plate shaker for 10-15 minutes at room temperature. The stop solution (50 µl)

was pipetted into each well. The plate was read on a microwell plate reader at 450nm within 20 minutes after addition of the stop solution.

Calculations

The mean optical density of each calibrator duplicate was calculated, a calibrator curve was drawn with absorbance on the Y-axis and the calibrator concentrations on the X-axis. The absorbance of each unknown duplicate (samples) was calculated. The values of the unknowns (samples) were read directly with calibration.

2.2.15 Determination of Serum Prostate Specific Antigen (PSA) Concentration

Enzyme immunoassay technique was used for the quantitative determination of prostate specific antigen as described by Stowell *et al*. (1991).

Principle

The PSA EIA test is a solid phase two-site immunoassay. Rabbit anti-PSA is coated on the surface of the microtiter wells and another anti-PSA monoclonal antibody labelled with horseradish peroxidase is used as the tracer. The PSA molecules present in the standard solution or serum are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen-antibody-enzyme complex, the unbound antibody-enzyme tracers are removed by washing. The horseradish peroxidase activity bound in the wells is then assayed by a colorimetric reaction. The intensity of the colour formed is proportional to the concentration of PSA present in the sample.

Procedure

The desired numbers of coated wells were secured in the holder. The standards, specimens, and controls (25 µl each) were dispensed into appropriate wells. Enzyme Conjugate Reagent (100 µl) was dispensed into each well and gently mixed for 5 seconds. The mixture was incubated at room temperature for 45 minutes. The incubation mixture was removed by emptying plate contents into a waste container. The microtitre wells were rinsed and emptied 5 times with washing buffer (1X). The wells were stroked sharply onto absorbent paper to remove residual water droplets. The TMB solution (100µl) was dispensed into each well and was gently mixed

for 5 seconds and then incubated at room temperature for 15 minutes. The reaction was stopped by adding 100µl of stop solution to each well. It was gently mixed for 30 seconds to make sure that the blue colour completely changed to yellow. Using a microtitre plate reader, the absorbance was read at 450nm within 15 minutes.

Calculations

The mean absorbance value was calculated (A450) for each set of reference standards, controls, and samples. A standard curve was plotted by using the mean absorbance obtained from each reference standard against its concentration in ng/ml. The absorbance values were placed on the vertical or Y-axis and concentrations on the horizontal or X-axis. The mean absorbance values for each specimen were used to determine the corresponding concentration of PSA in ng/ml from the standard curve.

2.2.16 Determination of Serum Dihydrotestosterone Concentration

Enzyme immunoassay technique was used for the quantitative determination of dihydrotestosterone concentration (Bassett, 1980).

Principle

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, control and samples) and an enzyme-labeled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stop solution. The absorbance is measured on a microtitre plate reader. The intensity of the color formed is inversely proportional to the concentration of DHT in the sample. A set of standards is used to plot a standard curve from which the amount of DHT in samples and controls can be directly read.

Procedure

Working solutions of the DHT-HRP conjugate and wash buffer were prepared. The required numbers of microwell strips were removed. The bag was Reseal and unused strips were returned

to the refrigerator. Each of the calibrator, control and specimen sample (50 µl) were pipette into correspondingly labelled wells in duplicate. The conjugate working solution (100 µl) was pipette into each well. The plate was incubated on a plate shaker (approximately 200 rpm) for 1 hour at room temperature. The stop Solution (50 µL) was added to each well. The liquid was mixed by tapping the side of the plate until colour change appeared uniformly. The TMB substrate (150 µl) was pipetted into each well. The plate was incubated on a plate shaker for 10-15 minutes at room temperature. The stop solution (50 µl) was pipetted into each well. The plate was read on a microwell plate reader at 450nm within 20 minutes after addition of the stop solution.

Calculations

The mean absorbance of each calibrator duplicate was calculated, a calibration curve was drawn with the mean absorbamce on the Y-axis and the calibrator concentrations on the X-axis. The mean optical density of each unknown duplicate (samples) was calculated. The values of the unknowns (samples) were read directly off the calibration curve.

2.2.17 Assay for Serum Acid Phosphatase (ACP)

The concentration of serum acid phosphatase (ACP) was assayed using the method of Tietz (1994) as outlined in Randox Kit, UK.

α-Naphthyl phosphate + H_2O $\longrightarrow$ α – Naphthol + Inorg. Phos

α-Naphthol + Fast Red TR $\longrightarrow$ Diazo Dry (Chromophore)

The α-Naphthol released from the substrate α-Naphthyl phosphate by acid phosphates is coupled with Fast Red TR to produce a coloured complex that absorbs light at 405 nm. The reaction can be quantitated photometrically because the coupling reaction is instantaneous. The L-tratrate inhibits prostatic acid phosphates but does not interfere with the reaction mechanism. Therefore, if testing is performed in the presence or absence of L-tratrate, the difference between the results of the two assays is the level of prostatic acid phosphates in the serum.

Procedure

The serum (100 µl) pipetted into the sample test tube and 100 µl was pipetted into the blank test tube. Then, 1 ml of reagent solution were added, mixed and incubated for 5 minutes at 37^0C. The

initial absorbance was read at 405 nm and the timer started simultaneously. The absorbances were read again 1, 2, and 3 minutes.

$$\text{Acid phosphatase (U/L)} = \frac{\text{Change in Abs/min. x 106 x 1.1}}{\text{12.9 x 103 x 1.0 x 0.1}}$$

10^6 = conversion of moles to millimoles

1.1 = total reaction volume

12.9 x 10^3 = molar absorptivity of α-Naphthol-Fast complex at 405 nm

1.0 = light path in cm

0.1 = sample volume (ml)

2.2.18 Assay for Oxidative Activity

2.2.18.1. Assay for Superoxide Dismutase Activity

Superoxide dismutase (SOD) activity in the prostate tissue was assayed according to the method of McCord and Fridovich (1969).

Principle

Illumination of riboflavin solution in the presence of EDTA causes a reduction of the flavin. It then re-oxidizes and simultaneously reduces oxygen to O_2^-, which is allowed to react with a detector molecule nitro blue tetrazolium (NBT), reduce the NBT to a formazan blue. The SOD in the sample will inhibit the formazan production.

Procedure

The homogenate (0.01 ml) was mixed with 0.2 ml of 0.1 M EDTA (containing 0.0015 % NaCN), 0.1 ml of 1.5 mM NBT and phosphate buffer (67 mM, pH 7.8) in a total volume of 2.6 ml. After adding 0.05 ml of riboflavin, the absorbance of the solution was measured against distilled water at 560 nm. All the tubes were illuminated uniformly for 15 min and absorbance of the blue colour formed was measured again. Percent of inhibition was calculated after comparing absorbance of sample with the absorbance of control (the tube containing no enzyme activity).

The volume of the sample required to scavenge 50 % of the generated superoxide anion was considered as 1 unit of enzyme activity and expressed in U/mg protein.

2.2.18.2. Assay for Catalase Activity

Catalase (CAT) activity in the prostate tissue was assayed according to the method of Beer and Sizer (1952).

Principle

Catalase catalyses the decomposition of H_2O_2. In the ultraviolet range, H_2O_2 shows a continual increase in absorption. The decomposition of H_2O_2 can be followed directly by the decrease in extinction at 240 nm.

$2\ H_2O_2 \longrightarrow 2\ H_2O + O_2$

Procedure

The tissue homogenate (0.1 ml which is approximately 0.1 mg protein) was mixed with 1.9 ml of the phosphate buffer (0.5 M, pH 7). The decrease in extinction was measured at 240 nm, 1 min interval for 3 min immediately after adding 1 ml of 11 mM H_2O_2 solution in buffer. A sample control was placed in the reference cuvette containing 0.1 ml of tissue homogenate and 2.9 ml of the buffer. The activity of catalase was calculated using the mmoles extinction coefficient 40 cm^{-1}.

2.2.18.3. Assay for Glutathione Peroxidase Activity

Glutathione peroxidase (GPx) activity in the prostate tissue was assayed according to the method of Hafemann *et al.* (1974).

Principle

The activity of GPx was determined by measuring the decrease in GSH content after incubating the sample in the presence of H_2O_2 and NaN_3.

$H_2O_2 + 2\ GSH \longrightarrow 2H_2O + 2\ GSSG$

Procedure

Tissue homogenate (approximately 0.5 mg protein) was incubated with 0.1 ml of 5mM GSH, 0.1 ml of 1.25 mM H_2O_2, 0.1ml of 25 mM NaN3 and phosphate buffer (0.05 mM, pH 7) in a total volume of 2.5 ml at 37ºC for 10 min. The reaction was stopped by adding 2 ml of 1.65 % HPO_3^{2-} and the reaction mixture was centrifuged at 1500 rpm for 10 min. Two (2) ml of the supernatant was mixed with 2 ml of 0.4 M Na_2HPO_4 and 1ml of 1mM DTNB. The absorbance of the yellow coloured complex was measured at 412 nm after incubation for 10 min at 37ºC against distilled water. A sample without the tissue homogenate processed in the same way was kept as non enzymatic reaction control. One unit of enzyme activity was defined as decrease in log GSH by 0.001/min after subtraction of the decrease in log GSH per minute for the non enzymatic reaction and is expressed as units/mg protein.

