HISTOSTEREOLOGICAL EFFECTS OF CRUDE METHANOLIC BARK EXTRACT OF *PRUNUS AFRICANUS* ON TESTOSTERONE-INDUCED BENIGN PROSTATIC HYPERPLASIA IN WISTAR RATS (*RATTUS NORVEGICUS*)

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Histostereological Effects of Crude Methanolic Bark Extract of *Prunus africanus* on Testosterone-Induced Benign Prostatic Hyperplasia in Wistar Rats (*Rattus norvegicus*)

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

I dedicate this thesis to my wife Patricia Wacu, my daughter Celine Wanjiru and my son Alpha Kanyoni for their prayers and encouragement.

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ABBREVIATIONS AND ACRONYMS

5-ARIs	5-alpha reductase inhibitors
AED	Animal Equivalent Dose
AFS	Anterior Fibromuscular Stromal
AR	Androgen Receptor
BOO	Bladder Outlet Obstruction
BPE	Benign Prostatic Enlargement
BPH	Benign Prostatic Hyperplasia
CE	Coefficient of Error
CMBEPA	Crude Methanolic Bark Extract of *Prunus africanus*
COHES	College of Health Sciences
CZ	Central Zone (of the prostate)
DF	Degree of Freedom
DHT	Dihydrotestosterone
DMSO	Dimethyl sulfoxide
ELISA	Enzyme Linked Immuno-sorbent Assay
FDA	Food and Drug Authority
fPSA	Free Prostatic Antigen
g	grams
JKUAT	Jomo Kenyatta University of Agriculture and Technology
Kg	Kilogram
LUTS	Lower Urinary Tract Symptoms
mg	Milligrams
PBR	Prostate Body Ratio
PKC	Protein Kinase C
PSA	Prostate Specific Antigen levels
PZ	Peripheral Zone (of the prostate)
SAFARI	Small Animal Facility for Research and Innovation
SC	Subcutaneous
SPSS	Statistic Package for Social Science
SURs	Systemic Uniform Random sampling

TP	Testosterone Propionate
TPI	Testosterone Propionate Induced
TPSA	Total Prostatic Antigen
TZ	Transitional Zone (of the prostate)
wt	Weight

DEFINITION OF TERMS

Benign Prostatic Hyperplasia	is a non-neoplastic condition characterised by abnormal cellular multiplication of smooth muscle, connective tissues and glandular epithelium of the prostate within the transition zone, and this diagnosis is made through histological analysis of the prostate sections.
Benign Prostatic Enlargement	is a condition characterised by increase of the size of prostate gland following cellular proliferation, this term is applied when the diagnosis is made through clinical examination.
Bladder Outlet Obstruction	is a condition characterised by the increase of detrusors muscle pressure and reduced urine flow.
Stereology	is quantitative method for determination volume and volume densities using two-dimensional cross sections to provide quantitative information about a three-dimensional tissue.

ABSTRACT

Benign prostatic hyperplasia (BPH) is the non-cancerous proliferation of epithelial and smooth muscle cells of the prostatic tissue surrounding the urethra in males causing urethral constriction, backflow of urine, acute kidney failure and associated complications. Today, BPH is a major killer of men above the age 45 years worldwide. Various treatment regimen for BPH have been advanced among them being ethnomedical oral use of crude bark extract of *P.africanus* by the local communities. Though data exist on its phytotherapeutic and ethnopharmacological activities in the management of BPH, there is paucity of anatomical histostereological data on its restorative and inhibitory effects in management of BPH. Further, data on whether the restorative and inhibitory effects *P.africanus* are dose-dependent is yet to be established. The broad objective of this study, therefore, was to examine the histostereological effects of *P.africanus* on testosterone induced BPH in Wistar rats. In carrying out the study, sixty Wistar were sourced from SAFARI animal house were used as the experimental model. The 60 rats were broadly divided into two study groups of 30 rats in the restorative group and 30 rats in the inhibitory group. Each group was further categorized into 5 control and 25 experimental rats. The experimental rats were further subdivided into 5 sub-groups based on varying doses of the crude methanolic bark extract of *P.africanus* (0 mg, 25 mg, 50 mg, 125 mg, and 200 mg). BPH in experimental animal was induced by subcutaneous injection of testosterone propionate (7.5 mg/kg) for 10 days. The findings of the study showed that the restorative group had a statistical significant dose dependent reduction of the prostate volume ($p=0.003$), the stromal ($p=0.000$) and epithelium volume ($p=0.000$) was observed. While in the inhibitory group a statistical significant dose-related inhibition, in the increase of the prostate volume ($p=0.000$), the stromal ($p=0.000$) and epithelium volume ($p=0.000$) was observed**.** In conclusion, the bark extract of *P.africanus* has both restorative and inhibitory effects to BPH and this restorative and inhibitory effects observed could be attributed to the phytochemical ingredients present in the extract that included triterpenes, flavonoids, saponins, glycoside and alkaloids. Further, the maximal restorative effect was observed to be up to 85.6% and at a dose of 200 mg, while the optimal inhibitory effects was observed to be between 66.7- 68.4% in the dose range of 100-200 mg/kg/bw. This study, therefore, recommends that future research using nonhuman primates need to be carried out to ascertain the findings of this study using the second order stereology to determine the effects of crude methanolic extract of *P.africanus* on the prostatic cell volumes, cellular characterization and cell densities in BPH as these nonhuman primates have closer genetic association to humans.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The prostate, an accessory gland of the reproductive system that is located in the inferior part of the urinary bladder around the prostatic urethral part in males is composed of both exocrine and endocrine parts (Sharma *et al.*, 2017; Standring, 2016). The exocrine part of the prostate usually undergoes a non-cancerous proliferation characterized by pathological cellular multiplication of epithelial and smooth muscle cells a condition referred to as Benign Prostatic Hyperplasia (BPH) (Roehrborn, 2005). This condition causes urethral constriction resulting is chronic end-stage bladder outlet obstruction that subsequently causes renal failure and uraemia (Briganti *et al.*, 2009; Kaplan *et al.*, 2019). Worldwide BPH is a leading cause of death in men of reproductive age above 45 years (Hassan *et al.*, 2013; Yeboah, 2016). This end-stage results in renal failure and uraemia make it one of the key causes of mortality and morbidity (Hassan *et al.*, 2013; Deori *et al.*, 2017). Many treatment options have been advanced for BPH including surgery, conservative medical therapy as well as the use of crude bark extracts of *P.africanus*. The use of *P.africanus* is gaining popularity due to its local availability as well as the associated cost and side effects associated with conventional method of BPH treatment (Jena *et al.*, 2017; David, 2018). Though there are claims of its effective inhibition and restorative effects on BPH, there is lack of data on its histostereological effects in management of BPH to substantiate these claims. At the same time, data on whether or not the restorative and inhibitory effects of crude methanolic bark extract of *P. africanus* (CMBEPA) are dose-dependent, is yet to be determined. Further, the active phytochemical ingredients of *P.africanus* and its toxicology profiles also need to be established as there exist a marked variability in the phytochemical concentrations of bark extracts in different geographical regions (Caroline *et al.*, 2012; Karani *et al.*, 2013). Locally *P africanus* is gaining popularity in management of BPH because it is affordable and available to many poor Kenyan men in rural areas compared to surgical procedure and medical therapies. Though bark extracts of *P.africanus* have been in use for decades and target either the prostatic

muscle tone relaxation or prostate gland mass reduction their outcomes is not guaranteed in the total cure of BPH (Jena *et al.*, 2017). Also the popularity gained in the use of *P.africanus* is based on the fact that it has been shown to be effective with less adverse effects and the wide availability in rural settings (Jena *et al.*, 2017).

The comparative embryology between the human and rat prostate show significant similarities in terms of embryological milestones, cellular differentiation, molecular control features, and embryological origin from the urogenital sinus (UGS), the endoderm structure present in both embryos at the ambisexual stage (Standring, 2016). Similarly, the morphogenetic process is achieved through well synchronized cellular activities that begin with ductal branching morphogenesis and canalization, mesenchymal and epithelial differentiation, and proliferation in both species (Sharma *et al.*, 2017).

There is marked variability between human and rodent prostates in gross morphology and histoarchitecture, whereby in the rats prostate there are distinct lobes, while in humans it is a compact single structure arranged in zones (Neill, 2015; Oliveira *et al.*, 2015). The peripheral zone (PZ) is homologous to the rodent dorsal lateral (DL) prostate lobes (Price, 1963; Fu *et al.*, 2014). The PZ is where 75-85% of adenocarcinomas of the prostate occur in patients (McNeal, 1968). The central zone (CZ) is equivalent to the human anterior posterior lobes (AP) lobes, which is a cone-shaped region that encircles the vas deferens, and comprises about 25% of the prostate volume. The transition zone (TZ), is a section that does not have a rodent equivalent and this section is where most of benign prostatic hyperplasia lesions occur in human, it also the tiniest zone (5-10% of prostate volume) and encircles the distal prostatic urethra (Viswanath *et al.*, 2013). As a result, the human urethra is completely encircled by the prostate gland. while the rodent is not (Barbara, *et al.*, 2014). More so in the human, the prostate gland is attached to the bladder pelvic floor and anterior to the rectum, while in rats the distal ends of each of the prostate lobes hangs freely in the pelvic cavity (Viswanath *et al.*, 2013).

The microscopic structure of the rat prostate depicts similar histological arrangements to that of the humans in that in it contains luminal acini and ductal system with epithelial cell types that include basal, neuroendocrine and columnar secretory cells.

Also the lumen cells in both rats and humans expresses a low molecular weight cytokeratin (CK) eight and eighteen, and androgen receptor (AR), while the basal cells in both species are characterized by expression of a high molecular weight p63 and CK5 (Liao *et al.*, 2010). Notably, the prostate specific antigen (PSA) is only secreted and expressed by human but not rat prostate lumen cells, which produces other proteins which are lobe specific (Karr *et al.*, 1995). The most marked histological variability is the stromal tissue, which is very well established and organised in humans as the anterior fibromuscular region, while in rat is scarce with few smooth muscle cells (Viswanath *et al.*, 2013).

However, despite these variances, the rat remains to be one of the most commonly used model for prostate disorders, owing to some of advantageous factors which include the ease of inducing BPH, short gestation period, small size of the body, and cost-effectiveness, resemblance of human genome and, more significantly the simplicity of the genetic manipulation. (Li *et al.*, 2018).

1.2 Statement of the Problem

Benign Prostatic Hyperplasia (BPH) is currently a major contributor to diseases burden in men aged above 45 years as it causes constriction of the urethra, leading to anatomic bladder outlet obstruction (BOO) (Shin *et al*., 2012). This anatomical bladder outlet obstruction results in urine retention in the bladder that leads to backflow of urine in the ureters then to the kidneys with concurrent associated urinary tract infections and kidney damage of which if left untreated ultimately leads to metabolic toxins accumulation in the body and death. Globally, BPH affects approximately 30 million men above 45 years of age, with a global prevalence of 26.2% (Wen *et al*., 2017). The risks of developing BPH has been shown to increases with advancing age (Shin *et al*., 2012). Many treatment options have been advanced and one among them being the oral intake of crude bark extracts of *P.africanus* that is currently being widely applied at local levels by many Kenyan communities (Wilt and Ishani, 2011; Wilt *et al.*, 2000; Edgar *et al.*, 2007). Though studies have shown there is a wide usage of crude of crude back extracts in management of BPH at community levels, there is paucity of anatomical histo-qualitative and histostereological data on its restorative and

inhibitory effects in BPH. Further, data on its safety profiles as well as its phytochemical constituents need to be established

1.3 Justification of the Study

Lack of a scientific data repository on the anatomical histo-qualitative and stereological data on both the restorative and inhibitory effects of crude bark extract of *P.africanus* in the management of BPH will continue denying men the benefits of a using locally available cheap options in management BPH. In addition, lack of data on the histostereological quantification of both the inhibitory and restorative effect of *P.africanus* in terms of its application in graded doses would lead to toxicity accumulation in the process of its usage as there is no guiding data to guide the applications of its most effective doses in attaining the optimal therapy. It is therefore imperative to have this data to give a clear picture on its reversal and inhibitory patterns to both the prostatic parenchymal volume densities, the proportionate volumes of the stromal tissue, the acinar glandular cells, as well as the total prostatic volume to guide the dose levels that would be applicable at different stages of BPH. This information would also guide in the possibilities of developing patented *P.africanus* medicines that would be cheap and safe for the treatment of BPH at local levels. Furthermore, the findings from the study will be useful in formulating predictive baseline dosages of *P.africanus* that can be used in future research work.

1.4 Research Objectives, Question and Hypotheses

1.4.1 Broad Objective

To evaluate histostereological effects of crude methanolic bark extract of *P.africanus* on testosterone-induced benign prostatic hyperplasia in Wistar rats

1.4.2 Specific objectives

1. To establish the qualitative phytochemical composition of crude methanolic bark extract *P. africanus*.
2. To determine the acute toxicity of crude methanolic bark extract of *P.africanus* in Wistar rats.

3. To evaluate the gross morphometric effects of crude methanolic bark extract *P. africanus* on testosterone-induced benign prostatic hyperplasia in Wistar rats.
4. To evaluate the histostereological restorative effects of varying doses of crude methanolic bark extract of *P. africanus* on testosterone-induced benign prostatic hyperplasia in Wistar rats.
5. To evaluate the histostereological inhibitory effects of varying doses of crude methanolic bark extract of *P. africanus* when administered concurrently with testosterone propionate in Wistar rats.

1.5 Research Question

What are the histomorphological and histostereological effects of crude methanolic bark extract of *P.africanus* on testosterone-induced benign prostatic hyperplasia in Wistar rats?

1.6 Null Hypotheses (Ho)

There is no statistical significant difference in the histostereological and histomorphological features in testosterone induced BPH treated with varying doses of crude methanolic bark extract of *P.africanus* when compared to BPH untreated group in Wistar rats.

1.7 Study Limitations

1. Quantification of free Prostatic Specific Antigen and total Prostatic Specific Antigen in Wistar rats to confirm BPH was challenging, due to availability and cost of reagents.
2. Some of the Wistar rats induced with testosterone propionate did not develop BPH, hence the rats had to be replaced and this prolonged the duration of the experiment.

CHAPTER TWO

LITERATURE REVIEW

2.1 The histological structure of the prostate gland

The prostate is the largest accessory gland in the male reproductive system that is located on the inferior surface of urinary bladder surrounding the prostatic urethra (Aaron, *et al.*, 2016). The histo-cyto-architecture of the prostate is composed of glandular acini that are separated by fibromuscular stromal tissues and smooth muscle cells (Aaron, *et al.*, 2016). The glandular exocrine part produces an alkaline fluid that contributes to about 30 to 50% of the seminal fluid volume that is composed of high amount of citric acid and proteolytic enzymes, and rich in fibrinolysins that liquefies the coagulated semen after it has been deposited in the vagina (Standring, 2016). This function is key to male fertility, as the changes in the prostatic fluid may affect the sperm function resulting in infertility (Mona, *et al.*, 2017).

As men progress in age, the prostate in some of them undergoes pathological noncancerous proliferation of the glandular epithelium as well as the smooth muscle cells in the transition zones leading to a condition called benign prostatic hyperplasia (BPH). This enlargement or the BPH results into bladder outlet obstruction, that is subsequently associated with lower urinary tract symptoms (LUTS) that includes increased frequency, weak stream, urge incontinence, nocturia, sexual dysfunction and dysuria. Today, BPH is a major public health problem among men above 45 years worldwide, it is the 2nd leading cause of mortality in men among the developed countries. An increase in life span of men has led to steady increase in the occurrence of BPH. Most of these patients use conventional drugs and invasive surgical interventions. Use of conventional drugs is associated with a lot side effects among them gynecomastia and erectile dysfunction (Lee *et al.*, 2012). Further, prostatectomy are associated with poor outcomes and high mortalities. Due to these side effects, people have embraced the use of phytotherapeutic agents which have been proven to be effective and among them is the bark extracts of *P.africanus* which is locally available (Nyamai *et al.*, 2016).

2.1.1 Prevalence and pathogenesis of Benign Prostatic Hyperplasia

The global prevalence of BPH, is estimated to be about 40% of the total disease morbidity in men aged between 50 to 60 years and between 80-90% in men aged between 80 to 90 years (Shin *et al.*, 2012). Benign prostate hyperplasia contributes to a high disease burden in men as it is ranked as the highest contributor to the disease burden in men worldwide affecting approximately 30 million men.

The trigger for the development of BPH is poorly understood though many studies have postulated multifactorial aetiologies that are thought to play a role in its pathogenesis and progression spanning from hormonal imbalances, genetic predisposition and inflammation (Roehrborn, 2005; Konwar., *et al* 2008). The commonest causes that have been described include systemic hormonal alteration, local hormonal perturbations and vascular disturbances to the gland (Roehrborn, 2005; Ribal, 2013). In addition, conditions like prostatic inflammation have also been linked with triggering BPH leading to abnormal cellular proliferation (Briganti *et al.*, 2009).

A study by Elkahwaji, (2012), has advanced an argument that BPH is also an immune inflammatory disease where it suggests that the initiation of the inflammation is by an unidentified aetiological stimulus producing inflammatory infiltrates. These inflammatory infiltrates are predominantly composed of activated T-lymphocytes release cytokines and growth factors creating a pro-inflammatory environment that supports fibro-muscular growth in the prostate gland. As a result of increased demand of oxygen by these proliferating cells, this creates a state of relative hypoxia which is a conducive environment that promotes and maintains the state of prostatic tissue inflammation (Briganti *et al.*, 2009; Ribal, 2013).

Concerning the local hormonal alterations that lead to the development of BPH, studies have shown that hormonal alterations cause an increase in 5α-reductase activity that causes a change in prostatic androgen metabolism leading to abnormal accumulation of dihydrotestosterone (DHT). DHT is a by-product of the breakdown of testosterone by the enzyme 5α-reductase and it is a potent androgen. DHT has higher affinity to androgen receptors than testosterone, and stimulates protein synthesis, differentiation, and prostate cell growth. DHT has a useful role in the development of prostate but has

negative effect in the adult prostate, because it propels the pathologic growth of the prostate (Carson and Rittmaster, 2003).

Further, the role of oestrogens in prostate is mediated by oestrogen receptors, and they appear to support the development of BPH .A rise in oestrogen/androgen ratio and increase in the synthesis of sex hormone binding globulin are also implicated in BPH formation. Oestrogens act synergistically with androgens enhancing the expression of aromatase that catalyses the peripheral conversion of androgens into oestrogens and the expression of oestrogen receptors at the transition zone of prostate tissue leading to BPH genesis (Ajayi and Abraham, 2018).

Other studies have recently shown that metabolic syndrome and in particular dyslipidaemia and central obesity, are the main culprit associated with BPH (Wang, *et al.*, 2016). Patient with metabolic syndrome are likely to suffer from hypogonadism and systemic inflammation which play an important role in induction of BPH (De Nunzio *et al.*, 2012; Afroz *et al.*, 2015).

On the other hand the enlargement of the prostate gland leads to the irritative and obstructive symptoms observed in BPH patients. The irritative symptoms include frequent nocturnal urination (nocturia), frequent diurnal urination (pollakisuria), urinary urgency, urinary incontinence, incomplete emptying of urinary bladder, difficulty and pain in urination (dysuria).The obstructive symptoms include weak urine stream, urine stammering, urinary retention (ischuria), terminal dribbling of urine, residual urine in the bladder and delayed initiation of micturition (Gacci *et al.*, 2015).

