Assessment of histological changes in rat prostate treated with Prunus Africana extract

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ASSESSMENT OF HISTOLOGICAL CHANGES IN RAT PROSTATE TREATED WITH PRUNUS AFRICANA EXTRACT

By

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled "Assessment of histological changes in rat prostate treated with *Prunus Africana* extract" in (partial) fulfillment of the requirements for the degree of Master of Sciences (Human Anatomy) of Muhimbili University of Health and Allied Sciences.

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Dr. D. Russa

(Supervisor)

Date _____

DECLARATION AND COPYRIGHT

I, **Felix Peter William HD/MUH/T.729 /2018** declare that this **dissertation** is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

Signed: ..... Date: …….

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DEDICATION

I am dedicating this dissertation to my beloved Mother, she meant and continues to mean a lot to me. Her love for me and encouragement throughout is limitless despite her difficulty and hardship she has passed through until now, I love her, and may God grant her a more blessed life. AMEN

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ABBREVIATIONS

AED:	Animal Equivalent dose
ANOVA:	Analysis Of Variance
ARI:	Adreno-Receptor Inhibitor
BOO:	Bladder Outlet Obstruction
BPH:	Benign Prostatic Hyperplasia
CRBD:	Completely Randomized Block Design
DHT:	Dihydrotestosterone
EGF:	Epidermal Growth Factor
bFGF:	Fibroblast Growth Factor
IGF-I:	Insulin Growth Factor
ITM:	Institute of Traditional Medicine
LUTS:	Lower Urinary Tract Symptoms
MUHAS:	Muhimbili University of Health and Allied Sciences
NBBSA:	N-Butylbenzenesulphonamide
TP:	Testosterone proprionate
TURP:	Trans Urethral Resection of Prostate
QoL:	Quality Of Life
USA:	United State of America

DEFINITION OF KEY TERMS

ACUTE INFLAMMATION: This is an innate, immediate, and stereotyped response that occurs in the short term following tissue injury, it is a series of processes initiated to limit damage to tissue.

APOPTOSIS: Is a form of programmed cell death that occurs in multicellular organisms.

CHRONIC INFLAMMATION: Referred to as slow, long-term inflammation lasting for prolonged periods of several months to years.

EFFICACY: The ability, especially of a medicine or a method of achieving something, to produce the intended result, the quality of being effective.

HISTOLOGY: Is the study of the tissues of the body and how these tissues are arranged to constitute organs

PHYTOCHEMICAL: Are chemical compounds produced by plants, generally to help them thrive or thwart competitors, predators, or pathogens

PHYTOSTEROL: Are groups of naturally occurring compounds found in plant cell membranes, structurally similar to the body's cholesterol

PHYTOTHERAPY: The use of plant-derived medications in the treatment and prevention of disease

PROSTATE: Is a gland that produces the fluid that carries sperm during ejaculation. It surrounds the urethra, the tube through which urine passes out of the body.

QUALITY OF LIFE: The degree to which an individual is healthy, comfortable, and able to participate in or enjoy life events.

TESTOSTERONE: Androgen hormone produced primarily by the testicles responsible for the development of male sexual characteristics.

THERAPEUTIC: The branch of medicine concerned with the treatment of disease and the action of remedial agents.

ABSTRACT

Benign prostatic hyperplasia (BPH) is the most common benign proliferative disease among men during aging, Prucan capsule contains an extract of *P. Africana*, and the extract has been used for the treatment of BPH since time immemorial. Therefore this study was designed to investigate the histological changes in rat prostates treated with *P .Africana* extract. Rats were divided into six groups, each group containing 7 rats, Negative Control group (rats were subcutaneously injected with vehicle agent olive oil and oral normal saline for four weeks), BPH group {BPH was induced by subcutaneous injection of testosterone propionate (TP) daily for four weeks},Treatment group (rats were injected with TP for four weeks followed by daily administration of crude *P.Africana* extract for four weeks), preventive group (where rats were given crude of *P. Africana* extract simultaneously with TP injection for four weeks), positive control group (rats were injected with TP for four weeks followed by daily administration of finasteride for four weeks) and Prucan group (where rats were administered with only crude *P. Africana* extract for four weeks. Ventral prostates were extracted, cleaned, and measured then processed for histological and histomorphometric examination. Prostate weight was significantly increased in the BPH group and significantly decreased in Prucan, preventive, positive, and treatment groups. Histologically and morphometrically, the BPH group showed epithelial hyperplasia, stromal expansion, and reduced acinar lumens these features were significantly improved in the preventive,Treatment, and positive control group, while the Prucan group showed a significant decrease of the epithelial area, acinar luminal area, and stroma area . Generally, this study has demonstrated that there is a restoration of histopathological findings in BPH induced rats following treatment with crude of *P. Africana* extract and that *P. Africana* extract seems to have a potentially ant-BPH and protective histological effect to rat prostate.

CHAPTER ONE

1.0 INTRODUCTION

1.1.1 Background

Benign prostatic hyperplasia (BPH) is the most common benign disease proliferative in nature among men as they get older.Its incidence is thought to increase by 42% in men between 40 and 50 years andup to 90% in those over 80 years(McVary, 2006; Zhong *et al.*, 2015). It is achronic condition that affects aging men predominantly worldwide regardless of their culture or ethnic origin (Barkin *et al.*, 2009). With the continuedaging of the general population, BPH will place a continuallyincreasing burden on healthcare resources in the future (McKELVIE *et al.*, 1993). Benign prostatic hyperplasia is a histological diagnosis associated with an unregulated proliferation of connective tissue, smooth muscle, and glandular epithelium within the prostatic transition zone(Auffenberg, Helfand, and McVary, 2009a).Stromal cells are key regulators of growth and differentiation in the adult human prostate, the prostatic stroma is composed of two major cell types, smooth muscle cells, and fibroblast(Peehl and Sellers, 1997).Histologically BPH is characterized by a progressive increase in the number of epithelial and stromal cells that develop initially in the periurethral area of the prostate gland (Yoshida *et al.*, 2011).