2.2.18.4 Determination of Reduced Glutathione (GSH) Concentration

Glutathione concentration in the prostate tissue was determined according to the method of Clifton (2012).

Principle

The acid soluble sulfhydryl groups (non-protein thiols of which more than 93 % is reduced glutathione) form a yellow coloured complex with dithionitrobenzene (DTNB). The absorbance of the coloured complex was measured at 412 nm.

Procedure

The tissue homogenate (0.5 ml) was mixed with 0.1 ml of 25 % TCA, kept on ice for few minutes. These were then subjected to centrifugation at 3000 g for few minutes to settle the

precipitate. Also, 0.3 ml of the supernatant was mixed with 0.7 ml of 0.2 M sodium phosphate buffer (pH 8) and 2 ml of 0.6 mM DTNB (prepared in 0.2 M buffer, pH 8). The yellow colour obtained was measured after 10 min at 412 nm against a blank which contained 0.1 ml of 5 % TCA in place of the supernatant. A standard graph was prepared using different concentrations (10-50 n moles) of GSH in 0.3 ml of 5 % TCA. The GSH content was calculated with the help of this standard graph and expressed as n moles/mg protein.

2.2.18.5 Determination of Tissue Malondialdehyde Concentration

The level of lipid peroxidation in the tissue was measured using TBARS assay. The TBARS assay was performed by standard method using malondialdehyde equivalents derived from tetramethoxypropane (Devasagayam *et al*., 1983).

Principle

Malondialdehyde (MDA) and other aldehydes have been identified as products of lipid peroxidation that react with thiobarbituric acid (TBA) to give a pink coloured chromophore at 532nm.

Procedure

The method involved heating of biological samples with TBA reagent for 20 min in a boiling water bath. The TBA reagent contains 20 % TCA, 0.5 % TBA, 2.5 N HCl and 6 mM EDTA. After cooling, the solution was centrifuged at 2,000 rpm for 10 min and the precipitate obtained was removed. The absorbance of the supernatant was determined at 532 nm against a blank that contained all the reagents except the biological sample. The concentration of TBARS was then calculated as MDA equivalents with the help of a standard curve prepared using different concentrations (1-10 n mole) of 1'1'3'3'-tetramethoxypropane in 1 ml distilled water and is expressed as n mole of MDA/mg protein. For correction of endogenous TBARS, fresh samples (without any treatment) were boiled with TBA and values were subtracted.

2.2.19 Prostate Tissue Histology

The prostatic tissue harvested from each animal group was fixed in 10 % formal saline solution for seven days. Before embedding in paraffin wax, the fixed prostate tissue was removed and

dehydrated in increasing concentrations of ethanol; (70 %, 80 %, 90 % and 100 %). The tissue was thereafter treated with acetone and then cleared in xylene for 30 min to enhance the tissue transparency followed by impregnation and embedding in paraffin wax. The embedded tissue was sectioned at 10 μm, mounted on a slide and stained with hematoxylin and eosin (H&E) stains (Mbaka *et al*., 2017). Each section was examined under a light microscope for structural changes and photomicrographs were taken. (Mbaka *et al*., 2017).

2.2.20 Statistical Analysis

The results were expressed as means ± SD and tests of statistical significance were carried out using one-way and two-way analysis of variance (ANOVA). The Statistical Product and Service Solutions (SPSS) IBM version 20 was used. $p < 0.05$ was considered significant.

CHAPTER THREE

RESULTS

3.1 Extraction Yield of Methanol Extract of *Duranta erecta*

$$\text{Percentage yield} = \frac{143.12\ (\text{g})}{837.11\ (\text{g})} \text{ x } 100 = 17.10\ \%$$

3.2 Phytochemical Composition of *Duranta erecta* Leaves

3.2.1 Qualitative Phytochemical Composition of Methanol Extract of *Duranta erecta* Leaves

The qualitative phytochemical analysis of methanol extract of ***Duranta erecta*** leaves shows the presence of flavonoids, alkaloids, tannin, saponin, steroids, terpenoids and Phenolics (Table 2).

Table 2: Qualitative phytochemical composition of methanol extract of *Duranta erecta* leaves

Phytochemicals	**Inference**
Alkaloids	+++
Flavonoids	+++
Phenolics	+++
Glycosides	ND
Saponins	++
Tannins	++
Terpenoids	++
Steroids	+

Key:

+ Present in low concentration
++ present in moderate concentration
+++ present in high concentration
ND = Not Detected

3.2.2 Quantitative Phytochemical Composition of Methanol Extract of *Duranta erecta* Leaves

The quantitative analysis of methanol extract of *Dutanta erecta* is represented in table 3. Flavonoid has the highest concentration of 24.20 ± 0.14 mg QE/g. Alkaloid (15.87 ± 1.71 mg/g), total phenol (12.73 ± 0.61 mg GAE/g), tannin (9.24 ± 0.03 mg Tannic acid/g), terpenoid (8.90 ± 0.96 mg/g), steroid (2.65 ± 0.55 mg/g) and saponin (5.55 ± 0.76 mg/g).

Table 3: Quantitative phytochemical composition of methanol extract of *Duranta erecta* leaves

Phytochemical constituent	Concentration (mg/g)
Total phenol	12.73 ± 0.61
Flavonoid	24.20 ± 0.14
Alkaloid	15.87 ± 1.71
Tannin	9.24 ± 0.03
Terpenoid	8.90 ± 0.96
Steroid	2.65 ± 0.55
Saponin	5.55 ± 0.76

Values are presented as mean ± SD (n = 3)

3.3 Antioxidant Activity

3.3.1 DPPH Radical Scavenging Activity of Methanol Extracts of *Duranta erecta.*

The ability of the extract to scavenge DPPH radicals was investigated at various concentrations of the extract. The addition of methanol extract of *Duranta erecta* to the DPPH solution caused a rapid decrease in absorbance at 518 nm. As shown in Table 4, the extract possessed substantial dose-dependent antioxidant activity and the activity which is comparable to that of L-ascorbic acid, which was used as a control antioxidant.

The percentage inhibitory activity of *Duranta erecta* leaves extracts was concentration dependent with a half maximal inhibitory concentration (IC_{50}) of 40.91 ± 0.19 µg/ml compared to standard (ascorbic acid) with IC_{50} of 26.88 ± 0.57 µg/ml.

Table 4: DPPH radical scavenging activity of methanol extracts of *Duranta erecta*

Concentration (µg/ml)	% Inhibition by extract	% Inhibition by standard (Ascorbic acid)
20	41.67 ± 0.76	48.11 ± 1.14
40	47.97 ± 1.33	58.64 ± 1.58
60	57.20 ± 1.14	61.74 ± 2.69
80	70.83 ± 1.14	86.74 ± 0.58
100	77.90 ± 2.31	89.37 ± 2.53
IC_{50} (µg/ml)	40.91 ± 0.19	26.88 ± 0.57

Values are presented as mean ± SD (n = 3)

3.3.2 Nitric Oxide Radical Scavenging Activity of Methanol Extracts of *Duranta erecta*

Table 5 shows a concentration dependent nitric oxide radical scavenging activity of the extract with a half maximal inhibitory concentration (IC_{50}) of 56.89 ± 3.39 µg/ml while the standard (ascorbic acid) has an IC_{50} of 32.16 ± 3.79 µg/ml. The highest percentage inhibition of nitric oxide activity was observed at the highest dose of 100 µg/ml (68.51 ± 1.44 %) while the lowest was at the concentration of 20 µg/ml (35.40 ± 2.11 %).

Table 5: Effect of methanol extracts of *Duranta erecta* on nitric oxide radicals

Concentration (µg/ml)	% Inhibition by extract	% Inhibition by standard (Ascorbic acid)
20	35.40 ± 2.11	35.43 ± 2.81
40	41.60 ± 2.61	57.93 ± 1.24
60	49.20 ± 0.80	71.72 ± 2.12
80	61.38 ± 3.45	76.55 ± 1.42
100	68.51 ± 1.44	84.21 ± 0.14
IC_{50} (µg/ml)	56.89 ± 3.39	32.16 ± 3.79

Values are presented as mean ± SD (n = 3)

3.3.3 Ferric Reducing Antioxidant Power of the Extract.

Table 6 shows a concentration dependent reducing power of the extract with a half maximal inhibitory concentration (IC_{50}) of 97.23 ± 0.68 µg/ml while the standard (gallic acid) has an IC_{50} of 60.64 ± 2.81 µg/ml. The highest percentage inhibition was observed at the highest dose of 100 µg/ml (52.58 ± 0.53 %) while the lowest was at the concentration of 20 µg/ml (30.86 ± 1.28 %).