2.1.2 Treatment Options in BPH Management

Many treatment options have been advanced in the management of BPH including surgery, use of conventional medicine as well as application of local ethnomedicines like the use of back extracts of *P. africanus*. The main goal of the treatment in BPH is to alleviate LUTS that result from prostatic enlargement. Currently, BPH treatments are focusing on the modification of disease progression as well as prevention of complications that are associated with lower urinary tract symptoms (LUTS). Varying classes of pharmacotherapeutic agents are currently being used including 5-alpha

reductase inhibitors, anticholinergics, alpha-adrenergic blockers and phytotherapeutic agents. Selection of the best medical treatment option for BPH is a complex process which is ever-changing (Papaioannou and Jena, 2017). Phytotherapeutic agents derived from fruits, seeds, roots, or bark of plants are now often used as first-line in management of BPH to alleviate LUTS and among them is the use of bark extract of *P.africanus*. The usage of bark extract of *P.africanus*, either as single therapy or in combination with conventional drugs is becoming progressively common globally (Cheetham, 2013).

The community interest in using ethnomedicinal phytochemical agent like *P. africanus* is chiefly due to side effects related to conventional agent and fear of morbidity and mortality associated with surgical procedures. Therefore phytotherapeutics provide one of the most favourable substitutes in the treatment of BPH (Jena, *et al.*, 2017).

2.1.3 A comparative histo-morphological structure of the prostate between human and the rat.

A comparative gross and histo-morphological structure of the prostate has demonstrated very similar characteristics. In humans, as it is with rats, the prostate is pyramidal shaped. The prostatic apex faces downward and is in contact with the urethra. The base is in contact with the bladder and faces upward. It lies at the base of the bladder and anterior to the rectum. The gland weighs about 20 gram in humans and about 1.5 gram in rats, its size measures 3 cm long x 4 cm wide x 2 cm thick in humans. There is considerable variation in the size from one person to the other as well as from one rat to the other. The prostate has an appended seminal vesicles lying on the basal part of the prostate (Hammerich, *et al.*, 2009).

On the histo-morphology, both the human and the rat prostate have a glandular part that is comprised of transitional zone, central zone, and peripheral zone in a normal healthy male, each prostate zone contains approximately 5%, 20%, and 70%-80% of gland tissue, respectively, but these percentages are altered greatly in the presence of BPH. The non-glandular part is comprised of anterior fibromuscular stromal (AFS) and the urethra (Barbara, *et al.*, 2014). This fibromuscular tissue is constituted of a rich

aggregation of smooth muscle cells admixed with fibroblasts, blood vessels, and nerves. There are no adipose cell in the prostate in both human and the rats (Barbara, *et al.*, 2014).

The prostate also contains a sheet of luminal epithelial cells comprised of basal, secretory luminal and neuroendocrine cells. These luminal cells are highly specialized in secretion of various substances into the glandular lumen, which forms the seminal fluid. These substances include prostate-specific antigen secreted by tall luminal cells that have cytoplasm which is clear to pale in colour. These cells are positive for prostate specific antigen staining (Barbara, *et al.*, 2014). The basal cells are adjacent to the basement membrane, are not well differentiated compared to the lumen cells and do not contain secretory substances such as PSA. PSA is an androgen-regulated serine protease (Balk, *et al.*, 2003). It is an affiliate to human tissue kallikrein gene family, located on chromosome 19q13.4. PSA is produced by the epithelial secretory cells in the ducts and acini of the prostate and is present at a concentration of 0.5–2.0 mg/ml in the seminal fluid. Its physiological function is to liquefy the seminal coagulum formed just after ejaculation by cleaving the semenogelins (Balk, *et al.*, 2003; Lai, *et al.*, 2010). After transcription and translation, PSA is secreted as an inactive pro-enzyme (pro-PSA) which is composed of 244 amino acids. Inside the prostate lumen, pro-PSA is transformed by prostatic protease to a 237 amino acid long active PSA. Approximately 30 % of the PSA in the seminal fluid is in the active form, while 5 % remains bound with protein C inhibitor. The remaining PSA go through internal cleavage to form catalytically inactive PSA. The active and cleaved PSA can go into the blood circulation. About 70% to 90% of PSA remain bound to protease inhibitor, mainly the alpha 1 anti-chymotrypsin, whereas 10 % to 30 % is the inactive, cleaved PSA which circulates as free PSA in the peripheral blood (Diamandis, 2000; Sasaki and Sugimura, 2018). The ratio of free PSA (fPSA) to total PSA (tPSA) is referred as the PSA index and is crucial in distinguishing between normal and diseased prostate in patients Genes associated with human PSA are only found in primates and nonhuman primate species, and not present in the murine genome. Therefore the rodent

animal models PSA levels are not useful in assessing BPH and prostate cancer (Karr *et al.*, 1995).

The neuroendocrine cells are haphazardly spread within the acini and ducts, with the highest percentage in the ducts. The prostate has the largest population of neuroendocrine cells than any other genitourinary organs. The main function of these cells is not well understood though speculation is that these cells are involved in paracrine release of peptide regulation. Prostatic ducts and acini are distinguished by architectural pattern at low power magnification. The prostate becomes more complex with ducts and branching glands arranged in lobules and surrounded by stromal tissue with advancing age.

2.2 Distribution of *Prunus africanus*

P.africanus is an ever green canopy tree that is distributed across the high mountain forests in the Africa continent, it is also referred as *Pygeum africanus*. Pygeum is a member of Rosaceae family (Papaioannou and Jena, 2017). The trees are well established in mountain forests of equatorial Africa in Kenya, Congo, Angola, Ghana, Cameroon, Mozambique, Ethiopia, Tanzania, Madagascar, South Africa, Malawi, Uganda, Zambia, and Zimbabwe. The trees usual height is 10 - 25 metres long, they have cylindrical straight trunk with a dense round crown. Their leaves have a glossy appearance and deep green. The flowers are small with colour ranging from white to whitish cream. The fruits look like cherry with their colour ranging from purplish-brown to red. The wood colour is pale red and when freshly cut it has a strong cyanide smell. The bruised leaves, bark, and fruits have a strong almond smell (Nsawir and Ingram, 2007). Due to its wide uses to-date the medicinal property has put this tree in danger of extinction (Nsawir and Ingram, 2007; Papaioannou and Jena, 2017).

2.2.1 Ethno-therapeutic uses of *P.africanus* in management of BPH.

In Kenya, communities have traditionally used bark extract of *P.africanus* for a number of medicinal purposes. For example its leaves have been used as inhalants and in the management of fever or as appetizers. Boiled water extracts of the pounded bark

has been used for the management of stomach ache, fever, malaria, chest pains or as a purgative for cattle (Bii *et al.*, 2010). The plant has also been used traditionally in the treatment of colorectal carcinoma, breast cancers and management of BPH in older men to alleviate its symptoms (Gachie *et al.*, 2012). The principal reason why communities prefer use of *P.africanus* is that the *u*se of conventional drugs is associated with a lots of side effects among them gynecomastia and erectile dysfunction. Many individuals prefer use of ethno-phytotherapy for the treatment of BPH to avoid these severe side effects. The *P.africanus* phytotherapy options (alternative medicine) has been shown to alleviate BPH related symptoms with less severe or no side effects (Nyamai *et al.*, 2016).

Concerning its application in the management of BPH, the bark extract of *P.africanus* have been traditionally been applied for decades and has been shown to restores the prostate gland physiological functions through re-establishing of the normal secretory patterns, inhibits pathological fibroblast proliferation, decreases hypersensitivity of the detrusor muscle, and has anti-inflammatory effects and even some estrogenic action. All these varied mechanisms of action are thought to enable this extract to provide a joint pharmacologic approach to the management of BPH (Edgar *et al.*, 2007).

2.2.2 Phytochemical Composition of Bark extract of *P. africanus*

Previous studies have shown that *P.africanus* derived from different parts of Kenya and in the rest of the world to have varied proportions of its phytochemical constituents (Jena, *et al.*, 2017; Caroline *et al.*, 2012). However all studies have shown that apart from varying in proportions they more or less have the same chemical constituents including phytosterols, saturated and unsaturated fatty acids, pentacyclic triterpenoids, alcohols, carbohydrates and anthocyanidins. The phytosterols includes the β-sitosterol, β-sitosteryl glucoside and β-sitosterone while the saturated and unsaturated fatty acids are composed of 12 -22 carbon structure, that include erucic acid, behenic acid, linolenic acid, arachidic acid, linoleic acid, nonadecanoic acid, stearic acid, heptadecanoic acid, margaric acid, palmitoleic acid, palmitic acid, pentadecanoic acid, myristic acid and lauric acid. Pentacyclic triterpenoids includes ursolic acid, 2α-

hydroxyursolic acid, oleanolic acid, crataegolic acid and friedelin. Alcohols are the n-tetracosanol and n-docosanol. Carbohydrates are triacontane (C_30H_{62}) and nonacosane ($C_{29}H_{60}$).

The study by Jena *et al*. (2017), has also shown that the bark extract of *P.africanus* is more potent as compared other Prunus species like *Prunus domestica* and *Prunus cerasoides*, this because it is rich in total sterol content, has increased antioxidant potential and membrane stabilization effects validating its use in management of BPH (Jena, *et al.*, 2017). A distinct variation in the phytochemical composition of bark extract was noted among different populations of *P.africanus*, this suggests genetic variations play role, also interestingly the size and age of tree was noted to affect the phytocomposition of this tree (Caroline *et al.*, 2012).

2.2.3 Mechanism of Action of the Bark Extract of *P.africanus*

The mode of action of *P.africanus* is thought to be mediated by its phytochemical constituents, which are pivotal in the treatment of BPH. These phyto-constituent are formed from long-chain fatty alcohols (n-docosanol, n-tetracosanol, & trans-ferulic acid esters), β-sitosterol, and its 3-O-glucoside, β-sitosterone, oleanolic, ursolic, crataegolic, and fatty acids. The ferulic acid esters of fatty acids decreases the prostatic cholesterol concentrations, restricting the synthesis of testosterone. While hypothetically the phytosterols, which includes the β-sitosterol, β-sitosterone, and campesterol compete with androgen precursors and prevent prostaglandin biosynthesis while docosanol decreases luteinizing hormone and plasma testosterone and upsurges secretion of adrenal steroid in rats (Schulz *et al.*, 2004).

In addition, *P.africanus* exhibits anti-inflammatory activity and inhibited fibroblast proliferation, specifically fibroblasts induced by prostatic growth factors, basic fibroblast growth factor, and epidermal growth factor. Although it appears unlikely that *P. africanus* inhibits either androgens or 5α-reductase, it may have direct protective effects by restoring structural and functional characteristics in the aging prostate (Dvorkin and Song, 2002). Further, *P. africanus* improves prostate histoarchitecture, re-establishes normal secretory patterns, inhibits pathologic fibroblast proliferation, reduces hypersensitivity of the detrusor muscle, and reduces

contractile dysfunction. These varied mechanism of action enables this extract to provide a joined pharmacologic approach to the management of BPH (Hatsuko *et al.*, 2012; Vazquez, *et al.*, 2012).

2.3 Oral Acute Toxicity of the Bark Extract of *P.africanus*

The oral acute toxicity of bark extract of *P.africanus* at a dose of 5000 g/kg for 6 days causes pronounced organ damage, clinical signs, and 50% mortality rate, while on the other hand daily doses of up to 1000 mg/kg for up to 8 weeks have been shown to be safe (Gathumbi *et al*., 2002). In addition, experimental studies have demonstrated the safety of *P.africanus*, as it is well tolerated with merely minor to moderate gastrointestinal system side effects, which include nausea, gastric pain, constipation and diarrhoea. There are no reported pharmacological interactions with other herbs, conventional medicine, food supplement or laboratory tests (Mahunnah and Masanja, 2007).

2.4 Histostereology Quantification of the Prostate

Though there is paucity of data on the histostereological or the quantification of both the parenchyma and stromal tissues following the treatment options that are currently being applied in the management of BPH, stereological quantification of the prostate parameters would provide a powerful tool for anatomical quantification of a two-dimension (2D) histological images of the prostate to a three-dimension (3D) structure and this offers a more practical methods to enable quantify the reversal and inhibitory effects of *P.africanus* in management of BPH. Thus, histostereology presents a very essential method for meaningful quantitative data for prostate researches in anatomy and histopathology field (Baak *et al.*, 1977; Suleyman and Kaplan, 2012; West, 2017).

Though data on prostate stereology is generally lacking, a lot of researchers are currently employing stereology for quantitative analysis in the discipline of oncology and radiology in monitoring cancer cells, tumour grade and prognosis of patients. (Baak *et al.*, 1977; West, 2017).

CHAPTER THREE
MATERIALS AND METHODS

4.1. Study Area/Site

In carrying out of this study the Small Animal Facility for Research and Innovation (SAFARI) in Jomo Kenyatta University of Agriculture and Technology (JKUAT) was used, while tissue processing for light microscopy and histostereology was done in the histology laboratory in the Department of Human anatomy, School of Medicine in the College of Health Sciences (COHES) of JKUAT.

3.1 The experimental Rats and Materials

In this study, a total of 60 adult male Wistar rats (weighing between 180±20 grams) were used. The Wistar rats were chosen and applied in this study because previous studies have established that they are the laboratory rats that are highly susceptible to the development of benign and atypical prostatic hyperplasia after treatment with exogenous testosterone in both intact and post-castrated males (Scolnik, *et al.*,1994). In this case they were considered appropriate for this study as they would provide a more accessible and efficient animal models in evaluation of the effectiveness of *P. africanus* in restoring or inhibiting development of BPH. Wistar rats were sourced from SAFARI animal house in JKUAT. They were housed in standard rat cages and exposed to 12 hour light/dark cycles under humid tropical conditions. Litter papers in the cage were changed on alternate days. Each cage was labelled with a cage card showing the experiment number, date of starting the experiment, dosage level, and age, number of rats, species and sex of the animal. The rats were allowed unrestricted access to standard rodent pellets obtained from UNGA Mills ltd and water *ad libitum* throughout the experimental period. The rats were handled in accordance with the guidelines for the care and use of laboratory rats.

3.2 The Study Design

The study design was as static group case control experimental laboratory-based study.

3.3 Induction of BPH in Wistar Rats

BPH was induced by daily subcutaneous injection of testosterone propionate injection. This steroid hormone was diluted using corn oil which served as the solvent. The stock solution was prepared by adding 19 mL of Corn oil to 1mL (25 mg) of testosterone. The rat were injected with 7.5 mg/kg of the stock solutions for 10 days (El-mehi and El-said, 2015).

3.4 Harvesting, preparation and extraction of *P. africanus*

The barks of *P. africanus* that was used in this study was sourced from Mt. Kenya forests at Mukurweini location of Nyeri County. A botanist specialized in plant taxonomy and with many years of experience dealing with studies of *P. africanus* assisted in identifying trees, debarking of the stems and bark collection, preservation and transportation of back extracts. A voucher specimen of *P. africanus* plant was deposited in Jomo Kenyatta university of Agriculture and Technology botanical Herbarium, voucher number Rosacea 0001. Sustainable harvesting was done by cutting the old branches and prunes i.e. "renewable plant sourcing" this preserved the source and kept it getting renewed (Tewari, *et al.*, 2016).

In harvesting of the barks for subsequent extraction of the crude extract, the *P. africanus* branches and stem were debarked using a sharp-edged knifes to obtain ten kilograms of the wet bark. The pieces of the wet bark were then transported in meshed plastic bag to Jomo Kenyatta University of Agriculture and Technology, where they were air dried at room temperature of 25^{o}C to a moisture content of about 10-15%. The dried bark was weighed (1.2 kg) and chopped into small pieces and grounded into fine powder using a laboratory mill (Christy and Norris Ltd., Chelmsford, England). One thousand grams of the powdered material was soaked in 2000 ml of 75 % methanol at room temperature for 24 hours. The methanol mixture was filtered through Whatman filter paper no. 1 (Whatman international, England). The organic solvent was evaporated to near dryness by vacuum evaporation using rotary evaporator (BUCHIR 200) (Harborne, 1985). After evaporation, 68 grams of the bark extract (pygeum) was obtained which was a greenish mass with almond Smell (Tewari, *et al.*,

2016). The extract was stored in air tight bijou bottles in a freezer at -20^{o} C prior to use.

The percentage yield of bark extract was calculated as follows;

$$Percentage\ yield = \frac{Weight\ of\ plant\ after\ extraction}{Weight\ of\ plant\ before\ extraction} \times 10$$

3.4.1 Phytochemical Determination of the constituents in the crude bark extract of *P.africanus*

In the determination of phytochemical constituents of the bark extract of the *P.africanus* the study adopted the procedure described by Tiwari *et al.* (2011). The criteria for grading was done on the basis of the intensity of colour produced from reactions observed in the test tubes and the amount of frothing. The reaction were denoted as follows; +++ for strong positive reaction, ++ for a positive reaction, + weak positive reaction and – for represented no observable reaction (Savithramma *et al*., 2015).

1. Determination for the presence of alkaloids in the CMBEPA

The 2 grams of CMBPE was dissolved in 20ml of dilute hydrochloric acid shaken for two minutes. The reaction mixture was filtered and the following reagents were added to determine the presence of alkaloids:-

(a) Mayer's reagent (Potassium Mercuric Iodide) 3 drops was added to 2 ml of reaction mixture and formation of a yellow coloured precipitate indicated the presence of alkaloids.

(b) Wagner's reagent (Iodine in Potassium Iodide) 2 drops was added to 2 ml of reaction mixture and formation of brown/reddish precipitate indicated the presence of alkaloids.

(c) The reaction filtrate (2 ml) was mixed with 2 drops of Dragendroff's reagent (solution of Potassium Bismuth Iodide) and formation of red precipitate indicated the presence of alkaloids.

(d) The reaction filtrate (2 ml) was mixed with 2 drops of Hager's reagent (saturated picric acid solution) and presence of alkaloids was confirmed by the formation of yellow coloured precipitate.

2. Determination for the presence of saponins in the CMBEPA

a) Froth Test

The CMBEPA (2 grams) was diluted with 20 ml of distilled water in a graduated test-tube and shaken vigorously for about 15 minutes and formation of 1 cm layer of foam indicated the presence of saponins.

b) Foam Test

The CMBEPA (0.5 g) was shaken with 2 ml of distilled water and foam persisting for ten minutes indicated the presence of saponins.

3. Determination for the presence of phytosterols in the CMBEPA

(a) Salkowski's Test

The CMBEPA (0.5 grams) was dissolved in 10 ml of anhydrous chloroform and filtered. The filtrates was treated with 1 ml of Concentrated Sulphuric acid, shaken and allowed to stand. The appearance of a golden yellow colour indicated the presence of triterpenes.

(b) Liebermann Burchard's Test

The CMBEPA (0.5 grams) was dissolved in 10ml of anhydrous chloroform and filtered. The filtrates was mixed with 2 drops of acetic anhydride, boiled and cooled. Concentrated Sulphuric acid (1 ml) was then added. Brown ring at the junction indicated the presence of phytosterols.

4. Determination for the presence phenols in the CMBEPA

The CMBEPA (5 mg) was mixed with 2 ml of 0.1% ferric chloride solution and formation of bluish black colour indicated the presence of phenols

5. Determination for the presence of tannins in the CMBEPA

The CMBEPA (5 mg) was reacted with 2 ml 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicated the presence of tannins.

6. Determination for the presence of flavonoids in the CMBEPA

The CMBEPA (5 mg) was mixed with 2 drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicated the presence of flavonoids.

(b) Lead acetate Test

The CMBEPA (5 mg) was mixed with 2 drops of lead acetate solution. Formation of yellow colour precipitate indicated the presence of flavonoids.

3.4.2 Determination of the Acute Oral toxicity of *P.africanus*

The first part of the experimentation was on the determination acute oral toxicity of the Crude Methanolic Bark Extract of *P.africanus* (CMBEPA) that would guide the safe dose limits and the modified Lorke's method (1984) was applied for this determination. This experimentation used fifteen male Wistar rats obtained from SAFARI animal house in JKUAT and was conducted in two phases as follows:

Phase I; had four groups each with 3 rats, group 1, 2 & 3 rats were administered with single oral dose of 10, 100 & 1000 mg/kg of the bark extract in 5% dimethyl sulfoxide (DMSO), respectively while Group 4 was the control group with three rats, which were given 5% DMSO in distilled water (5 ml/kg body weight).