Table 6 Effect of methanol extracts of *Duranta erecta* on ferric reducing antioxidant power

Concentration (µg/ml)	**% Inhibition by extract**	**% Inhibition by standard (Gallic acid)**
20	30.86 ± 1.28	39.28 ± 1.21
40	33.83 ± 0.32	43.01 ± 2.12
60	38.85 ± 3.85	47.98 ± 1.01
80	44.59 ± 1.06	56.90 ± 0.23
100	52.58 ± 0.53	62.02 ± 1.21
IC_{50} (µg/ml)	97.23 ± 0.68	60.64 ± 2.81

Values are presented as mean ± SD (n = 3)

3.3.4 Antioxidant Minerals Composition of Methanol Extract of *Dutanta erecta*

Table 7 shows the antioxidant minerals composition of the methanol extract of *Duranta erecta*. Selenium has a lower concentration (0.59 ± 0.04 mg/100g) compared with zinc having a concentration of 1.82 ± 0.03 mg/100g.

Table 7: Antioxidant minerals composition of methanol extract of *Dutanta erecta*

Mineral Constituents	Concentration (mg/100g)
Selenium	0.59 ± 0.04
Zinc	1.82 ± 0.03

Values are presented as mean ± SD (n = 3)

3.3.5 Antioxidant Vitamin Composition of Methanol Extract of *Duranta erecta*

Table 8 shows the antioxidant vitamin composition of the extract. It shows that the extract has lower concentrations of vitamin C (0.35 ± 0.01 mg/100g) and vitamin E (0.68 ± 0.07 mg/100g)

Table 8: Antioxidant vitamin composition of methanol extract of *Duranta erecta*

Vitamin	Concentration (mg/100g)
Vitamin C	0.35 ± 0.01
Vitamin E	0.68 ± 0.07

Values are presented as mean ± SD (n = 3)

3.4 Acute Toxicity (LD_{50}) Test of Methanol Extract of *Duranta erecta* Leaves

The acute toxicity test of the methanol extract of leaves of *Duranta erecta* showed neither death nor any sign of toxicity up to a dose of 5000 mg/kg body weight.

Table 9: Phase I and II of the acute toxicity (LD_{50}) test

	Dosage (mg/kg body weight)	Mortality
Phase I		
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Phase II		
Group 1	1600	0/3
Group 2	2900	0/3
Group 3	5000	0/3

3.5 Prostate Markers

3.5.1 Effect of Methanol Extracts of *Duranta erecta* Leaves on Prostate Weight and Prostatic Index.

The subcutaneous injection of testosterone propionate caused a significant ($p < 0.05$) increase in prostatic weight and prostatic index in the positive control group (1.41 ± 0.18 g, 5.71 ± 0.29 mg/g) when compared with the normal control group (0.20 ± 0.02 g, 0.91 ± 0.06 mg/g) as shown in table 10. There is significant ($p < 0.05$) reduction in the prostate weight and prostatic index in groups treated with 200 mg/kg of extract (0.69 ± 0.11 g, 3.10 ± 0.22 mg/g), 400 mg/kg of extract (0.53 ± 0.11 g, 2.50 ± 0.40 mg/g), 600 mg/kg of extract (0.45 ± 0.04 g, 1.65 ± 0.21 mg/g) and the standard control group (0.49 ± 0.02 g, 2.35 ± 0.10 mg/g) compared with the positive control (1.41 ± 0.18 g, 5.71 ± 0.29 mg/g).

Table 10: Effect of methanol extracts of *Duranta erecta* leaves on prostrate weight and prostatic index

Group	PW (g)	PI (mg/g)
Normal Control	0.20 ± 0.02^{a}	0.91 ± 0.06^{a}
Positive Control	1.41 ± 0.18^{d}	5.71 ± 0.29^{e}
Standard Control	0.49 ± 0.02^{b}	2.35 ± 0.10^{b}
BPH+200 mg/kg b.w of extract	0.69 ± 0.11^{c}	3.10 ± 0.22^{c}
BPH+400 mg/kg b.w of extract	0.53 ± 0.11^{b}	2.50 ± 0.40^{b}
BPH+600 mg/kg b.w of extract	0.45 ± 0.04^{b}	1.65 ± 0.21^{d}

Values are presented as mean ± SD (n = 5)

Groups with different superscripts down the column are significantly different from each other at $p < 0.05$.

Key:

BPH = Benign prostate hyperplasia

PW = Prostate weight

BW = Body Weight

PI = Prostatic Index

3.5.2 Effect of Methanol Extract of *Duranta erecta* on Serum Prostate Specific Antigen (PSA).

The subcutaneous injection of 3 mg/kg of testosterone propionate for 28 days caused a significant ($p < 0.05$) increase in serum prostate specific antigen (PSA) in all the induced groups (2.03±0.06ng/ml) compared with the normal control group (0.39±0.13ng/ml). Treatment with Finasteride (standard drug) and different doses of the extract (200 mg/kg, 400 mg/kg and 600 mg/kg b.w) for 21 days caused a significant ($p < 0.05$) decrease in PSA levels (0.98±0.12,1.48±0.16, 1.25±0.20 and 1.10±0.38ng/ml) compared with the untreated group (2.03±0.06ng/ml) (Table 11).

Table 11: Effect of methanol extract of *Duranta erecta* on serum prostate specific antigen (PSA)

Group	PSA (ng/ml) Before Treatment	PSA (ng/ml) After Treatment
Normal Control	0.40 ± 0.09^{a}	0.39 ± 0.13^{a}
Positive Control	1.94 ± 0.07^{b}	2.03 ± 0.06^{e}
Standard Control	$1.89 \pm 0.12^{b*}$	$0.98 \pm 0.12^{b*}$
BPH+200 mg/kg b.w of extract	$2.06 \pm 0.10^{b*}$	$1.48 \pm 0.16^{d*}$
BPH+400 mg/kg b.w of extract	$2.00 \pm 0.16^{b*}$	$1.25 \pm 0.20^{c*}$
BPH+600 mg/kg b.w of extract	$2.01 \pm 0.62^{b*}$	$1.10 \pm 0.38^{bc*}$

Values are presented as mean ± SD (n = 5)

Groups with different superscripts down the column are significantly different from each other at $p < 0.05$.

Groups bearing * across the row are significantly different from one another at $p < 0.05$.

3.5.3 Effect of Methanol Extracts of *Duranta erecta* Leaves on Serum Testosterone and Serum Dihydrotestosterone Levels.

Table 12 shows the effect of methanol extract of *Duranta erecta* leaves on serum testosterone and serum dihydrotestosterone level. The subcutaneous injection of testosterone significantly ($p < 0.05$) increased the serum levels of testosterone and dihydrotestosterone in the positive control group (3.95 ± 0.56 ng/ml; 18.59 ± 1.16 ng/ml) compared with the normal control group (1.39 ± 0.23 ng/ml; 8.35 ± 0.65 ng/ml). There was significant ($p < 0.05$) decrease in the levels of testosterone and dihydrotestosterone in all groups compared with the positive control group. There was however no significant ($p > 0.05$) increase in testosterone level in group treated with 600 mg/kg b. w of extract (1.72 ± 0.39 ng/ml) compared with the normal control (1.39 ± 0.23 ng/ml).

Table 12: Effect of methanol extracts of *Duranta erecta* leaves on serum testosterone and serum dihydrotestosterone levels

Groups	Testosterone (ng/ml)	Dihydrotestosterone (ng/ml)
Normal Control	1.39 ± 0.23^{a}	8.35 ± 0.65^{a}
Positive Control	3.95 ± 0.56^{e}	18.59 ± 1.16^{d}
Standard Control	2.23 ± 0.13^{bc}	14.35 ± 0.47^{c}
BPH+200 mg/kg b.w of extract	3.18 ± 0.59^{d}	14.63 ± 0.69^{c}
BPH+400 mg/kg b.w of extract	2.86 ± 0.77^{cd}	14.30 ± 1.02^{c}
BPH+600 mg/kg b.w of extract	1.72 ± 0.39^{ab}	12.60 ± 0.89^{b}

Values are presented as mean ± SD (n = 5)

Groups with different superscripts down the column are significantly different from each other at $p < 0.05$.

3.5.4 Effect of Methanol Extract of *Duranta erecta* Leaves on Acid Phosphatase Activity (ACP)

There was significant ($p < 0.05$) increase in serum acid phosphatase activity in the positive control group (42.23 ± 4.64) when compared with the normal control group (23.60 ± 1.12). There were however significant ($p < 0.05$) reduction in serum acid phosphatase in groups 3, 4, 5 and 6 which received 3 mg/kg of finasteride (31.75 ± 1.25), 200(30.39 ± 1.94), 400(33.14 ± 3.90) and 600 (30.70 ± 1.27) mg/kg of extract respectively when compared with the positive control group(42.23 ± 4.64) as shown in table 13.

Table 13: Effect of methanol extract of *Duranta erecta* leaves on acid phosphatase activity (ACP)

Group	ACP (U/L)
Normal Control	23.60 ± 1.12^{a}
Positive Control	42.23 ± 4.64^{c}
Standard Control	31.75 ± 1.25^{b}
BPH+200 mg/kg b.w of extract	30.39 ± 1.94^{b}
BPH+400 mg/kg b.w of extract	33.14 ± 3.90^{b}
BPH+600 mg/kg b.w of extract	30.70 ± 1.27^{b}

Values are presented as mean ± SD (n = 5)
Groups with different superscripts down the column are significantly different from each other at $p < 0.05$.

3.5.5 Effect of Methanol Extract of *Duranta erecta* Leaves on Prostate Antioxidant Enzymes

There was significant ($p < 0.05$) reduction in the activities of superoxide dismutase (22.50 ± 3.20), catalase (5.49 ± 1.02), glutathione peroxidase (13.79 ± 0.55) and reduced glutathione (12.50 ± 1.41) concentration in the prostate gland in the positive control group compared with the normal control group. Treatment with different doses of the extract and standard drug exhibited a significant ($p < 0.05$) increase in the activity of SOD, CAT, GPx and concentration of GSH when compared with the untreated control group (Table 14).

3.5.6 Effect of Methanol Extract of *Duranta erecta* Leaves on Prostate Lipid Peroxidation

Malondialdehyde (MDA) concentrations increased significantly ($p < 0.05$) in the prostate of the positive control group (7.76 ± 0.73) when compared with the normal control group (1.80 ± 0.26). Group 3, 4, 5 and 6 which were treated with 3 mg/kg of finasteride, 200, 400 and 600 mg/kg of extract respectively had significant ($p < 0.05$) reductions (3.04 ± 0.77, 3.07 ± 0.27, 2.99 ± 0.92 and 2.47 ± 0.57) in the MDA concentrations in the prostate when compared with the positive control group (7.76 ± 0.73) . Group 6 (treated with 600 mg/kg b.w of extract) however had a non significant ($p > 0.05$) difference in MDA concentration (2.47 ± 0.57) when compared with the normal control (1.80 ± 0.26) as shown in Table 14.

Table 14: Effect of methanol extract of *Duranta erecta* leaves on prostate antioxidant enzymes and lipid peroxidation

Group	SOD (U/mg protein)	CAT (U/mg protein)	GSH (n Mol/mg protein)	GPx (U/mg protein)	MDA (n Mol/mg protein)
Normal Control	42.28 ± 2.38^{d}	10.83 ± 0.78^{c}	21.66 ± 1.73^{c}	21.58 ± 0.81^{d}	1.80 ± 0.26^{a}
Positive Control	22.50 ± 3.20^{a}	5.49 ± 1.02^{a}	12.50 ± 1.41^{a}	13.79 ± 0.55^{a}	7.76 ± 0.73^{c}
Standard Control	32.87 ± 2.10^{b}	8.07 ± 0.86^{b}	17.45 ± 1.59^{b}	19.86 ± 1.12^{bc}	3.04 ± 0.77^{b}
BPH+200 mg/kg b.w of extract	36.05 ± 1.87^{c}	8.48 ± 0.36^{b}	19.17 ± 1.60^{b}	19.26 ± 2.08^{bc}	3.07 ± 0.27^{b}
BPH+400 mg/kg b.w of extract	32.02 ± 1.87^{b}	7.86 ± 0.60^{b}	18.83 ± 0.92^{b}	18.53 ± 0.97^{b}	2.99 ± 0.92^{b}
BPH+600 mg/kg b.w of extract	34.26 ± 1.32^{c}	8.25 ± 0.70^{b}	18.01 ± 1.07^{b}	20.44 ± 0.99^{cd}	2.47 ± 0.57^{ab}

Values are presented as mean ± SD (n = 5)
Groups with different superscripts down the column are significantly different from each other at $p < 0.05$.

Key:

BPH = Benign prostate hyperplasia

SOD = Superoxide dismutase
CAT = Catalase
GSH = Reduced Glutathione
GPx = Glutathione peroxidase
MDA = Malondialdehyde

3.6 Effect of Methanol Extract of *Duranta erecta* Leaves on the Histology of the Prostate Gland

The prostate tissues of rats in the normal control group (Plate 2) showed normal histological features characterised by adequate micro acini. It had small-sized acini with thin epithelium (EP), little or no prostatic secretion (S) was observed in the acini. The general feature of the tissue was adequate with no sign of dysfunction. The prostate of the positive control group (BPH induced and untreated) (Plate 3), showed ectasia (dialation) of the prostate associated with enlarged acini with thick epithelium (EP). The ectasia of the acini appeared cystic. Haemorrhagic (H) condition associated with blood vessel rupture was observed within the fibromuscular stroma which could lead to adenoma of the epithelium. More prostatic secretion was also observed.

Plate 4 showed the photomicrograph of the prostate tissue of the standard control group. It had small sized acini with thin epithelium. Little or no prostatic secretion was observed in the lumen. The prostate tissue of rats in group 4 (plate 5) showed reduced prostatic secretion (S) relative to the untreated group. Minor passive haemorrhages (H) (black arrow) were observed. Benign prostate hyperplasia group treated with 400 mg/kg b.w of extract (Plate 6) showed slightly large acini with little prostatic secretion. Plate 7 (BPH induced treated with 600 mg/kg b.w of extract) showed small-sized acini, moderate prostatic secretions and thin epithelium.

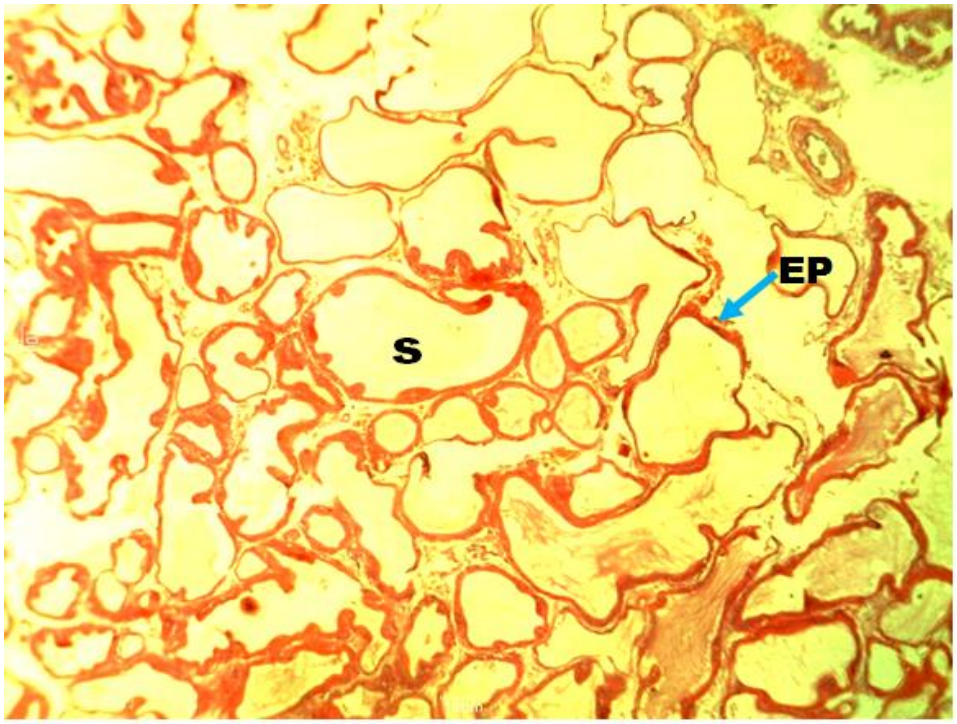

Plate 2: Photomicrograph of prostate gland of the normal control group. H&E. mag. 400X.