Phase II; had three rats and each received a single oral dose of 1600, 2900 and 5000 mg/kg of the bark extract in 5% DMSO respectively. The bark extract was administered orally using sterile gavage needles and prior to administration of the CMBEPA doses the rats were fasted overnight.

All the rats were monitored closely for signs of toxicity which are mortality, changes in gross appearance of the skin (piloerection) and fur, mucous membrane of the eye, respiratory distress, somatomotor activity, behaviour, and special attention was given to observation of tremors, salivation, diarrhoea, coma and convulsions, changes during the first 48 hours post dosing. The observation schedule was as follows; immediately, ½ an hour, 1 hour, 4 hour, 24 and 48 hours, the monitoring for signs of toxicity continued daily for 14 days. The body weights were monitored as follows; day 0 (initial weight), day 7 and day14 (terminal weight).

Terminal sacrifice of all surviving rats done on day 15th following fasting them overnight and euthanizing them with carbon dioxide and gross necropsies were performed. All Organs and tissue were harvested, grossly examined and weighed.

3.5 Sample Size Determination

The sample size in this study was determined using the modified "resource equation method" since there was no previous data on standard deviation from previous study on the histostereology of the prostate (Arifin and Zahiruddin, 2017). The sample size was determined separately for each of the 2 main groups; that is the restorative group and inhibitory group as follows:

$$n = \frac{DF}{k} + 1$$

$N = n \times k$

Where;

n – Number of animal per group.
DF-error of degree of freedom
k - Number of groups
N = total number of subjects

DF in ranges from minimum (10) and maximum (20) DFs to obtain the minimum and maximum numbers of rats per group:

Numbers of rats in the restorative group were calculate as follow;

$k = 6$ groups

$$= \frac{20}{6} + 1$$

$=4.333$

This was rounded up 5 rats per group

$=5 \times 6 = 30$

Inhibitory group had 6 groups

$$= \frac{20}{6} + 1$$

$= 4.333$

This was rounded up 5 rats per group

$=5 \times 6 = 30$

Total no animal is 60`

3.6 Groupings of the Study Rats

The grouping of animal was as follows.

The 60 rats were divided into the restorative group (Group 1) which was composed 30 rats and inhibitory group (Group 2) which was composed of 30 rats.

Group 1: was comprised of restorative group, the animal were randomly assigned into control group and five treatment subgroup.

Control group was comprised of 5 rats, which were fed on the rodent pellets (Mice Pellets UNGA Mills ltd and water *ad libitum*. They receive sham treatment i.e. corn oil subcutaneous (S.C) injection for first 10 days and then 5% DMSO through gastro gavage for 3 weeks (Normal control group).

In the treatment group all rats were injected with daily S.C injection testosterone propionate (7.5 mg/kg body weight) in corn oil for duration of 10 days to induce BPH. The testosterone propionate induced (TPI-BPH) rats were then assigned randomly into 5 sub-groups of 5 rats each.

i. Subgroup Ia -TPI-BPH rats administered with 5% DMSO only for 21 days (BPH control group).
ii. Subgroup Ib - TPI-BPH rats administered with 25 mg (2.5833 mg/kg body weight) CMBEPA in 5% DMSO for 21 days (TPI-BPH + CMBEPA 25 mg).
iii. Subgroup Ic -TPI-BPH rats administered with 50 mg (5.1667 mg/kg body weight) CMBEPA in 5% DMSO for 21 days (TPI-BPH + CMBEP.A 50 mg).
iv. Subgroup Id – TPI-BPH rats administered with 125 mg (12.9164 mg/kg body weight) CMBEPA in 5% DMSO (TPI-BPH + CMBEPA 125 mg)
v. Subgroup Ie – TPI-BPH rats administered with 200 mg (20.6667 mg/kg body weight) CMBEPA in 5% DMSO (TPI-BPH + CMBEPA 200 mg)

Group 2 was comprised of the inhibitory rats, which were randomly assigned into a control group (5 rats) and treatment group (25 rats). Control group received 5% DMSO through gastrogavage and corn oil S.C for 10 days. The treatment group were also randomly assigned into 5 sub-groups with 5 rats each, they received TP 7.5 mg/ kg body weight in corn oil / day S.C injection concurrently with varying dose of CMBEPA in for 10 days.

i. Subgroup 2a - received TP (7.5 mg/kg body weight in corn oil) concurrently with 5% DMSO only)

ii. Subgroup 2b – received T.P (7.5 mg/kg body weight in corn oil) concurrently with 25 mg (2.5833 mg/kg body weight) CMBEPA in 5% DMSO (TP + 25 mg CMBEPA).

iii. Subgroup 2c - received TP (7.5 mg/kg body weight in corn oil) concurrently with 50 mg (5.1667 Mg /Kg body weight) of CMBEPA in 5% DMSO (TP + 50 mg CMBEPA).

iv. Subgroup 2d – received TP (7.5 mg/kg body weight in corn oil) concurrently with 125 mg (12.9164 mg/kg body weight) of CMBEPA in 5% DMSO (TP + 125 mg CMBEPA).

v. Subgroup 2e - received TP (7.5 mg/kg body weight in corn oil) concurrently with 200 mg (20.6667 mg/kg) of CMBEPA in 5% DMSO (TP + 200 mg CMBEPA).

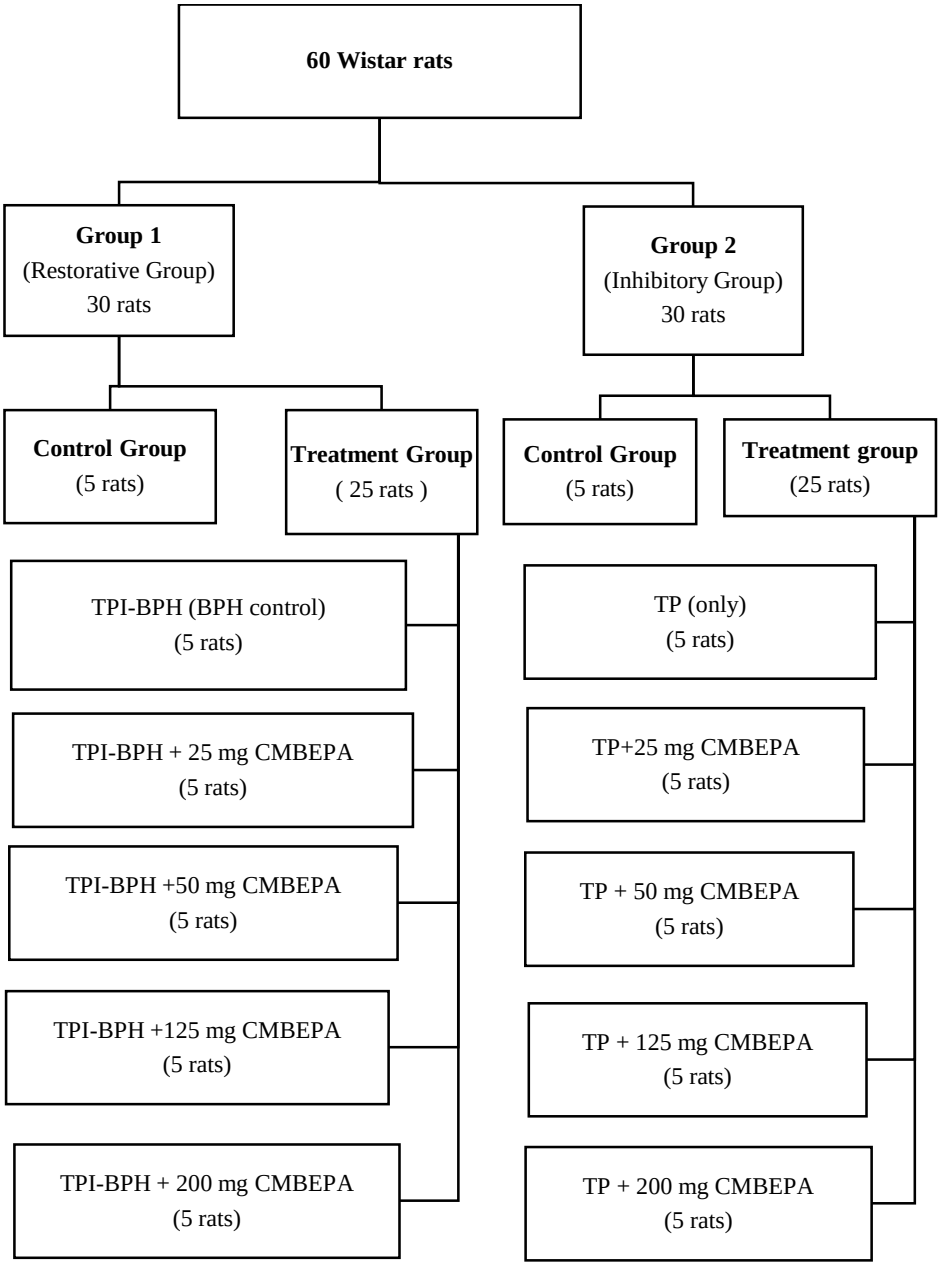

Figure 3.6.1: Shows how animal grouping were organized between experimental and controls group.

Key: *TPI-BPH - Testosterone Propionate induced benign Prostatic Hyperplasia, CMBEPA-Crude Methanolic bark extract of P. africanus, TP-testosterone propionate.*

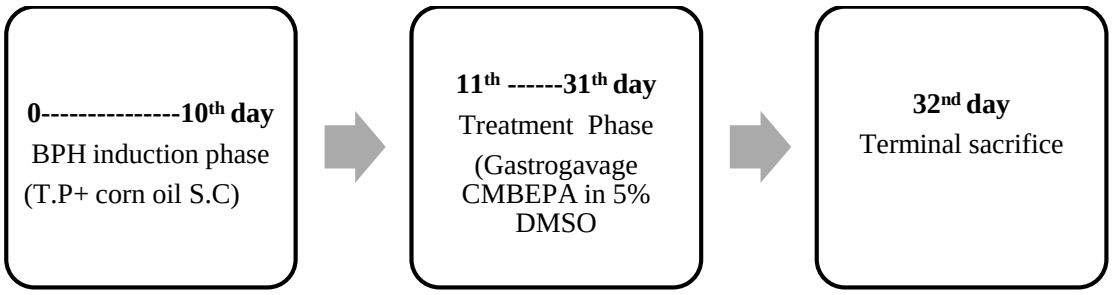

Figure 3.6.2: Shows the experimental procedure followed in the restorative group

Key: CMBE Crude methanolic bark extract of P. africanus extract; TP, Testosterone propionate

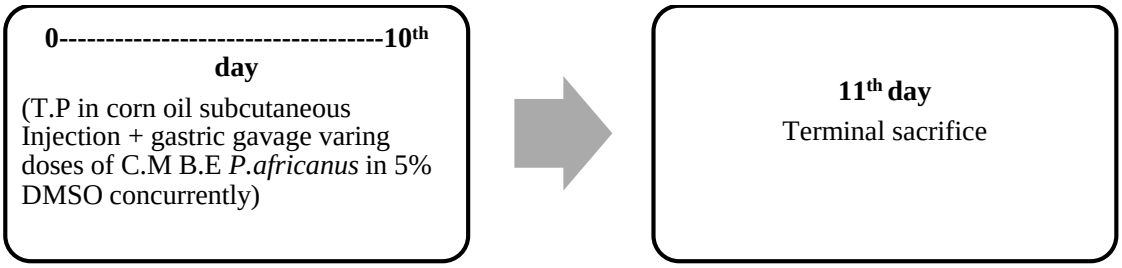

Figure 3.6.3: Shows the experimental procedure followed in the inhibitory group

Key: TP-Testosterone Propionate, C.M B.E -Crude Methanolic Bark Extract

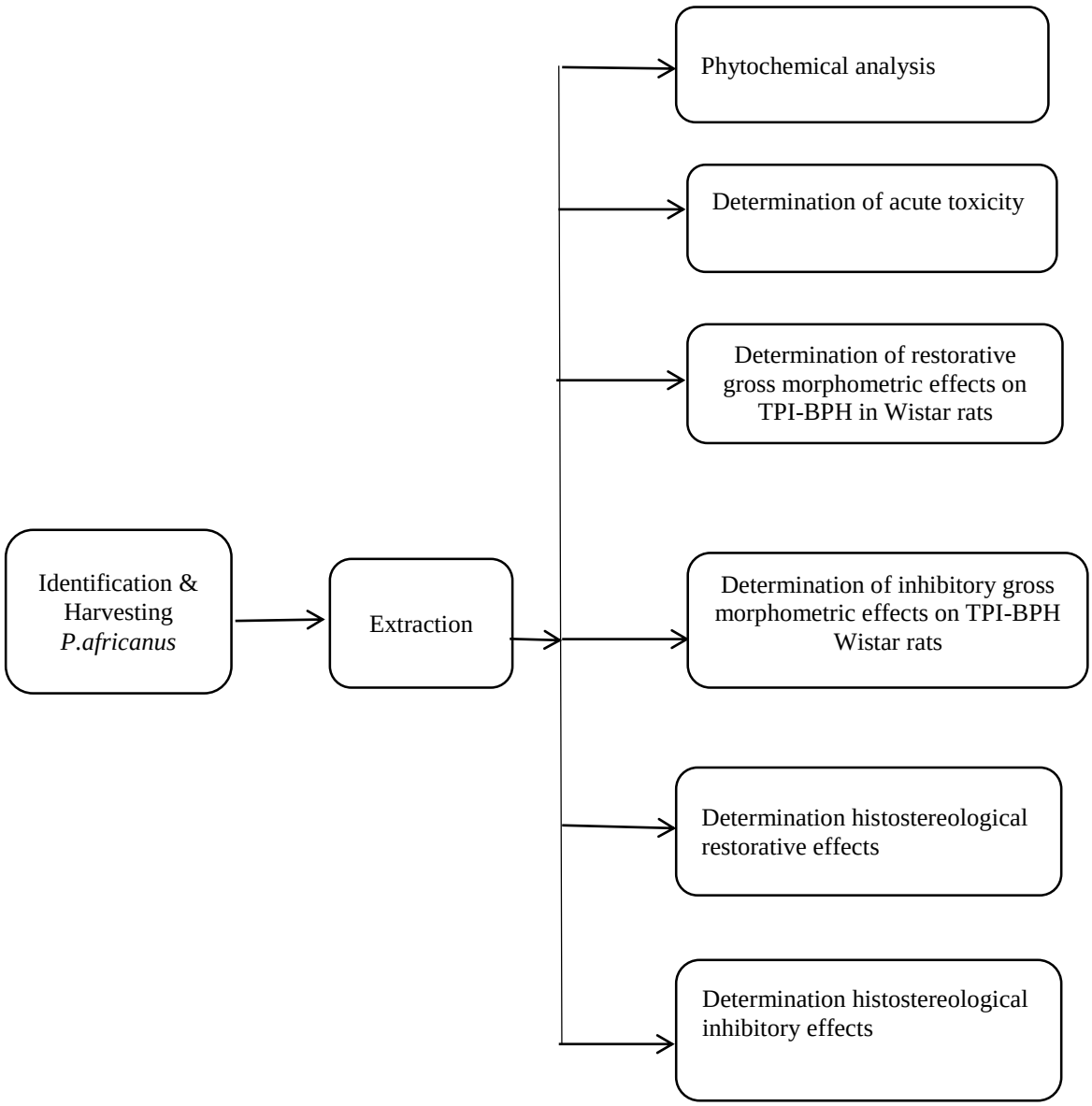

Figure 3.6.4: Shows the process followed from identification, extraction to experimentation with *P.africanus*.

3.7 Dilution and administration of crude bark extract of *P.africanus*

The dilutions of CMBEPA was done using 5% DMSO to make the stock solution. The administration of CMBEPA was done between 12:30 pm and 1:00 pm daily through gastric gavage. All the volumes of CMBEPA that were administered did not exceed the standard allowable daily oral volume of 3 ml per rat per day (OECD, 2002).

3.7.1 Determination of the *P.africanus* animal doses from the CMBEPA

The rationale for dose selection that were applied in this study were based on the reference standard Human dose equivalents of *P.africanus* that have been applied in human that ranged from 50 mg to 200 mg given once daily as described by Melo *et al.* (2002). Based on this therefore the animal equivalent doses (AED) were calculated from Human equivalent doses (HED) as follows, using the FDA guideline on human to animal dose conversion:-

The human dosages of bark extract *P.africanus* was converted as follows

i. 25 mg bark extract *P.africanus* was used as the lowest human dose
   Human dosage is first converted into mg/kg body weight considering human body weight of 60 kg.
   =25 mg ÷60=0.4167 mg/kg body weight
   Rat dosage in mg/kg = Human dose (mg/kg) x 6.2 (6.2 is a constant given in conversion of rat dosages based on surface area)
   = 0.4167 mg/kg x 6.2 =2.5833 mg/kg
   E.g. if average weight of Wistar rats is 110 mg
   0.11x 2.5833= 0.284163 mg
ii. 50 mg bark extract *P.africanus* was used as the lowest effective human dose documented.
   Human dosage is first converted into mg/kg body weight
   =50 mg ÷60=0.8333 mg/kg body weight
   Rat dosage in mg/kg = Human dose (mg/kg) x 6.2 (6.2 is a constant given in conversion of rat dosages based on surface area)

= 0.8333 mg/kg x 6.2 =5.1667 mg/kg

Rat dosage in mg/kg = 5.1667 mg /kg body weight

E.g. if Average weight of Wistar rats is 110 mg

0.11x 5.1665=0.5683 mg

iii. 125 mg of bark extract *P.africanus* was used as medium human dose (50 mg minimum effective dose + 200 mg maximum effective dose)÷2= 125 mg/kg

Human dosage is first converted into mg /kg body weight.

=125 mg÷60

=2.0833 mg/kg

Rat dosage in mg/kg = Human dose (mg/kg) x 6.2 (6.2 is a constant given in conversion of rat dosages based on surface area)

=2.0833 x 6.2

=rat dosage in mg/kg =12.9164 mg/kg body weight.

E.g. if average weight of Wistar rats is 110 mg

=12.9167 x 0.11

=1.4208 mg/kg body weight

iv. 200 mg of bark extract *P.africanus* was used as high dose as the maximum dose.

Human dosage is first converted into mg/kg body weigh

=200 mg ÷60

Human dosage in mg/kg =3.3333

Rat dosage in mg/kg = Human dose (mg/kg) x 6.2 (6.2 is a constant given in conversion of rat dosages)

= 3.3333 mg/kg x 6.2

Rat dosage mg/kg =20.6665

E.g. if Average weight of Wistar rats is 110 mg

=20.6667 x 0.11

=2.2733 mg/kg body weight

3.7.2 Assessing the Animal Body Weights

The rats were weighed daily using a high precision electronic weighing machine throughout the studies.

3.7.3 Procedure for anaesthetizing and harvesting the prostate gland

1. Carbon dioxide was introduced into a heavy bell jar with a tight fitting lid.
2. The male Wistar rats were then be put into the bell jar.
3. The rats were left for 5-10 minutes to be anaesthetized.
4. The animal was then be removed from the bell jar and mounted onto the dissection board using mounting pins with dorsal side on the board.
5. Using a pair of scissors and forceps the animal was cut through the ventral medial side from the symphysis pubis to the sternal angle of the thoracic cage.
6. Blood sample were collected from the left ventricle using a needle and a syringe for laboratory analysis.
7. A perfusion needle was inserted to the left ventricle of the heart while connected to the perfusion set containing 400 ml of normal saline
8. The blood was cleared from the animal using physiological saline (200 ml of 0.85 mol through the left ventricle of the heart (saline flowed by force of gravity from one of the drip set).
9. After sufficiently clearing the saline drip was removed and replaced with 10 % formal saline solution for *in situ* fixation.
10. The firmness of the tail was checked as a sign of effective fixation of the animal.
11. The drip was disconnected and the perfusion needle removed from the heart
12. The prostates was dissected and freed from the fascia, and were removed in *toto* (prostate gland, coagulating gland and the seminal vesicles) the urethra and urinary bladder were removed, and trimming was done. (Ingelheim *et al.*, 2003).
13. The prostate weights and dimensions (length, width, and thickness) were assessed using vernier calipers and rulers.