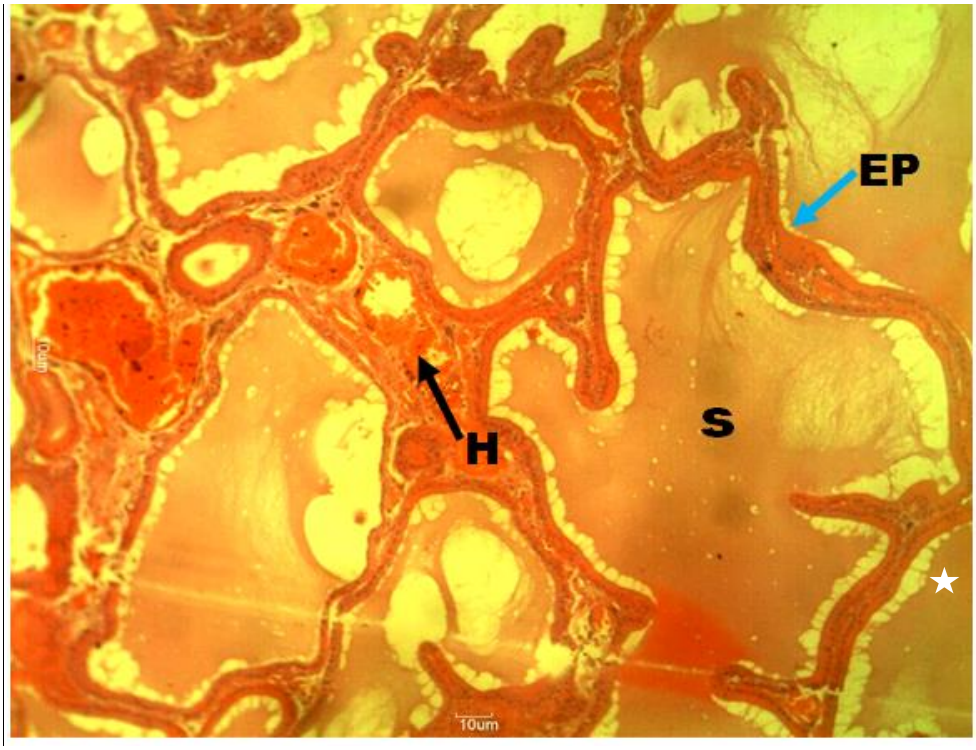

Plate 3: Photomicrograph of prostate gland of the positive control group. H&E. mag. 400X.

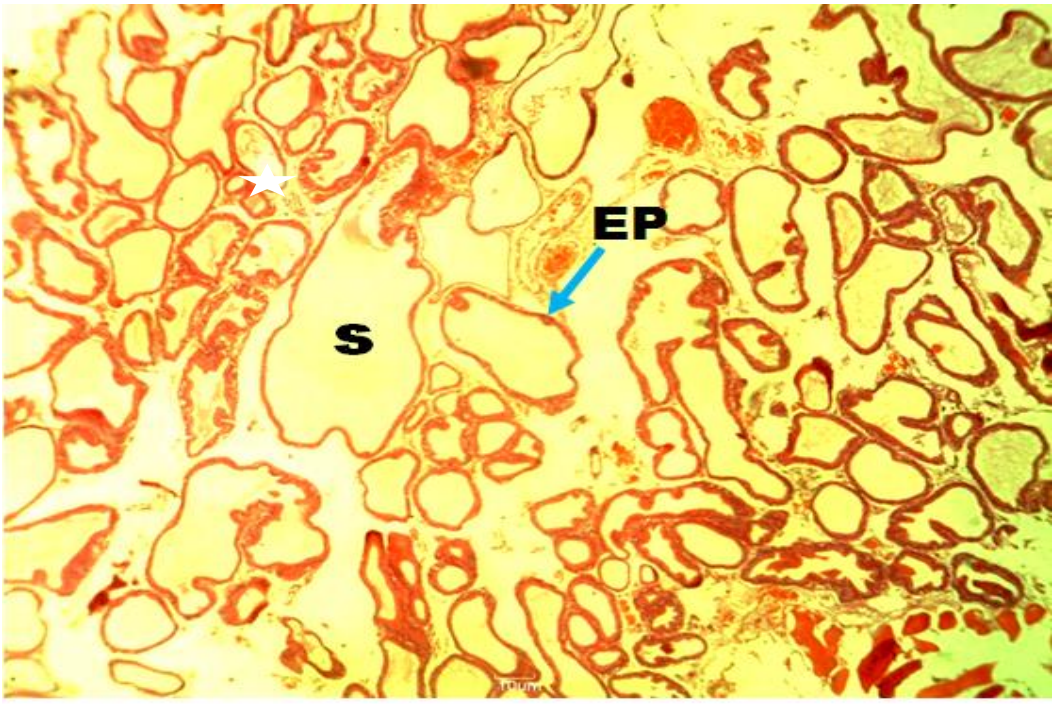

Plate 4: Photomicrograph of prostate gland of the standard control group. H&E. mag. 400X.

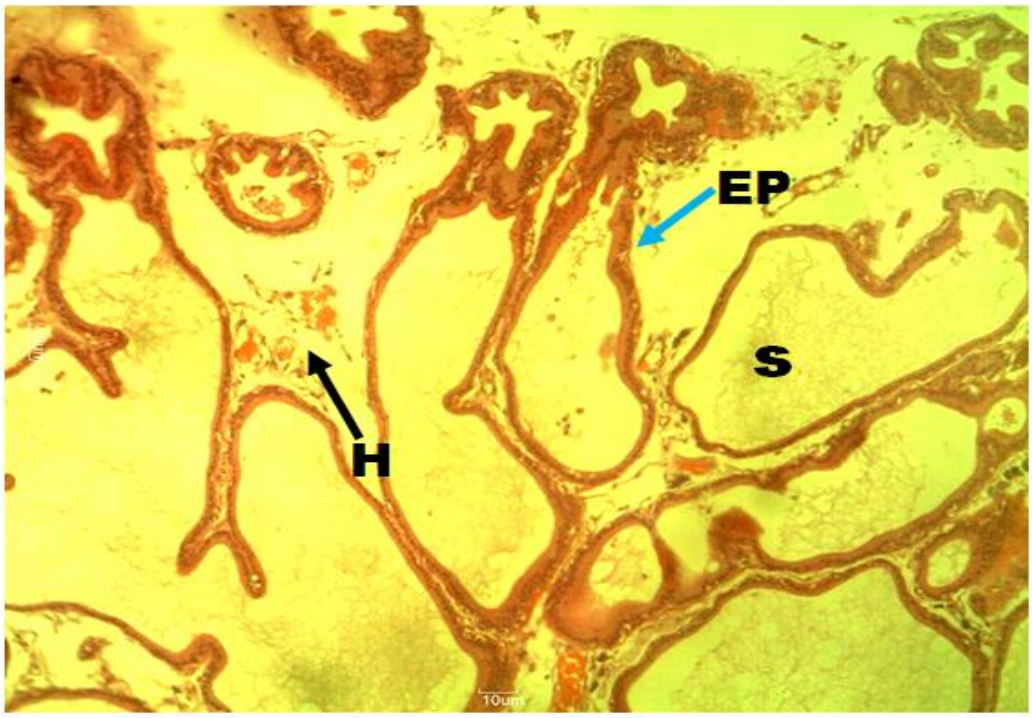

Plate 5: Photomicrograph of prostate gland of group treated with 200 mg/kg extract. H&E. mag. 400X.

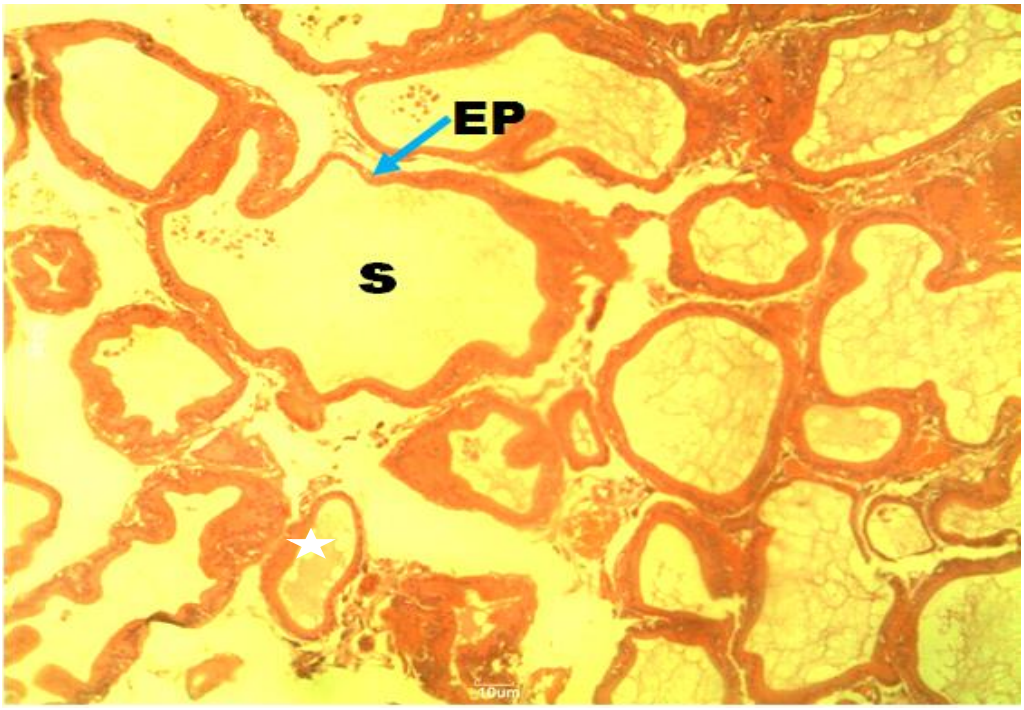

Plate 6: Photomicrograph of prostate gland of Group treated with 400 mg/kg b.w of extract. H&E. mag. 400X.