14. Estimation of prostate volume was done using Archimedes principle.

3.7.4 Estimation of prostate volume using Archimedes principle

The estimation of prostate volume was done using the Archimedes' principle so as to obtain independent prostate volume in all the rats. The Archimedes volumes were estimated by inserting the fixed prostate gland into graduated beakers containing normal saline, and the displacement was measured. The normal saline displaced by prostate represented the actual prostate volume (Scherle, 1970).

3.7.5 Estimation of prostatic index and percentage restoration

The prostatic body Ratio (%) and the percentage restoration of prostate gland weight by different treatments was calculated as previously described (Patil and Yadav, 2016)

$$Prostate\ body\ ratio\ \%\ (PBR) = \frac{Prostate\ weight(g)}{Terminal\ body\ weight(g)} \times 100$$

Further percentage of restoration was calculated as follows:

$$Percentage\ restoration = 100 - \left(\frac{(T - C)}{(B - C)} \times 100\right)$$

T - Mean prostate weights in the treatment group

C - Mean prostate weight in the control group

B – Mean prostate weight in the BPH group (BPH control group)

3.7.6 Tissue Processing for Light Microscopy and Histostereology

Prostate tissue processing for both light microscopy and histostereology was done by initially fixing the gland in Bouin's solution for 24 hours, followed by dehydration in an ascending concentration of alcohol (50%, 60%, 70%, 80%, 90%, 95% and 100 % each for one hour and cleared with xylene in 12 hours. The sections were then infiltrated with paraffin wax for 12 hours and then embedded in paraffin wax. Leitz sledge microtome was used to cut transverse and longitudinal thin sections 5-7µm thick, floated in water at 37^0 then stuck onto glass slides using egg albumin, applied as thin film with a micro-dropper. In each subgroup 40 slides were selected with systematic random sampling was then dried in an oven at 37^0 for 24 hours then stained with haematoxyline and eosin to demonstrate the general features of prostate gland in

all the lobes. Another 40 slides from each prostate were randomly selected and stained with haematoxyline and eosin stain to demonstrate cellular components.

3.7.7 Staining procedure for Histostereology

The prostate sections were stained using haematoxyline and eosin (H.E) (Cardiff *et al.*, 2014).

H.E Staining Procedure

1. The glass slides for holding the paraffin embedded sections of the prostate were placed in staining racks and the paraffin cleared in three dips of xylene at 2 minutes per change.
2. Hydration of the prostate samples was done by transferring the slides through three consecutive changes of 100%, 95% and 70% ethanol for 2 minute per change, then the slides were rinsed in running tap water at room temperature for 2 minutes.
3. The prostate samples were dipped in haematoxyline solution for 3 minutes.
4. The slides were placed under running tap water at room temperature for at least 5 min.
5. The samples were stained in working eosin Y solution for 2 minutes.
6. Dehydration of the samples was done by dipping the slides in 70%, 95% and 100% ethanol for 2 minutes per change for 20 times.
7. The samples were cleared in three changes of xylene for 2 minutes per change.
8. A drop of permount was placed over the tissue on each slide and a coverslip was added. The slides were now ready for viewing using a light microscope

3.8 Procedure for acquiring digital images for Prostate Histostereology

The digital images were acquired using the LABOMED iVu 3100 Digital Camera Software with PixelPro™ Image Analysis Software for capturing still images. The PixelPro software provided an easy way to navigate user interface designed for routine analysis by Quo path and STEPanizer which were applied for stereology quantification of prostate structures.

In addition, the digital images of the prostate tissue were captured using stereological sampling rules. The images were captured at the same magnification and saved in the

Joint Photograph Expert Group (jpeg) file format at adequate resolution. The picture heights were ensured to match the height of the computer monitor, both defined in pixels. The image series were named with an invariable prefix and consecutively numbered without leading zeroes and no whitespaces within the name sample as follows C111_1.jpg, C112_2.jpg, and C113_30.jpg.

The images captured were organized hierarchically according to the number of experimental groups and rats per group during storage. The images from one animal were saved in one folder.

A calibrated scale bar was added to one image of a batch to define the real dimensions of the structures under investigation, and placed on left hand side. Where stereological estimation required the use of a guard area they were set and not be changed in the course of the whole experiment and this ensured consistent results. The quantitative ultra structural analysis of rat prostate to assess both restorative and inhibitory effects of CMBEPA in the TPI- BPH Wistar rats was done using the following image analyser software, Quo path, image J and STEPanizer. These three software were complementary to each other (Bolender &Weibel, 1973).

3.9 Estimation of the Prostate Volume

Prostate volume estimation was done using Archimedes displacement method, Cavalieri point counting method and planimetry. These Archimedes volumes and planimetry were used as the gold standard (reference volumes) and was compared to cavalieri volumes. These were done prior the determination of prostate structure volumes (acinar, stromal and epithelium volume densities) to ensure accuracy of these parameters.

3.9.1 Estimation of prostate volume using Cavalieri principle.

The Cavalieri principle was used in the present study for practical estimation of prostate structure volumes and their volume densities, because they cannot be assessed using routine water displacement procedures. The volume estimation were done by applying the Cavalieri principle (Cruz-Orive, 1999).

The volume of the prostate and its anatomical structures were determined by combining the Cavalieri method of segmentation with point-counting. On evenly spaced prostate slices. The following steps were followed:-

1. Preparation of prostate Cavalieri sections.
2. Selection of the spacing for the point probe was done.
3. The point probe was tossed randomly onto each section.
4. The points that hit the region of interest were counted using STEPanizer stereology tool.
5. All sections were processed keeping a tally of counts per section.
6. The shape factor was finally estimated and the calculation of the volume and the Coefficient of Error (CE).

3.9.2 Cavalieri Point Counting for prostate structure volume determination.

In each prostate, 30 sections were sampled using systematic random sampling (SRS), to obtain section sampling fraction (ssf). The sampled sections were then subsampled by systematic random sampling using the microscope's stage Vernier and images were at magnification of X10. The prostate micrographs from the selected section, with a series of 5 um thickness were used to estimate the cavalieri volumes of the prostate structures. The micrograph were retrieved from the laptop where they had been saved. They were uploaded a STEPanizer stereology analyser tool, where a grid test system with d = between test points was introduced, randomly covering the entire image frame. Volume calculations were performed on each individual prostate section by using the interposing a test points (Figure 3.11.1). A point grid with 100 distances between two points was superimposed on the images and the point hitting points on the prostate were counted and then the volume of each component was calculated using the following formula:

$$V = \frac{t \times a(p) \times \Sigma P}{M^2}$$

Where;

V refers to volume component of interest,

t is the section thickness,

a (p) is the area of one point (359μ)

ΣP is the total number of point counted in the component of interest

M is the linear magnification (X40)

The estimation of volume density of the gland's epithelium, fibromuscular tissue and acinar, the systematically sampled sections were subsampled by systematic random sampling using the microscope's stage Vernier with images at magnification of 40x The volume density of the structures was estimated using STEPanizer a stereology software by the point-counting method and the following formula applied :

$$Vv(structure/prostate) = \frac{P(structure)}{P(prostate)}$$

Where;

P (structure) = the No, of points hitting the favoured histological structures

P (prostate) = the No, of points hitting the reference space, here whole prostatic sections. The absolute total volume of each structure was estimated by the volume density multiplied by the total volume of the prostate.

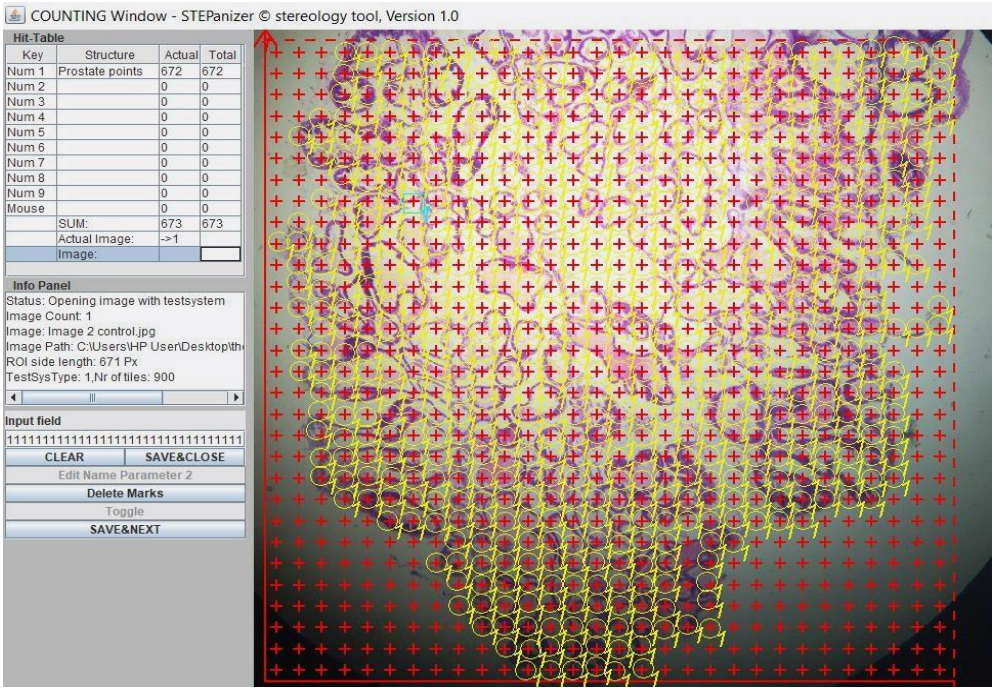

Figure 3.9.1: Prostate photomicrograph illustrating superimposed point-counting frame for cavalieri volume determination.

3.9.3 Correction for the prostate tissue shrinkage

The prostate tissue shrinkage was calculated according to Altunkaynak *et al.* (2007) as follows:

$$Volume\ shrinkage = 1 - [\frac{Volume\ after}{Volume\ before}]$$

Key:

Volume after *-Prostate volumes determined using Cavalieri method*

Volume before *-Prostate volume determined using Archimedes displacement method*

The tissue shrinkage caused by fixation and histological procedures is major setback of stereological estimation of the prostate volumes, therefore the final volume of the prostate volume and prostate structure volumes obtained from cavalieri were corrected accordingly after estimating the prostate shrinkage (Kannekens *et al.*, 2006; Christensen *et al.*, 2007).

3.9.4 Computation of Coefficient of Error (CE)

The coefficient of error was computed to ensure quality of quantitative estimates and the precision of stereological parameters. The CE was calculated to evaluate using the following formula (Gunders en *et al.*, 1999).

$$v = \frac{1}{\mathrm{ssf}} \mathrm{a_f\, t} \sum_{\mathrm{i=1}}^{\mathrm{n}} \mathrm{P_i} \quad \mathrm{a_{f=g^2}}$$

$$A = \sum_{i=1}^{n} P_i\, P_i \quad B = \sum_{i=1}^{n-1} P_i\, P_{i+1} \quad C = \sum_{i=1}^{n-2} P_i\, P_{i+2}$$

$$Var_{noise} = 0.0724.\left(\frac{b}{\sqrt{a}}\right)\sqrt{n\sum_{i=1}^{n} P_i}$$

$$Var_{SURS} = \alpha_i(3.(A - Var_{noise}) - 4.B + C)$$

$$\alpha_0 = \frac{1}{12} \quad \alpha_1 = \frac{1}{240}$$

$$CE = \frac{\sqrt{Var_{SURS} - Var_{noise}}}{\sum_{i=1}^{n} P_i}$$

Table 3.9.1: Showing slice interval, grid spacing and block advance for CE estimation for Cavalieri point counting method

Sampling Parameter	Value
Slice Interval	30
Grid spacing	349 microns
Block advance	5 microns

The slice sampling interval during tissue sectioning 30 (k^{th}) while the block advance was 5 microns, and grid spacing was 349 microns during cavalieri volume determination (Table 3.8.1)

3.9.5 Planimetry volumetry of prostate volume

Planimetry volumetry method was used as reference volume alongside Archimedes volume displacement for accurate prostate volume determination. The prostate images were transferred to a laptop and image analyses were performed using image analysis software, Qupath 0.1.2. The images were uploaded from the laptop to QuPath user interface where they were displayed in a standard image. The borders of the prostate epithelium, stromal tissue and acinar were manually traced on each section of the images using a hand-held mouse (Figure 3.11.2) and the cross-sectional surface area of interest within the image were determined (Gundersen *et al.*, 1988). The software computed the number of pixels within the traced area, and this process was carried out for each image. The area of the prostate is calculated in consecutive histological sections. The area is multiplied by the distance between the sections and the total volume determined by summation of all contributions(Aarnink *et al.*, 1995). This volume is calculated based on the pixel size and slice thickness using the following formula:

Volume= the sum of the areas (mm^2) X slice thickness (um) X No of slices.

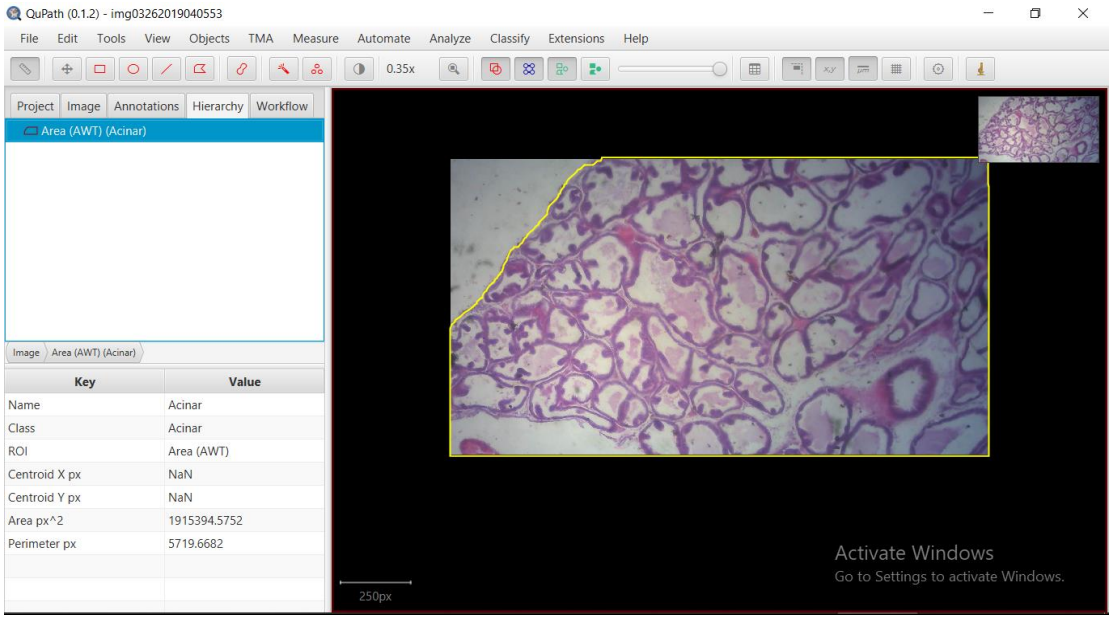

Figure 3.9.2: Prostate photomicrograph illustrating the probe for plainmetry volume determination.

3.10 Statistical Analysis

Data was analysed using statistical package for social sciences (SPSS) for Windows Version 25. For continuous data one way analysis of variance (ANOVA) was used to compare means between different treatment groups. Before applying ANOVA statistical analysis, the data were examined using Shapiro Wink normality and Levene test for homogeneity of variances as parametric test assumptions. Group means with a significant F-value ($p<0.05$) were further analysed usingTukey post hoc t-test.

To assess the level of agreement between the volume measurements of Archimedes' method, Cavalieri and plainmetry method, statistical agreement measures, including concordance correlation coefficient (CCC) and intraclass correlation coefficient (ICC) were applied.

3.11 Data Presentation

The quantitive finding were presented using tables and bar graphs

3.12 Ethical approval

The experimental protocol was approved by the Jomo Kenyatta University of Agriculture and Technology Animal ethics Committee (JKUAT AEC) approval

certificate number JKU/2/4/896A. The protocol followed Guidelines for Care and Use of Laboratory Rats in Biomedical Research, and the rats were only used once in the experiment. They were all sacrificed using humane end points at the end of the study (Leary *et al.*, 2013).

CHAPTER FOUR

RESULTS

4.1 The Qualitative Phytochemical Screening of the CMBEPA

4.1.1 The yields of CMBEPA

The total yields of solid *P.africanus* crude methanolic extract recovered was 68 grams. The extract was a greenish mass with almond smell. The percentage total yield of crude methanolic bark extract was found to be 6.8%

4.1.2 The qualitative phytochemical screening of the CMBEPA

The qualitative phytochemical screening identified several constituents of the methanolic bark extract *P.africanus*. As shown in table 4.1.1

Table 4.1.1: Shows Phytochemical Constituents found in the CMBEPA

Sample	**Phytochemical screened**							
Extract	carbohydrate	steroids	Triterpenes	Glycosides	Tannins	Flavonoids	alkaloids	Saponins
CMBEPA	++	-	+++	+	++	+++	++	+++

KEY: +++ = strong positive, ++ = positive, + =weak positive, - =absent

4.2 Acute Oral Toxicity Study of the CMBEPA

4.2.1 Mortality rate and behavior observations in acute oral toxicity determination of CMBEPA

There was no mortality observed neither within the first 48 hour nor the entire 14 days of observation in all groups administered with CMBEPA. On the other hand general behaviour of each animal observed for the first 30 minutes, 1 hour, 4 hours, 24 hours, 48 hours and daily for entire 14 days of acute toxicity were normal. They included pilo-erection, alertness, muscle tone on hind limbs, pain and feeding activity. However respiratory system signs of distress were noted at >2900 mg in the first 24 hours of administration and subsided thereafter.

Table 4.2.1: Shows observations for signs of acute toxicity and mortality rate after administration of CMBEPA

Experiment	Doses (mg)	Observation in hours							
		Immediate	0.5	1	4	24	48		
								Mortality	**Mortality rate (%)**
Phase I	10	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0/3	0
(B.E in 5% DMSO)	100	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0/3	0
	1000	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0/3	0
Control	0	Normal	Normal	Normal	Normal	Normal	Normal	0/3	0
[Distilled water + 5%DMSO]		activity	activity	activity	activity	activity	activity		
Phase II	1600	Normal	Normal	Normal	Normal	Normal	Normal	0/1	0
(CMBEPA in 5% DMSO)	2900	activity RD	activity RD	activity RD	activity RD	activity RD	activity Normal activity	0/1	0
	5000	RD	RD	RD	RD	Normal activity	Normal activity	0/1	0

Key: DMSO - dimethyl sulphoxide
B.E- bark extract of P.africanus
RD- Respiratory distress

4.2.2 Post-mortem results of gross pathology findings on the rats organs during acute toxicity study

The following post-mortem results of gross pathology on the organ of the rats were observed after administration of the CMBEPA, the lung abscess and hyperaemia was revealed in animal administered with 2900 mg and 5000 mg respectively, while liver congestion was observed in animal administered with 2900 mg and 5000 mg. While on the other organs which includes the kidney, spleen, brain, prostate and testis there were no obvious gross pathology observed (Table 4.2.2).

Table 4.2.2: Shows post mortem results of gross pathology findings on the rats organs against the various doses of CMBEPA during acute toxicity study.