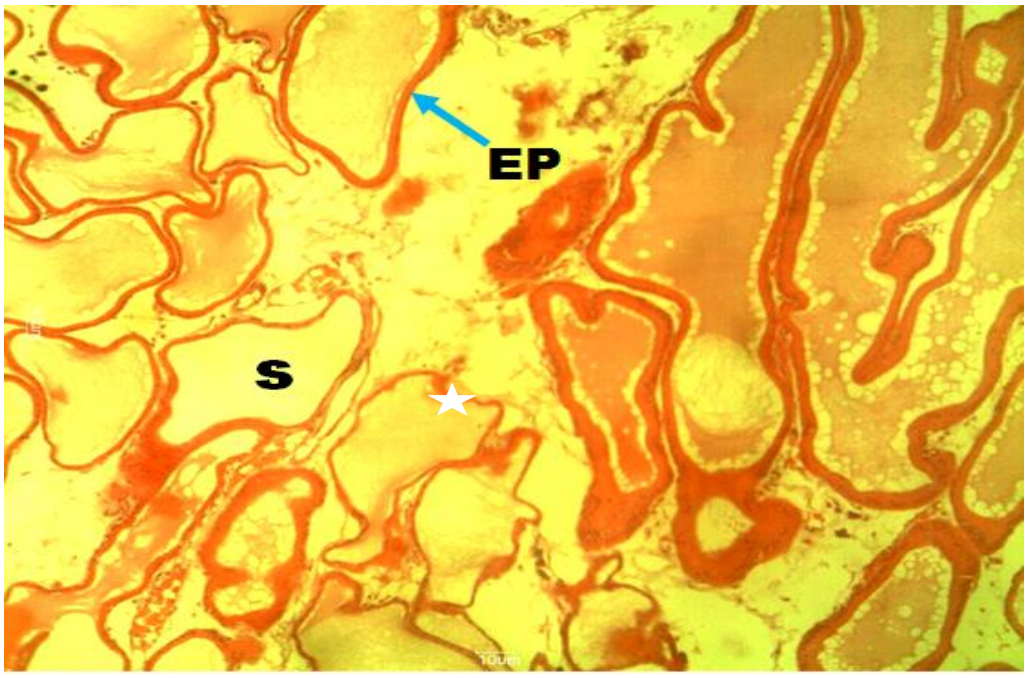

Plate 7: Photomicrograph of prostate gland of Group treated with 600 mg/kg b.w of extract. H&E. mag. 400X.

CHAPTER FOUR

DISCUSSION

Free radicals are involved in the initiation and progression of many diseases associated with ageing (Iweala, and Ogidigo, 2015). The activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione as well as phytochemicals naturally protects human against oxidative stress (Lobo *et al*., 2010). The present study investigates the antioxidant potentials of methanol extract of *Duranta erecta* and its effect on testosterone-induced benign prostatic hyperplasia.

The phytochemical screening of the plant extract revealed the presence of flavonoid (24.20 ± 0.14 mg QE/g), alkaloid (15.87 ± 1.71 mg/g), total phenol (12.73 ± 0.61 mg GAE/g), tannin (9.24 ± 0.03 mg Tannic acid/g), terpenoid (8.90 ± 0.96 mg/g), steroid (2.65 ± 0.55 mg/g) and saponin (5.55 ± 0.76 mg/g). The absence of glycoside was not consistent with the earlier report of Donkor *et al*. (2019) which stated that glycoside was present in *Duranta erecta* leaves extract. The result indicates that the leaves possess some biologically active components which could serve as antioxidants. Functional hydroxyl groups in flavonoids show their antioxidant effects by scavenging free radicals or by chelating metal ions. This helps in the prevention of radical generation that damage the biomolecules leading to oxidative stress and many diseases (Tiwari, and Husain, 2017). The antioxidant action of flavonoids includes suppression of ROS formation by inhibition of enzymes, by scavenging free radicals, and regulation of antioxidant defenses (Mishra *et al*., 2013). Flavonoids also protect the lipids of the biomembranes which are damaged due to lipid peroxidation (Tiwari, and Husain, 2017) by sugar substitution in the phenolic C ring (Plumb *et al*., 1999). Alkaloids have been shown to have antioxidant properties via alleviating H_2O_2-induced oxidative damage (Plumb *et al*., 1999). Tannins are known potent antioxidants, and this activity is achieved by chelating metal ions such as Fe (II) and interference with one of the reaction steps in the Fenton reaction thereby retarding oxidation (Donkor *et al*., 2019). Chen *et al*. (2014) reported that saponins possess antioxidant capability through scavenging of hydrogen peroxide. Evidence suggests that steroids also possess antioxidant and anti-inflammatory activities (Ju *et al*., 2004). An important bioactive compound; Beta-sitosterol has been identified and isolated from *Duranta erecta* leaves (Abou-Setta *et al*., 2007). Beta-

sitosterol, one of several phytosterols, has been shown to improve BPH symptoms (Kim *et al*., 2012). Beta-sitosterol, has anti-inflammatory effect and inhibits the stimulation and synthesis of prostaglandins, aromatase activity and 5-alpha- reductase activity (Nyamai *et al*., 2016). β-sitosterol helps reduce the elevated levels of prostaglandins in BPH patients (Nyamai *et al*., 2016). Terpenoids have anti-inflammatory properties. Two important triterpenes (oleanolic and ursolic acids) have been identified in *Duranta erecta* leaves (Kuo *et al*., 1996). There are reports stating that triterpenes such as oleanolic, and ursolic acid have anti-inflammatory activity in prostate connective tissue (Dvorkin and Song, 2002).

In the present study, the concentration of zinc and selenium in the methanol extract of *Duranta erecta* were 1.82 ± 0.03 mg/100g and 0.59 ± 0.04 mg/100g respectively. Selenium exists in ionic form, bound to methionine and cysteine. It has very high bioavailability and functions as a prosthetic group in enzymes which form cross-links in collagen and elastin formation of connective tissues (Nwaoguikpe *et al*., 2012). The mineral nutrient Selenium, is an important component of the enzyme, Glutathione peroxidase (an antioxidant enzyme), which targets hydrogen peroxide (H_2O_2) in the body and converts it to water. It is an antioxidant and plays vital roles in various organs of the human body (Brown and Arthur, 2001). Zinc is present at a reasonable quantity in the plant extract. The concentration of zinc in the plant extract is below the FAO/WHO permissible limit of zinc in both edible plants and medicinal plants which is 50 mg/kg (WHO, 2005). Zinc is an essential trace element. It acts as a co-factor for important enzymes involved in the proper functioning of the antioxidant defence system (Marreiro *et al*., 2017). In addition, zinc protects cell against oxidative damage, acts in the stabilization of membranes and inhibits the enzyme nicotinamide adenine dinucleotide phosphate oxidase (NADPH-Oxidase) (Cruz and Oliveira, 2015). Zinc also induces the synthesis of metallothioneins, which are proteins effective in reducing hydroxyl radicals and sequestering reactive oxygen species (ROS) produced in stressful situations (Andrews, 2000). *Duranta erecta* leaves extract containes vitamin C and vitamin E in the concentrations of 0.35 ± 0.01 mg/100g and 0.68 ± 0.07 mg/100g respectively. These vitamins have antioxidant and free radical scavenging activities (Nwaoguikpe *et al*., 2012).

The DPPH scavenging assay is an important assay to determine the antioxidant activity of the plant extracts in *in vitro* model (Alam *et al*., 2012). The DPPH is a free radical which reacts

rapidly with the antioxidant compounds. The anti oxidative compounds can donate a hydrogen atom to DPPH and change the colour. The intensity of colour is measured calorimetrically. The increasing intensity of colour is directly proportional to the inhibition of DPPH (Vijayakumar *et al*., 2015). In this study, the inhibition of DPPH radicals increased with increasing concentration of the extract. The highest inhibition was observed at a concentration of 100 µg/ml (77.90 ± 2.31 %). In the DPPH assay, the higher antioxidant activity is reflected in the lower half maximal inhibition concentration (IC_{50}) value. The IC_{50} value represents the concentration of the extract or standard that caused 50 % inhibition of the radical. It has been proposed that samples with IC_{50} lower than 50 µg/mL are very strong antioxidants, samples with IC_{50} of 50-100 µg/mL are strong antioxidant, those with 101-150 µg/mL are moderate, and samples with IC_{50} greater than 150 µg/mL are weak antioxidants (Fidrianny *et al*., 2018). The half maximal inhibition concentration (IC_{50}) for DPPH scavenging activity of methanol extract of *Duranta erecta* leaves was found to be 40.91 ± 0.19 µg/mL indicating that the extract is a very strong antioxidant. The DPPH scavenging activity of *Duranta erecta* extract is consistent with the earlier report of Donkor *et al*. (2019) which stated that *Duranta erecta* leaves extract effectively inhibited DPPH radicals.