	Gross pathology results					
Organ	**Dose (mg/ kg body weight)**					
	10	**100**	**1000**	**1600***	**2900***	**5000***
Kidney	None	None	None	None	None	None
Lungs	None	None	None	None	Lung abscess	Hyperaemia
Liver	None	None	None	None	Liver congestion	Liver congestion
Spleen	None	None	None	None	None	None
Brain	None	None	None	None	None	None
Prostate	None	None	None	None	None	None
Testis	None	None	None	None	None	None

Key: *n=3*
**Dose groups with single rat per group*

Table 4.2.3: Showing various doses of CMBEPA against mean body weight of the rats in day 0, 7 and 14 in acute toxicity study

Phases of experiment	**Dose in (mg/kg body weight)**	**Initial Weight (g) Day 0**	**Weight (g) Day 7**	**Terminal weight (g) Day 14**
Phase I	10	179.67±1.76	213.67±3.93	229.00±1.73
	100	167.67±1.53	204.00±3.06	228.00±1.00
	1000	157.33±2.03	185.00±2.52[a]	201.67±2.91[a]
Control	0	157.00±2.08	212.33±4.91[a]	230.67±3.92 [a]
Phase II	1600*	169.00	180.00	209.00
	2900*	155.00	172.00	189.00
	5000*	156.00	178.00	181.00

Key: *Comparison between groups was performed in columns. Values are presented as mean ± standard error of mean (n=3).*
[a] Significantly different from the control (p <0.05).
**Dose groups with single rat per group (n<3) were not compared due to absence of measure of variability*

In table 4.2.3 the mean rat weights were statistically significant different among the 4 groups of phase 1 administered with varying doses of CMBEPA in day 7 as determined by one-way ANOVA (F (3, 8) = 10.952, p = .003). Further, a Tukey post hoc test revealed that the rat mean weight was significantly lower after administration of CMBEPA at 1000 mg/kg body weight (185.00±2.52 g, p = .025) compared to the control group (212.33±4.91 g). There was no statistically significant difference between the other groups.

In addition, the terminal weights of rats on the 14th day in 4 groups of Phase 1 administered with varying doses of CMBEPA were found also to be statistically significant different using one way ANOVA (F (3,8)=7.534 (P=.010). Further, a Tukey post hoc test revealed that the rat mean weight was significantly lower in group

administered with CMBEPA at a dose of 1000 mg/kg body weight (201.67±2.91g, p=.005) compared to control group. There was no statistically significant difference between the other groups.

Table 4.2.4: Shows the mean absolute weight of organ in acute toxicity study rats against varying doses of CMBEPA

Organ	**Control**	**10 mg**	**100 mg**	**1000 mg**	**1600 mg***	**2900 mg***	**5000 mg***
Brain	1.42 ±0.01	1.32 ±0.0586	1.14 [a] ±0.0153	1.08 [a] ±0.1827	0.99	1.25	1.22
Kidney	2.21±0.0361	2.29±0.0656	2.11±0.0757	2.33±0.1677	2.84	1.88	2.29
Heart	1.19±0.0116	1.28±0.0153	1.12±0.0529	1.16±0.0625	1.21	1.02	0.96
Testis	5.58±0.0721	4.82±0.0833	4.35[a]±0.0802	4.54[a]±0.0462	5.25	4.41	4.82
prostate	0.57±0.05	054±0.0116	0.53±0.0252	0.54±0.0153	0.54	0.53	0.56
Liver	11.36±0.585	10.06±0.327	12.17±0.572	10.39±0.216	12.09	10.17	10.06
Spleen	1.07±0.0473	1.4[a]±0.0462	1.71[a]±0.0322	1.92[a]±0.095	1.95	1.93	1.89
Lungs	3±0.1609	2.78±0.0379	2.6±0.1115	2.01±0.0635	2.62	2.9	2.78

Key: *Comparison between groups was performed in row. Values are presented as mean ± standard error of mean (n=5).*
[a] indicates mean values that were significantly different (p <0.05) from the control using ANOVA inTukey test on post hoc t -test.
**Weight values of organs where n<3 were not compared due to absence of measure of variability.*

One-way ANOVA was conducted to compare the mean absolute weight of organs in rats after administering varying doses of CMBEPA in phase one groups during acute toxicity study (Table 4.2.4).

There was a significant difference of mean brain weights across the four groups, (F (3, 8) = 36.429, p =0.00). On further analysis usingTukey Post hoc t test the mean brain weight were found to decrease statistically in the group administered 100 mg (1.14 ±0.0153g, p=.000) and 1000 mg group (1.08 ±0.1827g, P=.000) when compared to control (1.42 ±0.01). The other comparisons were not significant.

Further, the mean testes weight of the 4 groups were statistically significant according to a one-way ANOVA, F (3, 8) =56.48, p =0.000. Pairwise comparisons of the means using Tukey's Honestly Significant Difference indicated only two significant comparison: the comparison showed that there was a decrease in mean testis weight which was statistically significantly higher in the group administered with CMBEPA

at 100 mg group (4.35±0.0802 g, p=.000) and 1000 mg group (4.54±0.0462 g, p=.000) when compared to control group (5.58±0.0721 g), with a 95% confidence interval of the difference between means. The other comparisons were not significant (Table 4.2.4)

The spleen mean weights of the 4 groups were unequal according to a one-way ANOVA, F (3, 8) = 40.395, p =000. Pairwise comparisons of the means using Tukey's Honestly Significant Difference procedure indicated mean spleen weight was statistically significant higher in the group administered with CMBEPA at 10 mg (1.4±0.0462 g, p=.019), 100 mg (1.71±0.0322 g, p=.000) and 1000 mg group (1.92±0.095 g, p=.000) when compared to the control group (1.07±0.0473 g), with a 95% confidence interval of the difference between means. The other comparisons were not significant (Table 4.2.4)

The mean weights of the following organs were found to have no statistical significant difference at a 95% confidence interval of the difference between means; kidney ((F 3,8)=.956, p=.459), heart ((F3,8)= 2.168, p=.123), prostate ((F3,8)= .340, p=.798), liver ((F3,8)= 3.783, p=.059) and lungs ((F3,8)= 2.447, p=.139) (Table 4.2.4).

4.3 The Gross Morphometric Effects in Restorative Group

4.3.1 The Prostate Dimensional changes in restorative group

The prostate dimensional changes in restorative group are reported in table 4.3.1

Table 4.3.1: Shows the mean prostate dimensions against the doses CMBEPA in restorative group

	Calliper Measurement (mm)					
	Control	**TPI-BPH**	**25 mg**	**50 mg**	**125 mg**	**200 mg**
Length (mm)	15.97±0.297	17.358±0.184[a]	16.828±0.231	16.814±0.365	16.232±0.26	16.12±0.24[b]
Width (mm)	8.688±0.183	9.606±0.118[a]	9.132±0.0652	9.162±0.218	9.118±0.16	8.658±.21
Thickness (mm)	4.188±0.161	4.884±0.067[a]	4.388±0.128	4.308±0.092[b]	4.132±0.064[b]	4.244±0.094[b]

Key: *Comparison between groups was performed in rows. Values are presented as mean ± standard error of mean (n=5).*

[a] indicates values that were significantly different (p <0.05) from the control using ANOVA inTukey test on post hoc t -test

[b] indicates values that were significant different (p <0.05) from the BPH group using ANOVA inTukey test on post hoc t –test

The mean prostate length among the 6 groups were statistically significant using one-way ANOVA, (F (5, 25) =3.906, p=.01), and following a Post hoc comparisons using the Tukey HSD test indicated that the mean prostate length in the TPI BPH control group (17.358±0.1836 mm, p=.036) was significantly high than the control group (15.972±0.297 mm), and comparison between treatment group administered with CMBEPA 200 mg (16.12±0.244 mm, P=.035) was also significantly lower than the TPI-BPH group (17.358±0.1836 mm). However there was no statistical significant difference between the control and 25 mg dose group, 50 mg and 200 mg, nor was there significant difference between the TPI-BPH group, 25 mg, 50 mg and 125 mg dose of the crude methanolic bark extract of *P.africanus* groups, (Table 4.3.1).

In addition, the mean prostate widths were also found to have a statistical significant difference across the six groups as determined by one-way ANOVA (F (5, 24) = 4.383, p = .006). A Tukey post hoc test revealed that TPI-BPH group (9.606±0.118 mm, p=.008) was statistically significantly higher compared to the control group width (9.606±0.118 mm), on the other hand the treatment groups found to be significant lower when administered with a dose 200 mg (8.658±.21 mm, p=.008) compared to TPI-BPH group (9.606±0.118 mm), with a 95% confidence interval of the difference between means. The other comparisons were not significant (Table 4.3.1).

Furthermore, a significant difference in mean prostate thickness among the six groups was noted (F (5, 24) = 6.563, p = .001), and further analysis on Post hoc comparisons using the Tukey t test indicated a statistical differences in 4 groups; There was a statistically significant increase in prostate thickness in TPI-BPH group (4.884±0.067 mm, p=.001) compared the control group (4.188±0.161 mm). On the other hand there was a statistically significant decrease in prostate thickness in group administered with CMBEPA at a dose 50 mg (4.308±0.092, p=.032) group, 125 mg group (4.132±0.064 mm, p=.001) and 200 mg (4.244±0.094 mm, p=.003) when compared with TPI-BPH group (4.884±0.067 mm). However there was no statistical significant difference between the control and treatment groups (Table 4.3.1).

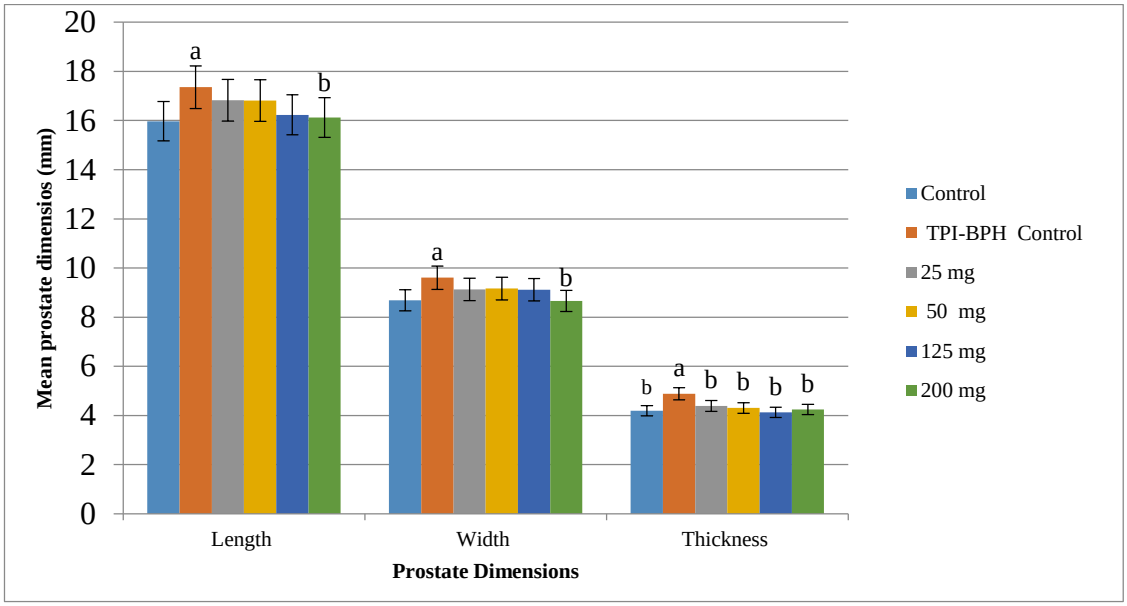

Figure 4.3.1: Mean prostate dimensional changes in restorative group

Key: *[a] indicates values that were significantly different (p <0.05) from the control using ANOVA in Tukey test on post hoc t -test*
[b] indicates values that were significant different (p <0.05) from the BPH group using ANOVA in Tukey test on post hoc t –test

From the figure 4.3.1: the treatment groups in (25 mg, 50 mg, 125 mg and 200 mg) CMBEPA showed a reduced prostate dimensions (length ,width and thickness).

Table 4.3.2: Shows mean prostate weights, prostate body ratio and percentage weight restoration against varying doses of CMBEPA in restorative group

	Control	TPI-BPH	25 mg	50 mg	125 mg	200 mg
Prostate weight (g)	0.416±0.015	0.498 ±0.012[a]	0.49±0.01[a]	0.488±0.011[a]	0.47±0.016	0.428[b]±0.03
PBR (%)	0.1518±0.005	0.1959±0.005	0.1922±0.003	0.1788±0.004	0.1745±0.006	0.171±0.01
Weight restoration (%)	100	0	9.756	12.195	34.146	85.366

Key: *Comparison between groups was performed in row. Values are presented as mean ± standard error of mean (n=5).*
[a] indicates values that were significantly different (p <0.05) from the control using ANOVA in Tukey test on post hoc t -test
[b] indicates values that were significant different (p <0.05) from the BPH group using ANOVA in Tukey test on post hoc t –test

The means prostate weights of the 6 groups were unequal according to a one-way ANOVA, (F (5, 24) = 4.902, p = 0.003) (Table 4.3.2). Pairwise comparisons of the means using Tukey's Honestly Significant Difference procedure indicated three significant comparison. The mean was found to be statistically significantly higher in

the TPI-BPH group (0.416±0.015 g, p=.013), 25 mg group (0.49±0.01 g, p=.001) and 50 mg group (0.488±0.011 g, p=.033) when compared to the control group (0.416±0.015 g). On the other hand treatment with CMBEPA significantly decreased the prostate weight at a dose 200 mg when compared with TPI-BPH group (0.416±0.015 g). All the other group comparisons were found not statistically different (Table 4.3.2).

In addition, the prostate body ratio means were found to be unequal among the six groups, F (5, 24) =7.466, P=.000). Following a post hoc HSD t test comparison prostate body ratio was significantly higher in the TPI-BPH group (0.1959±0.005 %, p=.013), 25 mg group (0.1922±0.003 %, p=.029) and 50 mg group (0.1788±0.004 %, p=.036), when compared to the control group, while on the other hand PBR was statistically significantly decrease in the treatment administered with CMBEPA at dose of (0.1518±0.005 %, p=.044) when compared to TPI-BPH group (0.1959±0.005 %), with a 95% confidence interval of the difference between means. The comparisons in other group were not statistically significant (Table 4.3.2).

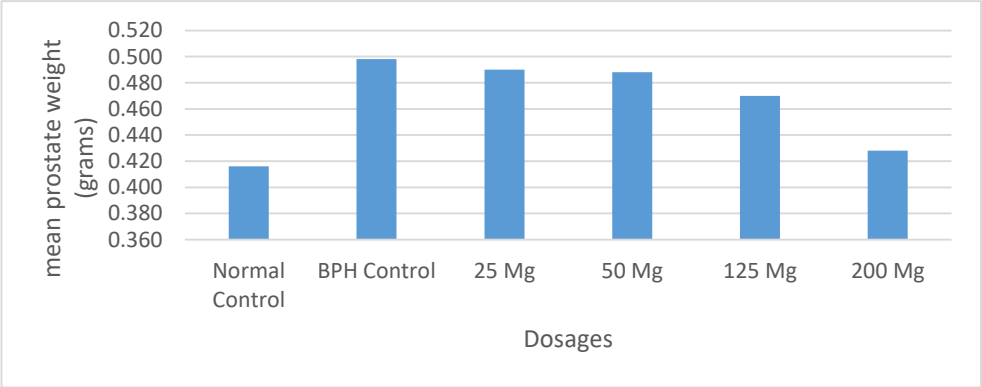

Figure 4.3.2: Bar graph showing the mean prostate weight against the dosages of CMBEPA in the restorative group

As shown in figure 4.3.2 the prostate weight is highest in TPI-induced group and the prostate weight decreases with increase of dose of the methanolic bark extract of *P.africanus*.

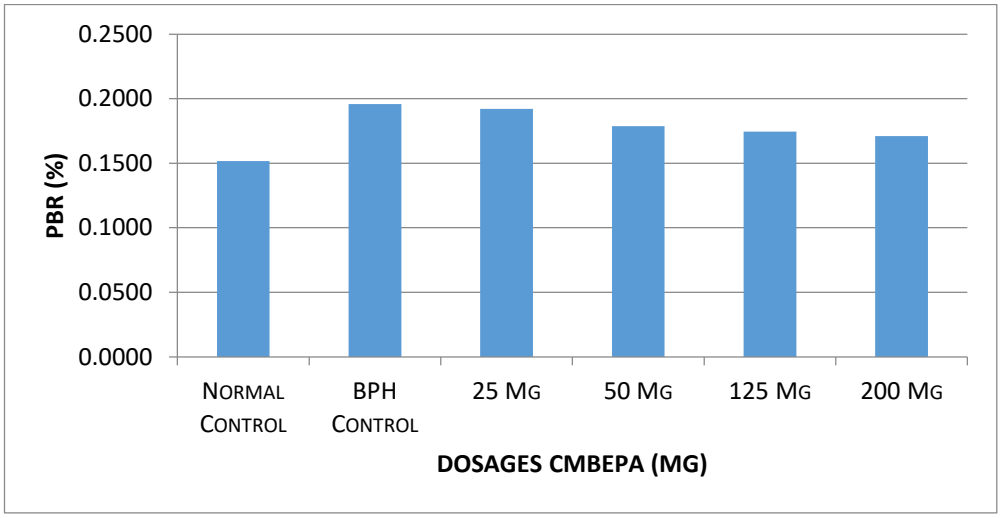

Figure 4.3.3: Bar graph showing the mean prostate body ratio against the varying doses of CMBEPA in restorative group

It was in figure 4.3.3 above the PBR was the highest in TPI-BPH group and lowest in control group, as the dose of CMBEPA increased the PBR decreased.

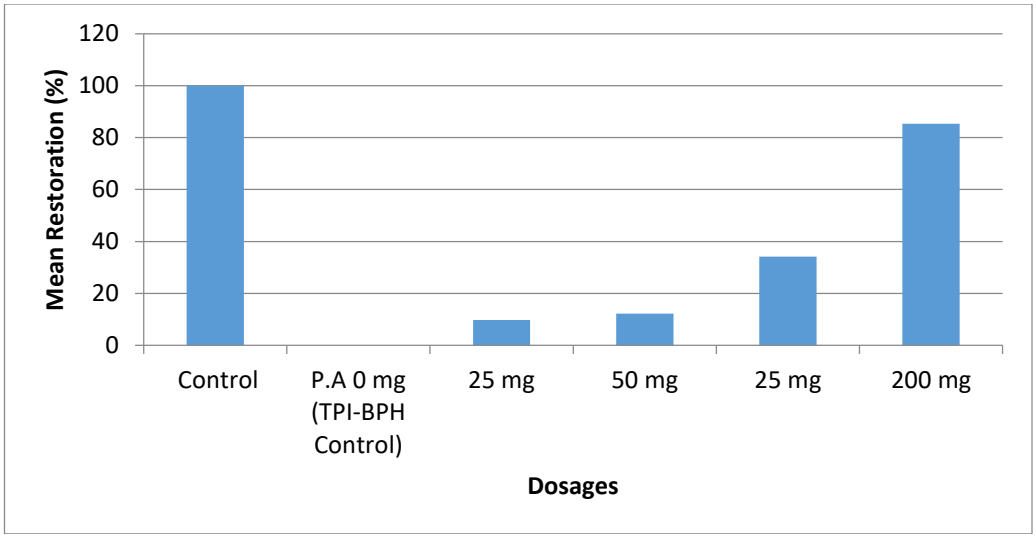

Figure 4.3.4: Bar graph showing percentage restoration of prostate weight against the various doses of CMBEPA in restorative group

From the figure 4.3.4, shows that there was no restoration of prostate weight in TPI-BPH group, while on the other hand CMBEPA treated groups (25 mg, 50 mg, 125 mg and 200 mg) showed that restoration increased with an increase of the dose of the CMBEPA as follows 9.756%, 12.195%, 34.146% and 85.366 % respectively**.**

4.3.2 The prostate dimensional changes in inhibitory group

Table 4.3.3: Showing mean prostate dimensions against the various doses of CMBEPA in the inhibitory group

	Calliper Measurement in mm					
	Control	**BPH**	**25 mg**	**50 mg**	**125 mg**	**200 mg**
Length	11.738±1.563	14.705±0.580[a]	13.795±1.121	13.445±1.612	11.78±1.383[b]	11.618±1.072[b]
Width	9.185±1.389	11.244±0.094[a]	10.975±0.685	10.993±1.357	9.845±0.823[b]	9.905±0.494[b]
Thickness	3.253±0.351	4.08±.436[a]	3.798±.341	3.408±0.706[b]	3.33±0.485[b]	3.308±0.524[b]

Key: *Comparison between groups was performed in row. Values are presented as mean ± standard error of mean (n=4)*
P.A-Prunus africanus; TP-Testosterone Propionate; TPI-BPH-Testosterone Propionate induced benign prostatic hyperplasia
[a] indicates values that were significantly different (p <0.05) from the control
[b] indicates values that were significant different (p <0.05) from the BPH group

When the mean lengths of the prostate among the 6 groups were tested using one-way ANOVA they were statistically different, (F (5, 24) = 3.849, p =.019). Pairwise comparisons of the means using Tukey's Honestly Significant Difference procedure indicated three statistical significant comparison: TPI- BPH group (11.738±1.563mm) were significantly higher than in the control group (14.705±0.580mm), TPI-BPH group lengths were significant higher than 125 mg, and so was 200 mg, at 95% confidence interval of the difference between means. The other comparisons were not significant (Table 4.3.3).

The means prostate widths in the 6 groups were unequal according to a one-way ANOVA, (F (5, 24) =3.52, p = .012). Pairwise comparisons of the means using Tukey's Honestly Significant Difference procedure indicate three significant comparison: TPI- BPH group (11.244±0.094 mm, p=.000) reported that the were significant increase in prostate width than in the control group (9.185±1.389 mm), also the width mean were significantly higher TPI-BPH group (11.244±0.094 mm) than the treatment group administered with 125 mg (9.845±0.823 mm, p=.034) and 200 mg, (9.905±0.494mm, p=.004) with a 95% confidence interval of the difference between means. The other comparisons were not significant (Table 4.3.3).

Mean prostate thickness were found not differ across the groups (Table 4.3.3).

Table 4.3.4: Shows the mean prostate weight, PBR and prostate weight against the varying dose CMBEPA in inhibitory group

	Caliper measurement					
	Control	**BPH**	**25 mg**	**50 mg**	**125 mg**	**200 mg**
Prostate wt (g)	0.236±0.0125	0.38±0.0389[a]	0.297±0.029[b]	0.272±0.57[b]	0.24±0.042[b]	0.231±0.011[b]
PBR (%)	0.1965±0.0012	0.24±0.005[a]	0.231±0.009[b]	0.208±0.003[b]	0.21±0.006[b]	0.211±0.002[b]
Wt. inhibition (%)	0	0	19.954	40.133	68.433	65.668

Key: *Comparison between groups was performed in row. Values are presented as mean ± standard error of mean (n=5).*

[a] indicates values that were significantly different (p <0.05) from the control using ANOVA in Tukey test on post hoc t -test

[b] indicates values that were significant different (p <0.05) from the BPH group using ANOVA in Tukey test on post hoc t –test

The mean prostate weight in the six group was statistically significant using one way ANOVA (F (5, 24) = 21.95, p = 0.000). Following a post hoc t test 5groups were statistically different; in the control group (0.236±0.0125g), was significantly lower than in the BPH group (0.38±0.0389g, p=.000), 25 mg (0.297±0.0289g, p=.031), group prostate was lower than BPH group, 50 mg (0.272±0.57g, p=.000) was lower than BPH group, 125 mg (0.24±0.042 g) was lower than BPH group, 200 mg (0.231±0.0113g) was lower than BPH group.

The mean PBR in the six groups was statistically different following one way ANOVA, F (5, 24) =7.466, P=.000). Following Tukey post hoc t test 4 statistical difference was note; PBR in control group (0.1965 ± 0.0012%, p=.000) was lower compared with the BPH control group (0.2399±0.005 %) , while on the hand TPI- BPH group was higher when compared with treatment groups, 25mg group (0.2312 ± 0.009%, p=.003) and 50 mg group (0.2084 ± 0.0033%, p=.032), 125 mg (0.2102 ± 0.006 %, p=.001), 200 mg (0.2114±0.002 %, p=.000).

Weight inhibition was found to decrease with increase with dose of CMBEPA as follows 19% in 25 mg group, 40.133% in 50 mg group, 68.433% in 125 mg and 65.668% in 200 mg group.

4.4 Histostereological Restorative effects of CMBEPA

4.4.1 Assessment of Precision on Stereological Parameters in the restorative group.

Coefficient of Error (CE) was calculated in order to ensure precision of quantitative stereological parameters. It was calculated for both restorative group (Table 4.4.1).

Table 4.4.1: Shows animal codes against values of coefficient of error (CE) for total prostate volume, epithelium stromal volume and acini volume in the restorative groups.

Animal codes	Total volume CE	Epithelium CE	Stromal CE	Acinar CE
In1	0.0290	0.0228	0.0255	0.0263
In2	0.0309	0.0225	0.0280	0.0289
In3	0.0312	0.0247	0.0267	0.0275
In4	0.0297	0.0239	0.0266	0.0274
In5	0.0306	0.0255	0.0284	0.0293
In6	0.0317	0.0348	0.0169	0.0174
In7	0.0317	0.0395	0.0141	0.0145
In8	0.0340	0.0418	0.0150	0.0155
In9	0.0339	0.0407	0.0153	0.0158
In10	0.0341	0.0420	0.0133	0.0137
In11	0.0267	0.0219	0.0245	0.0253
In12	0.0272	0.0222	0.0232	0.0239
In13	0.0313	0.0250	0.0271	0.0280
In14	0.0317	0.0258	0.0281	0.0290
In15	0.0265	0.0236	0.0215	0.0222
In16	0.0299	0.0217	0.0272	0.0280
In17	0.0328	0.0272	0.0280	0.0289
In18	0.0302	0.0288	0.0261	0.0269
In19	0.0272	0.0218	0.0246	0.0254
In20	0.0269	0.0230	0.0222	0.0229
In21	0.0330	0.0276	0.0289	0.0298
In22	0.0288	0.0232	0.0259	0.0267
In23	0.0293	0.0238	0.0254	0.0262
In24	0.0298	0.0239	0.0255	0.0263
In25	0.0301	0.0243	0.0281	0.0290
In26	0.0324	0.0272	0.0278	0.0287
In27	0.0311	0.0262	0.0258	0.0267
In28	0.0354	0.0294	0.0294	0.0304
In29	0.0336	0.0298	0.0278	0.0287
In30	0.0323	0.0265	0.0275	0.0284
Mean CE	**0.0308**	**0.0274**	**0.0245**	**0.0253**

As shown in table 4 .4.1 the upper limit for coefficient of error (CE) in total prostate volume, epithelium, stromal and acinar was 0.354, 0.42, 0.29 and 0.304 respectively, while the lower limit were 0.0265, 0.0236, 0.0215 and 0.0222 respectively. The mean CE for total prostate, epithelium, stromal and acinar was 0.308, 0.0274, 0.0245 and 0.0253 respectively.

4.4.2 Assessment of the level of agreement of the total prostate volumes

Table 4.4.2: Showing Intraclass correlation coefficient of Archimedes, Cavalieri and plainmetry methods used in determining total volume of the prostate

Inter-Item Correlation Matrix			
	Archimedes	Cavarieli	Plainmetry
Archimedes	1.000	.762	.543
Cavalieri	.762	1.000	.805
plainmetry	.543	.805	1.000

A high degree of reliability was found between Archimedes volume and Cavalieri volume measurements (.762). The average measure ICC was .585 with a 95% confidence interval from .356 to .774 (F (23, 46) = 5.233, p=.000) (Table 4.4.2).

Table 4.4.3: Showing prostate structure volumes against various doses of CMBEPA in Restorative group.

	Groups					
	Control	**TPI**	**25 mg**	**50 mg**	**125 mg**	**200 mg**
Epithelium Vol	0.114±.007	0.251±0.017[a]	0.138±0.013[b]	0.122±0.001[b]	0.128±0.005[b]	0.137±0.007[b]
Stromal Vol	0.11±0.005	0.193±0.012[a]	0.125±0.008[b]	0.114[b]±0.007	0.11[b]±0.004	0.118[b]±0 .006
Acinar Vol	0.138±0.003	0.147±0.019	0.162±0.015	0.142±0.005	0.144±0.005	0.147±0.003
Prostate Vol	0.362±0.011	0.592[a]±0.031	0.425[b]±0.036	0.378[b]±.019	0.382[b]±.012	0.402[b]±0.014

Key: Comparison between groups was performed in row.

P.A-Prunus africanus; TP-Testosterone Propionate; TPI-BPH-Testosterone Propionate induced benign prostatic hyperplasia; Vol - Volume

[a] indicates values that were significantly different (p <0.05) from the control

[b] indicates values that were significant different (p <0.05) from the BPH group

The mean prostate epithelium volume was found to have a statistical significant difference among the treatment groups. (F (5, 24) = 103.54, p=0.000), when Tukey post hoc test was performed the difference were found between: TPI group

(0.251±0.017mmm^3, p=.001), which was statistically higher when compared to control group (0.114±.007mm^3). While in treatment group there was a significantly decrease in epithelium volumes in group administered with CMBEPA at dose of 25 mg (0.138±0.013mm^3, p=.00), 50 mg (0.122±0.001mm^3, p=.000), 125 mg (0.128 ± 0.005 mm^3, p=.000) and 200 mg (0.137±0.007 mm^3) (Table 4.3.3).

In addition, the mean prostate stromal volume were found to be statistically significantly different among the group (F (5, 24) = 302.548, p=0.000), when Tukey post hoc test was performed the TPI group (0.193±0.012 mm^3, p=.000) was greater compared to control (l0.11±0.005 mm^3, p=.000). While in the treatment group there was a significant decrease in volumes as the CMBEPA increased (Table 4.3.3).

It was also revealed that the total prostate volume was statistical significant difference across the group (F (5, 24) = 56.82, p=0.000). Post hoc tests also revealed that the prostate volume in control group (0.362±0.011 mm^3, p=.000) was significantly lower when compared to TPI, also in treatment group it was observed that prostate volume decreased as the as CMBEPA increased as follows; 25 mg (0.425±0.036 mm^3 p=.000), 50 mg (0.378±.019 mm^3, p=.000) 125 mg (0.382±.012 mm^3, p=.00 and 200 mg (0.402±0.014mm^3, p=.001), these groups were also found to have significantly lower volumes when compared to TPI group (0.402 mm^3±0.014).

Furthermore, the prostate acinar volume was found not be statistically significant across the groups (F (5, 24) = 0.5, p=0.551) (Table 4.4.3).

Table 4.4.4: Showing Prostate structures volume densities against of various doses of CMBEPA in the restorative group.

	Control	BPH-TPI	25 mg	50Mg	125Mg	200Mg
Epithelium Vv %	31.527±1.145	42.521±2.593[a]	32.47±0.509[b]	32.27±1.151[b]	33.576±0.74[b]	34.03 ±1.007[b]
Stromal Vv %	30.353±0.933	32.623±1.574[a]	29.357±0.689	30.177±0.497[b]	28.857±0.188[b]	29.31 ±0.955[b]
Acinar Vv %	38.12±0.854	24.856±2.11[a]	38.173±0.6 [b]	37.553±0.946 [b]	37.567±0.767 [b]	36.662±0.51 [b]

Key: *Comparison between groups was performed in row*
P.A-Prunus africanus; TP-Testosterone Propionate; TPI-BPH-Testosterone Propionate induced benign prostatic hyperplasia
[a] *indicates values that were significantly different (p <0.05) from the control*
[b] *indicates values that were significant different (p <0.05) from the BPH group*

In Table 4.4.4 the mean epithelium volume density was found to be statistically significant difference among the 6 groups, (F (5, 24) = 36.76, p = 0.000), following

the post hocTukey t test the differences found in TPI group (42.521±2.593%), which was significantly higher than that control group (31.527±1.145%). On the other hand it was also noted that in treatment group the mean epithelial volume densities were significantly lower as the dose of CMBEPA increased; 25 mg (32.47±0.509%), 50 mg (32.27±1.151%), 125 mg (33.576 ±0.74%), and 200 mg (34.03 ±1.007%), when compared with TPI-BPH group (42.521±2.593%).

There was a significant effect on prostate stromal volume density across the group, F (5, 24) = 8.76, p = 0.000. Post hoc comparisons using theTukey HSD test indicated that in control group (30.353±0.933%) lower when compared to TPI (32.623±1.574, p=.000%), this was also noted in treatment groups, 50 mg (30.177±0.497%), 125 mg (28.857±0.188, p=.000%), 200 mg (29.31 ±0.955, p=.000%) when compared to TPI. However, 25 mg (29.357±0.689, p=.000%) it did not significantly differ from TP group (Table 4.4.4).

The mean acinar volume density was found to be statistically significant across the groups F (5, 24) = 90.33, p=0.000). Post hoc comparisons using the Tukey HSD test indicated that in control group (38.12±0.854%, p=.000) lower when compared to TPI (24.856±2.11%, p=.001), this was also noted in treatment groups, 25 mg (38.173±0.604%, p=.001), 50 mg (37.553±0.946%, p=.000), 125 mg (37.567±0.767, p=.000 %), 200 mg (36.662±0.506%, p=.008) when compared to TPI (24.856±2.11%) (Table 4.4.4).

4.4.3 The qualitative histomorphological restorative effects of CMBEPA

A segment of normal prostate tissue in control group (Figure 4.4.1a) stained with HE showed minimal thickness of the intraglandular smooth muscle fibres, and a well-organized simple glandular columnar epithelium with minimal papillary folds , also a few luminal acini had corpora amylacea. The photomicrograph of TPI BPH group with no treatment (Figure 4.4.1b) showed extensive glandular hyperplasia with thickened intraglandular epithelium. The interglandular smooth muscle exhibited a thin outline with minimal vacoulation. A considerable number of acini showed corpora amalycea. (Figure 4.4.2 d) histology of prostatic tissue treated with a dose of 50 mg of CMBEPA. The section indicated an extensive shrinkage of interglandular smooth muscle tissue

with decreased number of convolutions in the acini. The number of corpora amylacea appeared decreased in terms of number and size. The photomicrograph of prostatic tissue (Figure 4.4.3 e) treated with a dose of 125 mg of CMBEPA indicated a significant decrease in thickness of interglandular smooth muscle tissue. The intraglandular epithelium appeared thin with reduced convolution while corpora amylacea. Marked reduction in the interglandular stromal thickness and the glandular epithelial lining showed a marked shrinkage with no convolution while the luminal acini appeared enlarged (Figure 4.4.3f).

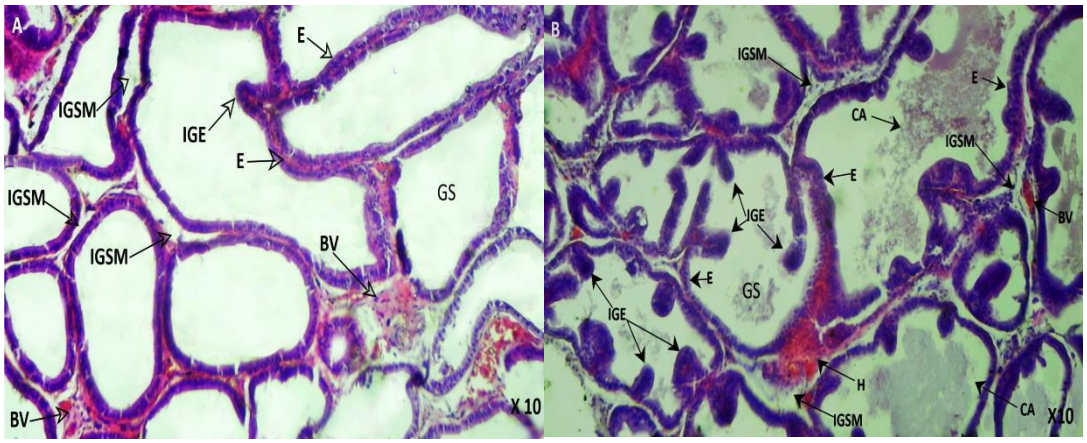

Figure 4.4.1: Photomicrograph A and B showing histomorphology of the prostate of normal control and BPH control respectively in Restorative group.

KEY: IGSM- intraglandular smooth muscle fibre, IGS-intraglandular stromal.CA- Corpora amylacea. IGE- intraglandular epithelium

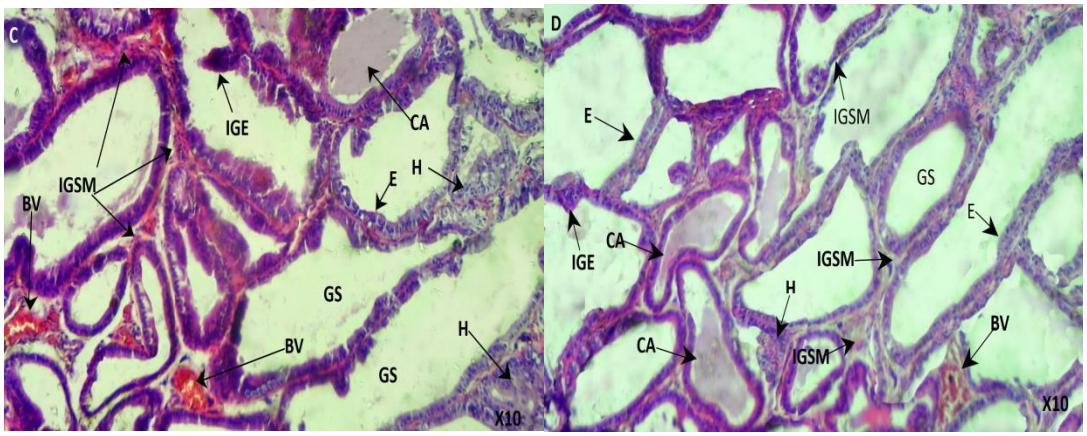

Figure 4.4.2: Photomicrograph C and D showing the histomorphology of the prostate in rat administer with dose 25 mg and 50 mg of *P.africanus* respectively in restorative group control.

KEY: IGSM- intraglandular smooth muscle fibre, IGS-intraglandular stromal.CA- Corpora amylace. IGE- intraglandular epithelium

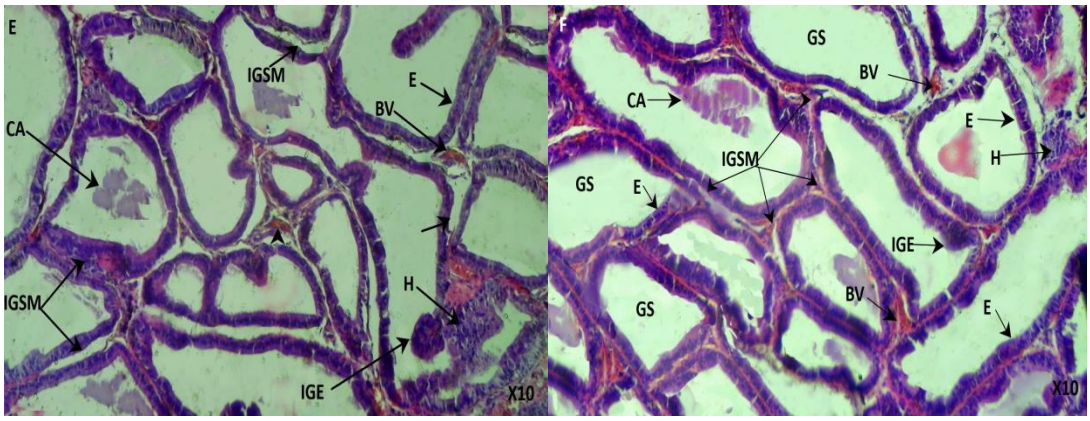

Figure 4.4.3: Photomicrograph E and F showing histomorphology of the prostate in rat administer with dose 25 mg and 200 mg *P.africanus* respectively in restorative group control.