Nitric Oxide is a free radical which is formed from sodium nitroprusside and it reacts with oxygen to form nitrite. The antioxidant activity was measured by the inhibition of the nitrite formation by the plant extract (Marcocci *et al*., 1994). Despite the potential health benefits of nitric oxide radical, its role in causing oxidative damage is becoming progressively prominent (Hou *et al*., 2019). In this work, the nitric oxide radical scavenging effect of methanol extract of *Duranta erecta* was studied. The result showed that the extract in the test range of 20-100 µg/mL exhibited nitric oxide radical scavenging activity in a dose dependent manner. The IC_{50} of the standard (ascorbic acid) was 32.16 ± 3.79 µg/ml while that of the extract was 56.16 ± 3.39 µg/ml. The result indicates that the *Duranta erecta* extract might serve as an excellent nitric oxide radical scavenger due to the high level of flavonoids. It has been reported that flavonoids are very powerful nitric oxide radical scavengers (Hou *et al*., 2019).

The reducing power is related to electron transfer ability of the plant extract. This assay was used to measure the transferring capacity of Fe^{3+} to Fe^{2+} (Vijayakumar *et al*., 2015). The result from this study showed that the plant extract exhibited ferric reducing power in a dose dependent

manner. From the result, it could be concluded that the extract may be effective in minimizing oxidative damages in tissues. The acute toxicity study of methanol extract of *Duranta erecta* leaves showed neither death nor any sign of toxicity at a high dose of 5000 mg/kg indicating that the leaves of *Duranta erecta* are safe for oral consumption.

The effect of methanol extract of *Duranta erecta* leaves on experimentally induced prostatic hyperplasia was evaluated on male albino rats in this study. The subcutaneous injection of rats with testosterone propionate for 28 days caused a significant ($p < 0.05$) increase in the serum levels of prostate specific antigen (PSA) in the test animals when compared with the normal rats confirming the induction of benign prostatic hyperplasia. The serum PSA levels which were elevated following BPH induction were observed to have decreased significantly ($p < 0.05$) after 21 days of administration of methanol extract of *Duranta erecta* and finasteride. The PSA is a glycoprotein found in the serum and it serves as a quantitative indicator of prostatic cancer and also predictor of BPH (Mbaka *et al*., 2017). However, much remains unknown about the interpretation of PSA levels as it pertains to test's ability to discriminate cancer from benign prostate conditions, and the best course of action following a finding of elevated PSA. The PSA level is noted to increase in both benign and malignant lesions of the prostate but is usually marked in prostatic cancer (Andriole *et al*., 2009).

Increase in prostate weight and prostatic index is used as one of essential markers of BPH (Cam, 2011; Ishola *et al*., 2017). The BPH is characterized by stromal and epithelial cells hyperplasia, resulting in prostate enlargement (Nahata and Dixit, 2011). The present study showed a significant ($p < 0.05$) increase in prostate weight and prostatic index in the BPH untreated (positive control) group whencompared with the normal control group whereas animals treated with different doses of the extract and finasteride had a significant ($p < 0.05$) reduction in prostate weight and prostatic index when compared with the positive control.

The level of testosterone in the blood is considered to be pivotal in BPH progression. Testosterone is known to promote the proliferation of prostate cells by the activity of type II 5α-reductase, an enzyme responsible for the conversion of testosterone to a more potent androgen dihydrotestosterone (DHT) (Griffiths and Denis, 2000; Mbaka *et al*., 2017). Androgens play important role in BPH pathogenesis. The DHT can easily bind to ARs due to its higher affinity to

the androgen receptor (AR), which stimulates the growth for the epithelial and stromal cells in the prostate (Cai *et al*., 2018). Therefore, DHT is basically responsible for prostatic epithelial and stromal cell hyperplasia (Mizokami *et al*., 2009). In this study, it was observed that the positive control group had elevated levels of testosterone and dihydrotestosterone while those treated with the extract had an appreciable decrease in the hormonal levels. This shows that the extract enhanced the mopping up of testosterone in the system to prevent its conversion to the more potent DHT by 5α-reductase. The reduction in the levels of DHT could also be due to the inhibition of the activities of 5-alpha reductase by β-sitosterol.

Acid phosphatase (ACP) is one of the prostatic secretions used as a marker of benign prostatic hyperplasia and as an indicator of treatment progress (Ejike, and Ezeanyika, 2011). Elevations in ACP levels have been reported in animals treated with testosterone and may be due to increased lysosomal activity (Jeyaraj *et al*., 2000). In the present study, there was elevated level of serum acid phosphatase in the positive control (BPH untreated) group compared with the normal control group indicating prostatic hyperplasia. There was however a significant ($p < 0.05$) reduction of ACP in the animals treated with the plant extract.

Oxidative stress is considered as one of the mechanisms that initiate the development and progression of benign prostatic hyperplasia (Eleazu *et al*., 2017; Mbaka *et al*., 2017; Cai *et al*., 2018; Ugwu *et al*., 2018; Chang *et al*., 2019). This is especially true as the human prostate tissue is vulnerable to oxidative DNA damage due to more rapid cell turnover and fewer DNA repair enzymes (Udensi and Paul, 2016). In the present study, testosterone propionate caused a significant ($p < 0.05$) increase in malondialdehyde (MDA), and reduction in superoxide dismutase, catalase, reduced glutathione and glutathione peroxidase in the positive control group when compared with the normal control group. Interestingly, treatment of rats with methanol extract of *Duranta erecta* leaves and finasteride attenuated lipid peroxidation, reduction in superoxide dismutase, catalase, reduced glutathione and glutathione peroxidase when compared with BPH untreated group (positive control).

Several studies in literature have shown higher levels of oxidants or lower levels of enzymatic or non-enzymatic antioxidants in patients with BPH compared to normal persons. Studies have also demonstrated the presence of oxidative stress in BPH (Miniciullo *et al*., 2015). The cause of

enhanced oxidative stress could be the overproduction of free radicals or decrease in the activities of free radical scavenging enzymes like SOD, CAT, GPx and glutathione levels in their circulation, or both (Bostanci *et al*., 2013). Some studies on animal models of BPH showed significant elevation of prostatic lipid peroxidation with concomitant significant reduction of the prostatic levels of GSH, SOD, and catalase activities of BPH untreated rats and which parameters were significantly improved following treatment with finasteride (Kalu *et al*., 2016; Ugwu *et al*., 2018).

Humans are naturally protected against free radical damage by anti oxidative enzymes and proteins such as superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) as well as phytochemicals. The GSH serves as a redox buffer by removing toxic peroxides via reactions catalysed by glutathione peroxidase. The ratio between the reduced and oxidized glutathione disulfide (GSSG) forms of glutathione is often used as an indicator of the cellular redox state, reflecting the balance between the capacity of the defence response for regeneration of GSH and the extent of neutralization by oxidants (Cimino *et al*., 2014). Superoxide dismutases (SODs) are a class of closely related enzymes present in almost all cells and in the extracellular fluids (Abe *et al*., 2011). They catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide. Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in protecting the cell from oxidative damage by ROS (Ugwu *et al*., 2018). These enzymes work synergistically in counteracting the deleterious effect of free radicals.

A wide variety of phytochemicals; flavonoid, polyphenols, steroids and alkaloids (Ishola *et al*., 2017), antioxidant minerals; zinc and selenium (Brown and Arthur, 2001; Marreiro *et al*., 2017) as well as antioxidant vitamins are believed to be responsible for the attenuation of oxidative stress in animals treated with the extract. The *in vitro* antioxidant assay showed the potential of the extract in scavenging of DPPH and nitric oxide radicals as well as reductive capability. It also revealed a substantial amount of zinc and antioxidant phytochemicals.

Testosterone has effects on several body organs including the prostate gland (Acheampong *et al*., 2019). Therefore the impact of hypertestosteronemia on histology of the prostate gland was examined. The positive control group (BPH untreated) recorded more histological disruption

than the finasteride and extract treated groups. The histological changes observed could be due to testosterone stimulated oxidative stress-mediated damage. The finasteride and the extract treated groups recorded a reduced serum testosterone level relative to the untreated group, thereby minimizing testosterone-mediated histological changes.

4.2 Conclusion

Findings from this study revealed that the methanol extract of *Duranta erecta* leaves showed antioxidant properties and reduced the effect of testosterone-induced BPH possibly through enhancement of antioxidant defense mechanisms and inhibition of inflammatory mediators. Thus, *Duranta erecta* could be a potential phytotherapeutic agent in the management of BPH in men.