KEY: IGSM- intraglandular smooth muscle fibre, IGS-intraglandular stromal.CA- Corpora amylace. IGE- intraglandular epithelium.

4.5 The Histostereological Inhibitory Effects of CMBEPA

Table 4.5.1: Shows animal codes against values of coefficient of error (CE) for total prostate volume, epithelium stromal volume and acini volume in the inhibitory group.

Animal code	Total volume CE	Epithelium CE	Stromal CE	Acinar CE
R1	0.02571	0.02004	0.02457	0.024121
R2	0.02736	0.01978	0.02700	0.026509
R3	0.02765	0.02169	0.02570	0.025236
R4	0.02635	0.02100	0.02562	0.025152
R5	0.02708	0.02237	0.02737	0.026873
R6	0.02809	0.03058	0.01628	0.015988
R7	0.02809	0.03464	0.01356	0.013309
R8	0.03012	0.03668	0.01446	0.014194
R9	0.03004	0.03570	0.01472	0.014448
R10	0.03018	0.03688	0.01280	0.01257
R11	0.02370	0.01923	0.02367	0.023236
R12	0.02408	0.01948	0.02236	0.021952
R13	0.02772	0.02195	0.02616	0.025685
R14	0.02810	0.02260	0.02711	0.026618
R15	0.02348	0.02068	0.02078	0.0204
R16	0.02651	0.01909	0.02619	0.025709
R17	0.02909	0.02391	0.02701	0.026521
R18	0.02680	0.02525	0.02515	0.024691
R19	0.02407	0.01916	0.02369	0.023261
R20	0.02381	0.02022	0.02141	0.021018
R21	0.02920	0.02422	0.02786	0.027358
R22	0.02548	0.02038	0.02496	0.024509
R23	0.02595	0.02085	0.02448	0.024036
R24	0.02639	0.02094	0.02462	0.02417
R25	0.02669	0.02133	0.02707	0.026582
R26	0.02870	0.02385	0.02680	0.026315
R27	0.02757	0.02298	0.02491	0.024461
R28	0.03139	0.02578	0.02836	0.027842
R29	0.02981	0.02614	0.02678	0.026291
R30	0.02863	0.02330	0.02651	0.026024
Mean CE	**0.02726**	**0.02402**	**0.02360**	**0.02317**

As shown in table 4. 5. 1 the upper limit for coefficient of error (CE) in total prostate volume, epithelium, stromal and acinar was 0.031, 0.026, 0.029 and 0.028 respectively, while the lower limit were 0.024, 0.019, 0.02236 and 0.02195 respectively. The mean CE for total prostate, epithelium, stromal and acinar were 0.02726, 0.02402, 0.02360 and 0.02317 respectively.

4.5.1 The histostereology Inhibitory effect of CMBEPA

Table 4.5.2: Showing histostereological prostate structure volumes against the dosages of CMBEPA in the inhibitory group.

	Groups					
	Control	**TPI-BPH**	**TP+CMBPA 25 mg**	**TP+CMBEPA 50 mg**	**TP+CMBEPA 125 mg**	**TP+CMBEPA 200 mg**
Epithelium Vol (mm)	0.075±0.005	0.166±0.006[a]	0.097±0.007[b]	0.088±0.006[b]	0.081±0.004[b]	0.079±0.002[b]
Stromal Vol (mm)	0.071±.002	0.124±0.003 [a]	0.086±0.003[b]	0.085±0.005[b]	0.068±0.004[b]	0.067±0.004 [b]
Acinar Vol (mm3)	0.091±0.004	0.093±0.006	0.115±0.004 [a]	0.105±0.007	0.091±0.006	0.086±0.009
Prostate Vol (mm3)	0.236±0.011	0.38±0.011[a]	0.297±0.004[b]	0.272±0.042[b]	0.24±0.014[b]	0.231±0.009[b]

Key: Comparison between groups was performed in row
P.A-Prunus africanus; TP-Testosterone Propionate; TPI-BPH-Testosterone Propionate induced benign prostatic hyperplasia
[a] indicates values that were significantly different (p <0.05) from the control
[b] indicates values that were significant different (p <0.05) from the BPH group

The study that found that the mean epithelial volume were statistically significantly different among the groups after performing a one way ANOVA (F (5,24) =51.22, P=.000), following a Post hoc comparisons, using the Tukey HSD test it indicated that the mean epithelial volume were statistically significant higher in TPI group (0.166±0.006 mm^3, p=.000) when compare with the control (0.075±0.005 mm^3). On the other hand the mean epithelium volume was observed to decrease in group administered with CMBEPA at a dose groups 25 mg (0.097±0.007 mm^3, p=.000), 50 mg (0.088±0.006 mm3, p=.000), 125 mg (0.081±0.004 mm^3p=.000) and 200 mg (.079±0.002 mm^3 ,p=000), when compared with TPI group (table 4.5.2).

In addition, the stromal volume was found to have a significant difference among the group, (F (5, 24) =36.75, P=.000), following a Tukey post hoc t test the differences were found to be significant high in TP group (0.124±0.003 mm^3, p=.000) when compared with the control group (0.071±.002 mm^3). On the other hand the mean stromal volume was found have statistical significant decrease in the treatment group administered CMBEPA at a dose of 25 mg (0.086±0.003 mm^3, p=.000), 50 mg group (0.085±0.005 mm^3, p=.000), 125 mg, (0.068±0.004 mm^3, p=.000) and 200 mg group (0.067±0.004 mm^3, p=.000) when compared with TP group.

In the acinar volume it was found to have a significant difference across the groups, (F (5, 24) = 4.71, P=.000), following a Tukey post hoc t test the differences were only

found between one group; 50 mg group (0.105±0.007 mm, p=.000) which was statistically lower when compared with control (0.091±0.004), while the other group were found not be statistically significant.

The mean prostate volume was found to have a significant difference across the groups (F (5, 24) = 51.22, p = .000). Following a Post hoc comparisons, using theTukey HSD test indicated that in control group (0.236±0.011 mm^3) was lower when compared to TPI (0.38±0.011 mm^3), these volumes were also noted to be low in treatment groups 25 mg (0.297±0.004 mm^3), 50 mg (0.272±0.042 mm^3), 125 mg (0.24±0.014 mm^3) and 200 mg (0.231±0.009 mm^3), when compared to TPI.

Table 4.5.3: Showing the prostate structure densities against the dosages of CMBEPA in the inhibitory group.

	Control	TPI-BPH	TP + 25 mg CMBEPA	TP+50 mg CMBEPA	TP +125 mg CMBEPA	TP+200 mg CMBEPA
Epithelium Vv %	31.923±2.13	44.012±2.145 [a]	32.38±0.564 [b]	31.863±1.841 [b]	33.603±0.82 [b]	34.105±1.12 [b]
Stromol Vv %	29.787±1.51	32.208±2.162	28.954±0.75 [b]	30.497±1.562	28.383±0.21 [b]	28.876±1.067 [b]
Acinar Vv %	38.291±0.98	23.78±2.497 [a]	38.666±0.67 [b]	37.64±0.351 [b]	38.014±0.84 [b]	37.02±0.552 [b]

Key: Comparison between groups was performed in row
P.A-Prunus africanus; TP-Testosterone Propionate; TPI-BPH-Testosterone Propionate induced benign prostatic hyperplasia; Vv- Volume density
:[a] indicates values that were significantly different (p <0.05) from the control
[b] indicates values that were significant different (p <0.05) from the BPH group

The mean epithelial volume density was found to have a significant difference across the groups in the control group F (5, 24) = 40.36, p = 0.000. Following a Post hoc comparisons, using theTukey HSD test indicated that in control group (31.923±2.13 %) was lower when compared to TPI (44.012±2.145%, p=.000), these volumes were also noted to be low in treatment groups 25 mg (32.38±0.564 %), 50 mg (31.863±1.841 %), 125 mg (38.014±0.84%, p=.000) and 200 mg (37.02±0.552 %, p=.000), when compared to TPI (44.012±2.145%, p=.000).

In the stromal volume density was found to have a significant difference across the group, F (5, 24) =7.68, P=.000). Following a Tukey post hoc t test the differences were found to be significant low in the control group (29.787±1.51, p=.000%) compared with the TP (32.208±2.162%) group, this was also case in in 25 mg (38.666±0.67,

p=.000%), 50 mg group (37.64±0.351, p=.000%) 125 mg, (38.014±0.84, p=.000%) and 200 mg group (37.02± 0.552, p=.000%).

In the acinar volume density it was found to have a significant difference across the group, F(5,24) = 117.4, P=.000). Following a Tukey post hoc t test the differences were found to be significantly (low) in the control group (23.78±2.497%, p=.000) compared with the TP group (38.666 ± 0.67%, p=.000), this was also noted to increase in the following treatment groups in 25 mg (37.64±0.351%, p=.451) ,50 mg group (38.014 ± 0.84%, p=.000) 125 mg, (38.014±0.84%, p=.000) and 200 mg group (37.02±0.552, p=.000%).

4.5.2 Qualitative histological findings in the inhibitory Group

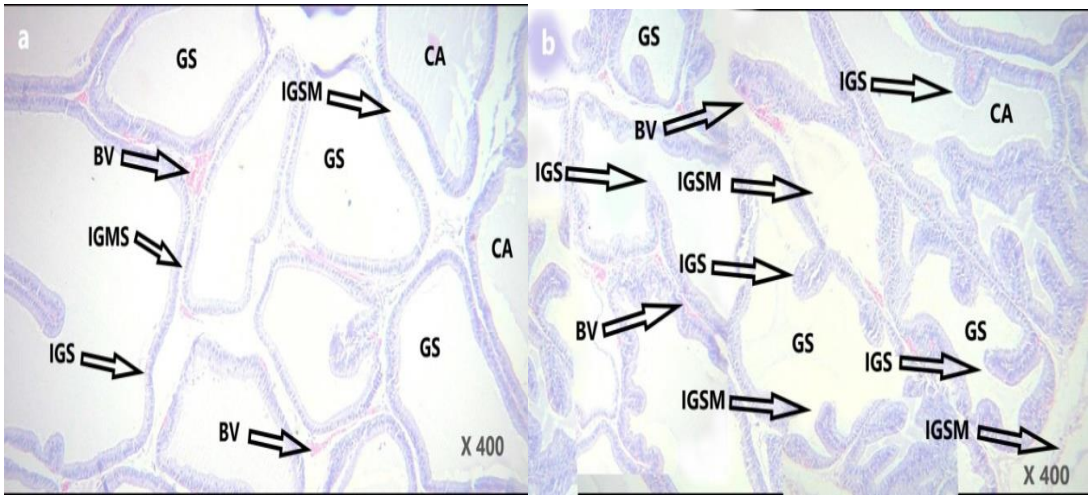

Figure 4.5.1: Photomicrograph a and b showing histomorphology of the prostate of normal control and BPH control respectively in the inhibitory group.

(a) IGSM- intraglandular smooth muscle fibres in control group exhibiting minimal thickness. IGS-intraglandular stromal in control group with minimal folding and composed of simple columnar epithelium.CA- Corpora amylacea control group is minimal. (A) SM- intraglandular smooth muscle fibres in normal control group

(b)IGSM-interglandular smooth muscle tissue in TPI BPH group exhibiting decrease in thickness, IGS-intraglandular epithelium is thickened with decreased number and length of the papillary folds in the acini. CA-corpora amylacea observed considerable number of acini (Figure 4.5.1).

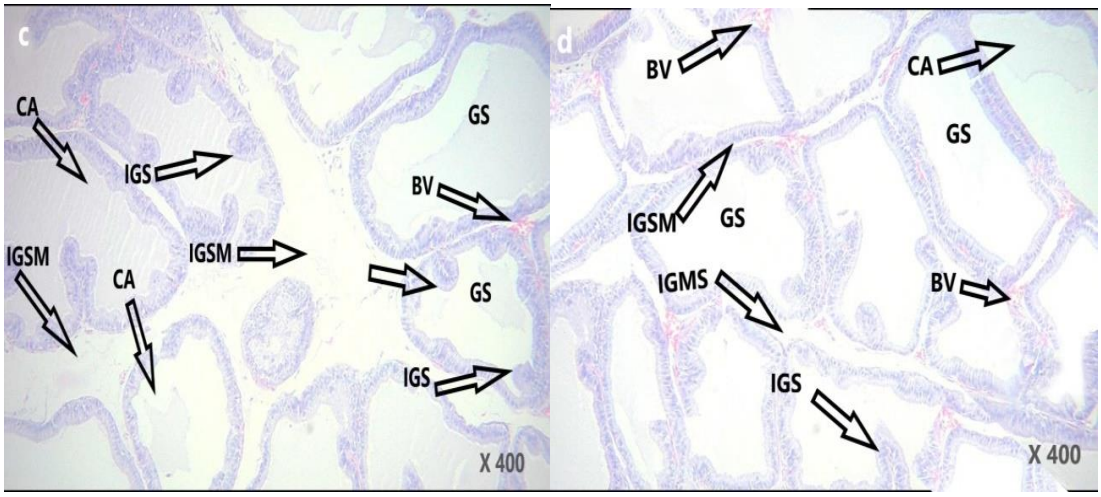

Figure 4.5.2: Photomicrograph c and d showing prostate histomorphology of the group administered with CMBEPA at a dose 25 mg and 50 mg respectively in the inhibitory group.

(c)IGSM-interglandular smooth muscle tissue in CMBEPA 25 mg group exhibiting some increase in thickness, IGS-intraglandular epithelium is thickness reduced with considerable decrease in number and length of the papillary folds in the acini. CA-corpora amylacea observed only on few number of acini. (Figure 4.5.2 c)

(d)IGSM-interglandular smooth muscle tissue in CMBEPA 50 mg group exhibiting decrease in thickness, IGS-intraglandular epithelium is thickness decreased with reduced number and length of the papillary folds in the acini.CA-corpora amylacea observed in a few acini. (Figure 4.5.2 d)

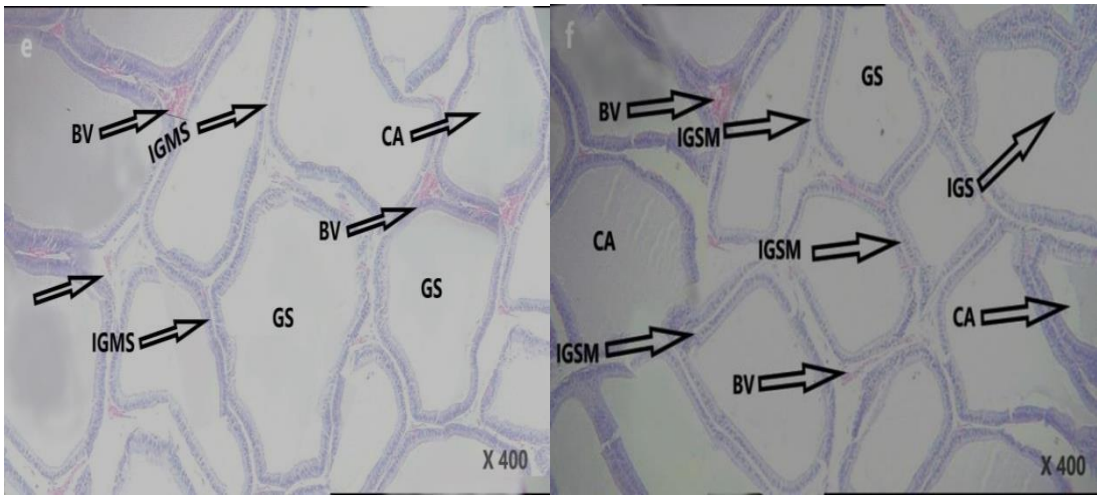

Figure 4.5.3: Photomicrograph e and f showing prostate histomorphology of the group administered with CMBEPA at a dose 125 mg and 200 mg respectively in the inhibitory group.

(e) IGSM-interglandular smooth muscle tissue in CMBEPA 125 mg group exhibiting a decrease in thickness, IGS-intraglandular epithelium is thin with reduced number and length of the papillary folds in the acini. CA-corpora amylacea in a few numbers of acini (Figure 4.5.3 e).

(f) IGSM-interglandular smooth muscle tissue in P.A 200 mg group exhibiting decrease in thickness, IGS-intraglandular epithelium is decreased with no papillary folds in the acini. CA-corpora amylacea exhibited in a number of acini (Figure 4.5.3 f).

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 The qualitative phytochemical constituents of the CMBEPA

This study established qualitative phytochemical constituents of the crude methanolic bark extract of *P.africanus* obtained from Mukurwe-ini in Mt. Kenya region. The various proportions of phytochemical constituents was determined by amount of frothing and the colour intensity of test reactions. The qualitative phytochemical screening in this study established that triterpenes, flavonoids and saponins reactions were strong positive, while alkaloids, tannins and carbohydrates were positive, these results conformed to a previous studies which reported the presence of tannins, triterpenes, glycosides, alkaloids, saponins, flavonoids and carbohydrates in *P.africanus* bark (Chepkoech, 2014; Komakech and Kang, 2019; Kumar *et al.*, 2012). The reaction colour intensity in triterpenes was found to be strong positive in this study, these findings were corncordant with a study by Nyamai *et al.* (2015), whose findings established concentration of triterpenes to be strong positive in Mt. Kenya region. The high amount of triterpenes could be associated with restorative and inhibitory effects established in the current study. These restorative and inhibitory effects could be attributed to the activity of triterpenes on the glucosyl transferase which has anti-edematous effects to the prostate gland.

In this study the proportion of the flavonoids were also observed to be relatively higher as compared to alkaloids based on the amounts of deepness in the colourations. This high proportion of flavonoids is in tandem with a previous study by Nyamai *et al*. (2015), whose findings demostrated high proportions of flavonoids of bark extract of *P.africanus* from Mt. Kenya region. These restorative and inhibitory activity observed in this study could be attributed to flavonoids which have been shown to have antioxidant and anti-BPH properties, this is due to its ability to inhibit several cyclin dependent kinases by blocking the cell cycle (Kadu *et al.*, 2012).

The saponins in the current study were also found to have a strong positive reaction, this concurred with a previous study by Kadu *et al.* (2012), which indicated present of saponins in high amounts. Previous studies have shown that saponins have antioxidant,

antitumor and antiproliferative effects in body cell hence it's likely that the saponins extracted from *P.africanus* extract inhibited and restored BPH (Guclu-Ustundag and Mazza, 2007).

The tannins were found to be present in high proportion in current study, this was in tandem with previous studies finding (Frutos *et al.*, 2004; Jeronimo *et al.*, 2013). The tannins are believed to have antioxidant effects which inhibit BPH and impede the activation of inflammatory agents such as interleukins and cyclooxygenase, which are crucial in protection of the prostate gland and this could be attributed to inhibitory and restorative effects established in this study.

In the current study alkaloid were present, and are believed to have antiproliferative effects, anti-tumour activity, and antispasmodic pain reliever effects. It is likely that the alkaloids identified in the methanolic bark extract of *P.africanus* are responsible for its restorative and anti-tumoral activity on BPH (Kadu *et al.*, 2012; Matsuura and Fett-Neto, 2015; Komakech and Kang, 2019).

5.2 The Acute Oral Toxicity of the CMBEPA

The acute oral toxicity of the CMBEPA study showed no mortality for the entire period of 14 days even after administration of the highest dose of CMBEPA which was 5000 mg/kg body weight. This concurs with the findings of study done by Karani *et al.* (2013), which showed no mortality during the entire period of fourteen days. In addition, the study also found that there was respiratory distress at a dose 2900 & 5000 mg/kg bwt in the first 24 hour which disagree with a study done by Karani *et al*. (2013). The respiratory distress observed during the study could be attributed to high level saponins which would have led to haemolysis and consequently leading to transient respiratory distress.

In addition, the study also found the following gross pathology after administration of CMBEPA at a dose of 2900 & 5000 mg/kg bwt the lungs were hyperaemia, the liver was congested and lung abscess. This was contrary to study findings by Nabende *et al.* (2015), which showed no pathologies at highest dose of 5000 mg/kg body weight. The gross pathologies findings in liver and lungs in this study could be attributed to saponins in bark extract. It is also possible that with the increase in dose of saponins

the toxicity to the lung increased. In addition, study findings by Igweh and Onabanjo, (1989), and Ayinke *et al.* (2012), established that saponins have a harmful haemolysing effect on circulating erythrocytes. Further, the liver congestion can also be attributed, to one of its roles which is biotransformation of xenobiotic (Abass *et al.*, 2012). The lung abscess observed in one of the rats at a dose of 2900 mg/kg body weight could be as result of accidental aspiration of bark extract in the lung.

Further, the study showed statistical significant decrease in the rat weight during day 7 post administration of CMBEPA at a dose of 1000 mg/kg bwt (p=.025) when compared to control.

A dose-dependent mean weight loss was recorded in day 7 and day 14 which was statistically significant at dose of 1000 mg/kg body weight (p=.003) and (p=.010) respectively when compared with the control group. This was in disagreement with the study findings of Nabende *et al.* (2015), which indicated normal weight of rat treated at dose of 1000 mg/kg bwt when compared with the control. The decrease in mean weight at higher doses could be attributed to the presence of tannins and phenolics which are believed to hinder the absorption of nutrients in intestines making them inaccessible and thereby reducing the voluntary feed intake even though the rats were provided with free access to feeds and water (Frutos *et al.*, 2004).

The findings of the study on the absolute mean organ weights of spleen was shown to significant increase in a dose-dependent manner after administration of CMBEPA at a dose 100 mg (p=.000) and1000 mg (p=.000) when compared with the control group, this contradicts to a previous study by Gathumbi *et al.*(2000), which reported normal spleen weights at highest dose of 1000 mg/kg bwt. This splenomegaly findings in the study could be attributed to high concentration of saponins in bark extract which are known to have haemolytic effects to red blood cell. In addition, there was a significant decrease in the mean absolute brain weight in group administered with CMBEPA at a dose of 100 mg (p=.000) and 200 mg (p=.000) when compared with the control. This contradicted the study findings by Gathumbi *et al.* (2000), which reported normal brain weights after administration of the bark extract of *P.africanus*. The decrease in the brain weights could be attributed to high levels of saponins found in the CMBEPA.

In addition, the testicular weight were also found to significantly decrease as the dose of CMBEPA increased, this contrary to a previous study by Gathumbi *et al.* (1995), whose finding showed that there were no effects of crude extract of *P.africanus* on testicular weight at dose of 1000 mg/kg bodyweight. This could have been attributed haemolytic effect of high amount of saponins in the bark extract. The 5000 mg/kg body weight was found to be safe as proposed by Lorke, 1984. This is also supported by lack of mortality among rats in all the dose groups during the entire 2 weeks of acute toxicity experimention.

5.3 The gross morphometric restorative and inhibitory effects of CMBEPA

5.3.1 The gross morphometric restorative effects of CMBEPA

The gross morphological findings of the study established that administration of CMBEPA in testosterone induced BPH Wistar, resulted in statistical significant dose dependent restoration of prostate weight (p=.003), prostate length (p=.01), prostate width (p=.006) and prostate thickness (p=.001) when compare with TPI-BPH control group. These findings agreed with a study carried by Jena *et al.* (2016), and Mandarim and Sol (2017), which reported that administration of crude bark extract *P.africanus* significantly ameliorates TPI-BPH in rat models. These restorative effects found in the prostate are thought to be due to the active phytotherapeutic constituents such as pentacyclic, phytosterols, ferulic acid esters and triterpenoids (Nyamai *et al.*, 2015). The agreement between the two studies could be attributed to the similar experimental protocols between the two studies. On the other hand the TPI- BPH model rats exhibited significant increase in PBR (P=.01) when compared with the control group and this agree with a previous study by Li *et al.* (2018), whose finding showed an increase in both PBR and prostate weight. The agreement between the two studies could be attributed to the fact that the same protocol for BPH induction was applied, further, this finding indicates that testosterone propionate successfully induced BPH in Wistar rat model**.**

In addition, the administration of CMBEPA in testosterone induced BPH for twenty one days exhibited a dose dependent (25 mg, 50 mg, 125 mg, and 200 mg) restoration of 9.756%, 12.196%, 34.146% and 85.366 % respectively of the prostate weights.

5.3.2 The gross morphometric inhibitory effects of CMBEPA

The gross morphometric inhibitory effects of CMBEPA was established by concurrently administered with testosterone propionate with CMBEPA. A statistical significant inhibition in the PBR (p=.001), prostate weight (p=.000), prostate length (p=.019) and prostate width (p=.026) was observed when compared with the control group. This agrees with previous studies by Jena *et al.* (2016), and Wilt *et al.* (2002), whose finding showed prostate dimensional decrease as well as a decrease in PBR when crude methanolic bark extract was administered in TPI-BPH rat model. On the other hand, the TP-induced BPH without treatment had marked significant increase, in PBR (p=.000) as well as prostate dimensional changes when compared with normal control. This was consistent with a previous study by Yang *et al.* (2014), whose study findings showed increase in PBR as well as prostate dimensions. The concordance between the two studies could be attributed to the use of similar protocol for induction of the BPH, therefore exogenous S.C injection of TP induces BPH successfully.

5.4 The histostereological restorative effects of CMBEPA

The histostereological findings of the study established that, when CMBEPA was administered for 21days in testosterone induce BPH group a dose dependent restoration was exhibited. The significant difference was found in the prostate structure volumes which includes the epithelium (p=.000), stromal (p=.000) and total prostate (p=.000). These findings were similar to studies by Yablonsky *et al.* (1997) and McNicholas and Kirby, (2011), which showed restorative histostereological effects in prostate volumes and volume densities. This similarity could be attributed due to the application of similar protocol in the experiment. In addition, the restoration can be attributed to its potent inhibition of the rat prostatic fibroblast proliferation through direct activation of protein kinase C (PKC). PKC activation appears to be an important growth factor-mediated signal transduction for *P.africanus.* On the other hand, in the TPI BPH group without treatment showed an increase in total prostate, stromal and epithelial. This concur with previous studies by Li *et al.* (2018), and Mbaka *et al.* (2019), whose findings reported an increase in prostate volume structure volume after exogenous exposure to testosterone propionate in the rats. The concordance could be

attributed similarities in methods to induce BPH. In addition, these study findings on histostereological increase in prostate structure volumes and densities substantially could be attributed to role of exogenous testosterone in inductive interactions in stromal and epithelial lining growth.

The study applied various methods during quantification of histostereology to ensure satisfactory precision which included Coefficient of Error (CE) whose values in the restorative and inhibitory group animal were calculated and they were found to be below 0.05 and this was considered to be satisfactory in delivering a precise quantitative information in stereology (Gundersen *et al.*, 1988). In addition, the study showed a high level of agreement between the gold standard (Archimedes volume) and cavalieri method which was 76%. This was examined using intraclass correlation at 95 % confidence intervals for volume differences.

5.4.1 The qualitative histomorphological effects of CMBEPA

The qualitative histomorphological finding of the study showed that after administered of varying doses CMBEPA in TPI induced rats there was marked improvement of the glandular morphology and reduction of epithelial proliferation. This is evidenced by reverting of epithelium to the simple columnar form, great reduction of the intraluminal papillary projection and amount of fibromuscular stromal in comparison to the testosterone-induced BPH group. This was in tandem with previous studies findings by Wilt *et al.* (2002), and Keehn *et al.* (2016). Which showed substantial restoration effects in the glandular epithelium and the interglandular smooth muscle after administration of crude extract *P.africanus*, this concordance could be attributed to the similar BPH induction procedure. In addition, in the TPI- BPH group the acini lumen illustrated many papillary folds and the epithelial lining cells were arranged haphazardly in many layers and were extensively separated by thick fibromuscular stromal that showed areas of cellular infiltration with also massive dilatation of prostate acini full of secretion, when compared to the compared to the control group. This agrees with previous study findings by Li *et al.* (2018), Kang *et al.* (2018), (Li *et al.* (2018), and Mbaka *et al.* (2019), which found an increase papillary projections in acini lumen and thick fibromuscular layer after subcutaneous injection of testosterone

propionate. This agreement could be attributed due to use of similar BPH induction protocol.

5.5 The Histostereological inhibitory effects of CMBEPA.

The histostereological inhibitory effects of CMBEPA in this study was established by administration of Testosterone propionate concurrently with CMBEPA. A dose dependent inhibitory effects on the following prostate structure volume were found to be statistically significant; epithelium (p=.000), and stromal (p=.000), when compared with testosterone propionate group. This was concordant with studies by Shen *et al.* (2017), and Jena *et al.* (2016), whose findings showed bark extract of *P.africanus* successfully inhibits increase in prostate structure volumes and densities when co-administered with testosterone propionate in the rat models. These histostereological inhibitive effects of the CMBEPA treated rats may be attributed to the inhibitive activity of 5α-reductase in CMBEPA, thereby preventing conversion of testosterone to dihydrotestosterone (DHT). DHT is potent androgen that is crucial in proliferation of stromal and epithelial cells subsequently leading to BPH development (Shen *et al.*, 2015; Wang *et al.*, 2017).

5.5.1 The Qualitative Histomorphological findings in the inhibitory group.

The qualitative histoquantative findings of this study established that when CMBEPA was administered concurrently with testosterone propionate there was dose dependent decrease in intraluminal papillary projections and interglandular smooth muscle when compared with TPI-BPH group rats. This was similar to previous study findings by Levin and Das, (2000), and Gerber, (2002), which showed *P.africanus* has ameliorative effects in prostate gland histoarchicture, these reversal effects observed in the current study could be attributed to phytochemical constituents in the CMBEPA. On the other hand, in the TP administered group without CMBEPA treatment, the histoarchitecture disruption of the prostate epithelium was evidenced by substantial thickening and hyperplasia with increased papillary projections in the lumen of the acini, also broadening of the luminal diameter was noted, when compared control which depicted normal histoarchitecture the epithelium, the acini and the stromal

tissue. This was in agreement with study finding of Shen *et al.* (2015), which showed increase in papillary projections in luminal acini and thickening interglandular smooth muscle in rat administered with testosterone propionate. These finding on the changes in histoarchitecture of the prostate indicates that subcutaneous injection of testosterone propionate induces BPH successfully.

5.6 CONCLUSION

The following were the conclusive findings of the study:-

1. The study established that the bark extract of *P.africanus* from Mt. Kenya contains flavonoids, triterpenes, glycosides, tannins, alkaloids and saponins which are thought to exert antiproliferative and antioxidant effects inhibiting the development and restoration of BPH.
2. The safe oral intake of CMBEPA at dose was established to be less than or equal to 5000 mg/kg body weight, which is harmless based on Loomis and Hayes classification of toxicity, hence it's usage as a traditional remedy is safe.
3. The inhibition and restorative histostereological effects of CMBEPA had an inverse dose-response relationship with prostate structures volume. The maximal dose-related restoration and inhibition of total prostate volume, stromal epithelium and acinar prostate volume being achieved at dose of 200 mg and 125 mg/kg bwt respectively.
4. The optimal restorative and inhibitory effects was *P.africanus* was maximum at 85.6% on BPH which was obtained at a dose of 200 mg/kg bwt. Therefore, this study strongly supports chemoprevention use of CMBEPA in the management of BPH.

5.7 RECOMMENDATIONS

This study therefore recommends the following;

1. The crude methanolic bark extract of *P.africanus* has both inhibitory and restorative effects that can be used in the management of BPH.

2. A study on long term effects of CMBEPA on histostereological parameters of prostate should be established through carrying out experiments using non-human primate.
3. Further research need to be carried out on restorative and inhibitory effects using second order stereology to determine the cellularity, the cell volumes and densities following administration of CMBEPA using non- primate animal

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APPENDICES

Appendix I: Copy of the Approval letter by Ethic Review Committee

JOMO KENYATTA UNIVERSITY
OF
AGRICULTURE AND TECHNOLOGY
P. O. Box 62000-00200 Nairobi, Kenya Tel 0675870225 OR Extn 3209
Institutional Ethics Review Committee

April 19th, 2018

REF: JKU/2/4/896A

James Mwangi Kanyoni,
Department of Human Anatomy.

Dear Mr. Kanyoni,

RE: HISTOMORPHOMETRIC EFFECTS OF *PRUNUS AFRICANUS* BARK EXTRACT ON TESTOSTERONE-INDUCED BENIGN PROSTATIC HYPERPLASIA IN WISTAR RATS.

The JKUAT Institutional Ethics Review Committee has reviewed your responses to issues raised regarding your application to conduct the above mentioned study with you as the Principal Investigator.

The is to inform you that the IERC has approved your protocol. The approval period is from April 19th 2018 to April 19th 2019 and is subject to compliance with the following requirements:

a) Only approved documents (informed consent, study instruments, study protocol, etc.) will be used.
b) All changes (amendments, deviations, violations, etc.) must be submitted for review and approval by the JKUAT IERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the IERC immediately.
d) Any changes, anticipated or otherwise that may increase the risks to or affect the welfare of study participants and others or affect the integrity of the study must be reported immediately.
e) Should you require an extension of the approval period, kindly submit a request for extension 60 days prior to the expiry of the current approval period and attach supporting documentation.
f) Clearance for export of data or specimens must be obtained from the JKUAT IERC as well as the relevant government agencies for each consignment for export.
g) The IERC requires a copy of the final report for record to reduce chances for duplication of similar studies.

Should you require clarification, kindly contact the JKUAT IERC Secretariat.

Yours Sincerely,

DR. PATRICK MBINDYO
SECRETARY, IERC

J.K.U.A.T
DIRECTOR,
RESEARCH DEPARTMENT (RPE)
P O Box 62000-00200
NAIROBI

Setting Trends in Higher Education, Research and Innovation

Appendix II: A Copy of Journal Publication

IOSR Journal Of Pharmacy www.iosrphr.org
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Volume 8, Issue 12 Version. I (December 2018), PP. 39-45

The phytochemical components and acute toxicity of methanolic stem bark extract of *Prunus africana*

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[2](School of Medicine (SoMed), Jomo Kenyatta University of Agriculture and Technology, Kenya)
[3](College of Health Sciences (COHES), Jomo Kenyatta University of Agriculture and Technology, Kenya)
Corresponding Author: Kanyoni J. Mwangi

Abstract: currently there is a growing interest in use of medicinal plants. This has led to amplified need of scientific analysis of their safety and extractive phytochemical component, thus providing health care workers with adequate knowledge regarding the plants, and this in turn will assist patients make informed choice on their utilization. The bark extract of Prunus africana (P.africana) has been used traditionally for decades in the treatment of various conditions such as abdominal upset, decreased appetite, fever, malaria, prostate cancer, and benign prostatic hyperplasia. The stem bark of P.africana was evaluated for its phytochemical constituents and acute toxicity effect on fifteen female wistar rats. The bark extract was collected in Mukurweini in Nyeri County, Kenya.

The bark extract of P.africana was soaked in methanol. The mixture was filtered and the organic solvent was evaporated to near dryness by vacuum evaporation using rotary evaporator. The bark extract was subjected to a phytochemical screening where extractive protocols were applied to detect majority of molecules present. The evaluation of acute toxicity of methanolic extract of the bark followed the modifiedLorke's model.

The phytochemical screening of the methanol bark extract revealed carbohydrates, flavonoids, tannins and saponins. The methanolic bark extract of P.africana at dose less than or equal to 5000 mg/kg body weight was found to be safe, therefore it isrelatively harmless based on Loomis and Hayes classification of acute toxicity.

Date of Submission: 13-12-2018 Date of acceptance: 29-12-2018

I. INTRODUCTION

Prunus africana, also known as *Pygeum africana*is ever green canopy tree native in Africa countries[1]. It is mainly wellestablished on tallhighlands forests across the Africa continent[2].Pygeum belongs to a member of Rosacea family[3].In Africa the tree are found in the rainforests of equatorial region, in Angola, Congo, Cameroon, Ghana, Kenya, Ethiopia, Madagascar, Mozambique Malawi, South Africa, Uganda, Tanzania, Zimbabwe and Zambia. The trees are usually 10 to 25 meters long but can grow up to 45 meters, their trunk is straight cylindrical with a dense round crown. The leaves have a deep green and glossy appearance. The flowers are small and the colour ranges from white to whitish cream. The fruits resembles cherry and their colour ranges from red to purplish-brown. The wood is pale red in color and has a strong cyanide smell when freshly cut. The bark, bruised leaves, and fruits have a strong and a bitter-almond smell[4].The *P.africana*bark extract has been used for several medicinal purposes; leaves has been used as inhalant for fever or drunk as appetizer, water extract from powdered bark has been used as a remedy for stomach ache or as a purgative for cattle[5]. *P.africana*bark, bruised leaves, and fruits have a strong and a bitter-almond smell. *P.africana*,it been crucial in the clinical management of BPH for numerous decades[1].The concentration of most compounds used in treatment of BPH in these trees either from the wild or domesticated habitats do not vary significantly, but some phytochemicals concentration vary hence the need of a phytochemical analysis[6].The extensive usages of *P.africana* to-date these trees are at the verge of extinction [3,4]

Appendix III: A Copy of the Letter of Approval of Research Proposal and Supervisors Allocation

JOMO KENYATTA UNIVERSITY
OF
AGRICULTURE AND TECHNOLOGY
DIRECTOR, BOARD OF POSTGRADUATE STUDIES

P.O. BOX 62000
NAIROBI – 00200
KENYA
Email: director@bps.jkuat.ac.ke

TEL: 254-067-52711/52181(6114)
FAX: 254-067-52164/52030
Mobile:0708-602225

REF BPS/HSM301-4002/2016 **1st November, 2017**

JAMES MWANGI KANYONI
C/o SOM
JKUAT

Dear Mr. Kanyoni,

RE: APPROVAL OF RESEARCH PROPOSAL AND SUPERVISORS

Kindly note that your MSC. research proposal entitled: **"Histomophometric effects of prunus africanus bark extract on testosterone induced benign prostatic hypeplasia in wistar rats."**has been approved by the Board of Postgraduate Studies. The following are your approved supervisors:-

1. Dr. Joseph Kweri
2. Dr. Ruben Thou
3. Dr. George Kibe

PROF. MATHEW KINYANJUI
DIRECTOR, BOARD OF POSTGRADUATE STUDIES
Copy to: Dean, SOM

Appendix IV: Classification of acute oral toxicity based on Loomis and Hayes

or less	Extremely toxic
1–50	Highly toxic
50–500	Moderately toxic
500–5000	Slightly toxic
5000–15,000	Practically non-toxic
More than 15,000	Relatively harmless

Appendix V: Data Capture sheet for histostereology of the prostate

rat No.	t µ	Length (mm)	No. Sections	A(p)	T Epith (P)	TStro (P)	T Acin (P)	M value	Epith V	Strol V	Acinar V	prostate volume	Epith Vv	Strol Vv	Acinar Vv

Appendix VI: Data capture sheet for gross morphometry of the prostate and Archimedes prostate volume

RAT No.	Length(mm)	Width(mm)	Thickness (mm)	Archimedes volumes