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APPENDICES

Appendix I: Homogeneous Subsets (Duncan) for Prostatic Activities

Duncan for Prostatic Weight (PW)

PW

	Group	N	Subset for alpha = 0.05			
			1	2	3	4
Duncan[a]	Normal Control	5	.2000			
	600 mg/kg b.w of extract	5		.4540		
	Standard Control	5		.4980		
	400 mg/kg b.w of extract	5		.5340		
	200 mg/kg b.w of extract	5			.6960	
	Positive Control	5				1.4160
	Sig.		1.000	.260	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Duncan for Prostatic Index (PI)

PI

	Group	N	Subset for alpha = 0.05				
			1	2	3	4	5
Duncan[a]	Normal Control	5	.9100				
	600 mg/kg b.w of extract	5		1.6540			
	Standard Control	5			2.3520		
	400 mg/kg b.w of extract	5			2.5020		
	200 mg/kg b.w of extract	5				3.1040	
	Positive Control	5					5.7120
	Sig.		1.000	1.000	.332	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Duncan for PSA before Treatment

PSA

	GROUP	N	Subset for alpha = 0.05	
			1	2
Duncan[a]	Group 1= Normal Control	4	.3950	

	Group 3 = Standard Control	4		1.8875
	Group 2 = Positive Control	4		1.9400
	Group 5 = BPH + 400 mg/kg b.w of extract	4		1.9975
	Group 6 = BPH + 600 mg/kg b.w of extract	4		2.0075
	Group 4 = BPH + 200 mg/kg b.w of extract	4		2.0575
	Sig.		1.000	.148

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Duncan for PSA after Treatment

PSA

	GROUP	N	Subset for alpha = 0.05				
			1	2	3	4	5
Duncan[a]	Group 1= Normal Control	4	.3950				
	Group 3 = Standard Control	4		.9825			
	Group 6 = BPH + 600 mg/kg b.w of extract	4		1.1025	1.1025		
	Group 5 = BPH + 400 mg/kg b.w of extract	4			1.2450		
	Group 4 = BPH + 200 mg/kg b.w of extract	4				1.4775	
	Group 2 = Positive Control	4					2.0275
	Sig.		1.000	.242	.168	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

T-test for PSA in Different Groups before and after Treatment

T-test {Group 1 = Normal Control}

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means					
	F	Sig.	t	Df	Sig. (2-tailed)	Mean Differen	Std. Error	95% Confidence Interval of the Difference

						ce	Differ ence	Lower	Upper
PSA	Equal variances assumed	1.073	.340	.000	6	1.000	.00000	.0803 1	-.19652	.19652
	Equal variances not assumed			.000	5.490	1.000	.00000	.0803 1	-.20103	.20103

T-test {Group 2 = Positive Control}

Independent Samples Test

	ene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	T	df	. (2-tailed)	Mean ifference	td. Error ifference	% Confidence Interval of the Difference	
								Lower	Upper
al variances umed	.022	.888	-2.013	6	.091	-.08750	.04347	-.19387	.01887
al variances not umed			-2.013	5.808	.092	-.08750	.04347	-.19472	.01972

T-test {Group 3 = Standard Control}

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	T	df	. (2-tailed)	Mean ifference	td. Error ifference	% Confidence Interval of the Difference	
								Lower	Upper
al variances umed	.002	.965	10.441	6	.000	.90500	.08667	.69291	1.11709
al variances not umed			10.441	5.992	.000	.90500	.08667	.69285	1.11715

T-test {Group 4 = BPH + 200 mg/kg b.w of extract}

Independent Samples Test

	ene's Test for Equality of Variances	t-test for Equality of Means

	F	Sig.	T	df	. (2-tailed)	Mean ifference	td. Error ifference	% Confidence Interval of the Difference	
								Lower	Upper
al variances med	.009	.926	4.622	6	.004	.58000	.12548	.27296	.88704
al variances not med			4.622	5.790	.004	.58000	.12548	.27023	.88977

T-test {Group 5 = BPH + 400 mg/kg b.w of extract}

Independent Samples Test

	ene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	T	Df	. (2-tailed)	Mean ifference	td. Error ifference	% Confidence Interval of the Difference	
								Lower	Upper
al variances med	.146	.716	5.659	6	.001	.75250	.13297	.42713	1.07787
al variances not med			5.659	5.929	.001	.75250	.13297	.42618	1.07882

T-test {Group 6 = BPH + 600 mg/kg b.w of extract}

Independent Samples Test

	ene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	. (2-tailed)	Mean ifference	td. Error ifference	% Confidence Interval of the Difference	
								Lower	Upper
al variances med	.082	.785	8.534	6	.000	.90500	.10605	.64551	1.16449
al variances not med			8.534	5.850	.000	.90500	.10605	.64389	1.16611

Duncan for Testosterone

Testosterone

	Group	N	Subset for alpha = 0.05				
			1	2	3	4	5
Duncan[a]	Normal Control	5	1.3920				

	BPH treated with 600 mg/kg b.w of extract	5	1.7240	1.7240			
	Standard Control	5		2.2340	2.2340		
	BPH treated with 400 mg/kg b.w of extract	5			2.8620	2.8620	
	BPH treated with 200 mg/kg b.w of extract	5				3.1800	
	Positive Control	5					3.9520
	Sig.		.300	.117	.057	.321	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Duncan for Dihydrotestosterone

DHT

	Group	N	Subset for alpha = 0.05			
			1	2	3	4
Duncan[a]	Normal Control	5	8.3540			
	BPH treated with 600 mg/kg b.w of extract	5		12.5960		
	BPH treated with 400 mg/kg b.w of extract	5			14.3020	
	Standard Control	5			14.3520	
	BPH treated with 200 mg/kg b.w of extract	5			14.6300	
	Positive Control	5				18.5920
	Sig.		1.000	1.000	.570	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Duncan for Acid Phosphatase

ACP

	Group	N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	Normal Control	5	23.6011		
	BPH treated with 200 mg/kg b.w of extract	5		30.3880	
	BPH treated with 600 mg/kg b.w of extract	5		30.7018	
	Standard Control	5		31.7486	

	BPH treated with 400 mg/kg b.w of extract	5		33.1394	
	Positive Control	5			42.2309
	Sig.		1.000	.158	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Appendix II

Homogeneous Subsets (Duncan) for Oxidative Stress Activities

Duncan for Superoxide Dismutase (SOD)

SOD

	Group	N	Subset for alpha = 0.05				
			1	2	3	4	5
Duncan[a]	Positive Control	5	22.4995				
	Standard Control	5		26.5092			
	BPH treated with 400 mg/kg b.w of extract	5			32.0203		
	BPH treated with 600 mg/kg b.w of extract	5			34.1080	34.1080	
	BPH treated with 200 mg/kg b.w of extract	5				36.0481	
	Normal Control	5					42.3843
	Sig.		1.000	1.000	.120	.147	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Duncan for Catalase

CAT

	Group	N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	Positive Control	5	5.4935		
	Standard Control	5	6.0835		
	BPH treated with 400 mg/kg b.w of extract	5		7.8587	
	BPH treated with 600 mg/kg b.w of extract	5		8.2474	

	BPH treated with 200 mg/kg b.w of extract	5		8.4796	
	Normal Control	5			10.8257
	Sig.		.202	.204	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Duncan for Reduced Glutathione

GSH

	Group	N	Subset for alpha = 0.05			
			1	2	3	4
Duncan[a]	Positive Control	5	12.4981			
	Standard Control	5		15.6494		
	BPH treated with 600 mg/kg b.w of extract	5			18.0194	
	BPH treated with 400 mg/kg b.w of extract	5			18.8277	
	BPH treated with 200 mg/kg b.w of extract	5			19.1669	
	Normal Control	5				21.6610
	Sig.		1.000	1.000	.221	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Duncan for Glutathione Peroxidase

GPx

	Group	N	Subset for alpha = 0.05				
			1	2	3	4	5
Duncan[a]	Positive Control	5	13.7858				
	Standard Control	5		15.9051			
	BPH treated with 400 mg/kg b.w of extract	5			18.5333		
	BPH treated with 200 mg/kg b.w of extract	5			19.2586	19.2586	
	BPH treated with 600 mg/kg b.w of extract	5				20.4402	20.4402
	Normal Control	5					21.5799
	Sig.		1.000	1.000	.345	.130	.143

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Duncan for Malondialdehyde (MDA)

MDA

	Group	N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	Normal Control	5	1.7994		
	BPH treated with 600 mg/kg b.w of extract	5	2.4743	2.4743	
	BPH treated with 400 mg/kg b.w of extract	5		2.9872	
	Standard Control	5		3.0409	
	BPH treated with 200 mg/kg b.w of extract	5		3.0698	
	Positive Control	5			7.7579
	Sig.		.106	.188	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.