1.1.2 Pathogenesis of BPH

Current theories assert BPH is a multifactorial process involving interactions between prostatic cells, the endocrine system, neural input, heredity, and environmental influences (Auffenberg, Helfand and McVary, 2009b). Despite the prevalence of BPH, its pathogenesis has remained controversial. The androgenic hormones testosterone and dihydrotestosterone have a significant role in this process (Auffenberg, Helfand and McVary, 2009b; Zhong *et al.*, 2015). Dihydrotestosterone (DHT), a primary metabolite of testosterone, is the principal intracellular androgen in the prostate and plays a key role in normal and hyperplastic prostatic growth, the conversion of testosterone to DHT is mediated by the enzyme 5α-reductase located in the prostate(Anderson *et al.*, 2001). Growth factors and other hormones including estrogens may also have a function in the pathogenesis of BPH (Zhong *et al.*, 2015).

It has been shown that acute and chronic inflammation also contributes to the development of BPH, by stimulating cellular growth through various pathways particularly oxidative stress,

or by affecting apoptotic mechanism whereby the disruption ofapoptotic equilibrium due to inactive apoptosis may lead BPH tissues to a state of epithelial and stromal hyperplasia (Ammar *et al.*, 2015; Chung *et al.*, 2016).

1.1.3 Signs and Symptoms of BPH

The enlarged gland is thought to lead to disease manifestations via two routes, i) static component, direct bladder outlet obstruction (BOO) from enlarged tissue, and ii) dynamic component, from increased smooth muscle tone and resistance within the enlarged gland (Auffenberg, Helfand and McVary, 2009b). This most common manifestation of BPH is the collection of symptoms described as lower urinary tract symptoms (LUTS), LUTS are any combination of urinary symptoms, including obstructive symptoms (hesitancy, weak stream, intermittency, terminal dribbling, and feeling of incomplete emptying) and irritating symptoms which are frequency, urgency, and nocturia (Auffenberg, Helfand and McVary, 2009b; Chung *et al.*, 2016). The obstructive and irritating lower urinary tract symptoms reduce an individual's quality of lifeby altering normal daily activities and sleep patterns. Although uncommon, seriouscomplications of BPH may occur including acute urinary retention, renal insufficiency, urinary tract infections, hematuria, bladder stones, and renal failure, these complications may be triggered or worsened by inadequate management of BPH (Yoshida *et al.*, 2011; Chung *et al.*, 2016).Traditionally, the primary goal of treatment has been to alleviate bothersome LUTS that result from prostatic enlargement (McVary, 2002; Wilt, Howe and MacDonald, 2002; Auffenberg, Helfand and McVary, 2009b). Treatment options include non-pharmacological `watchful waiting' or life-style modification pharmacological over-the-counter herbal preparations or prescriptionmedications and surgical procedures (Wilt, Howe and MacDonald, 2002; Yoshida *et al.*, 2011).

1.1.4 Treatment of Symptomatic BPH

1.1.4.1Surgical interventions for clinical BPH

The most common surgical procedure for BPH has been transurethral resection of the prostate (TURP) with open prostatectomy typically reserved for men with very large prostates, the indications for surgery are still not universally accepted and vary by type of surgical procedure. The agreement is greater for the absolute indications, which include hydronephrosis with the threat of urosepsis or renal failure, and anatomical bladder disorders, such as large diverticuli. It is generally accepted that older patients with increased

comorbidity or with renal insufficiency are at higher operative risk for complications and mortality, and may not be good candidates for surgery (15)

1.1.4.2 Medical therapy for symptomatic BPH

Prescription medications include 5α-reductase inhibitors to reduce prostate size and α-1-adrenoceptor antagonists to decrease smooth muscle tone in the prostate and bladder (Wilt, Howe, and MacDonald, 2002; Auffenberg, Helfand and McVary, 2009b; Pagano *et al.*, 2014).

α-Adreno- receptor antagonists are the most frequently used prescription medication that blocks prostate and urethra α-1-A adrenergic receptors results in the relaxation of the prostate and urethra smooth muscles. These agents include terazosin, doxazosin, prazosin, alfuzosin, and tamsulosin. Side-effects consisting mainly of tiredness,dizziness,hip fractures, and occasionally postural hypotension occur in around 10% of patients (McVary, 2002; Wilt, Howe, and MacDonald, 2002; Yoshida *et al.*, 2011). Finasteride and dutasteride are examples of available 5-ARIs, these compounds virtually eliminate the production of DHT, in turn inhibiting prostate growth.The most common side effects of these agents are impotence, loss of libido, ejaculatory dysfunction, and gynecomastia (Auffenberg, Helfand and McVary, 2009b). Combination therapy has been also used and the therapy is both safe and the most effective therapy for patients with LUTS secondary to BPH, additionally, combination therapy is the best option in preventing disease progression (Auffenberg, Helfand and McVary, 2009b).

1.1.5 Prucan capsule

Prucan capsule is a brand name for *Prunus Africana* extract which is produced by the institute of traditional medicine at Muhimbili University of Health and Allied Sciences, it is prepared from the Stem bark extract of *P. Africanum.* At MUHAS-ITM it is sold to clients as a complete dose for four weeks, each capsule contains 100mg, its indication includes management of mild to moderation BPH.

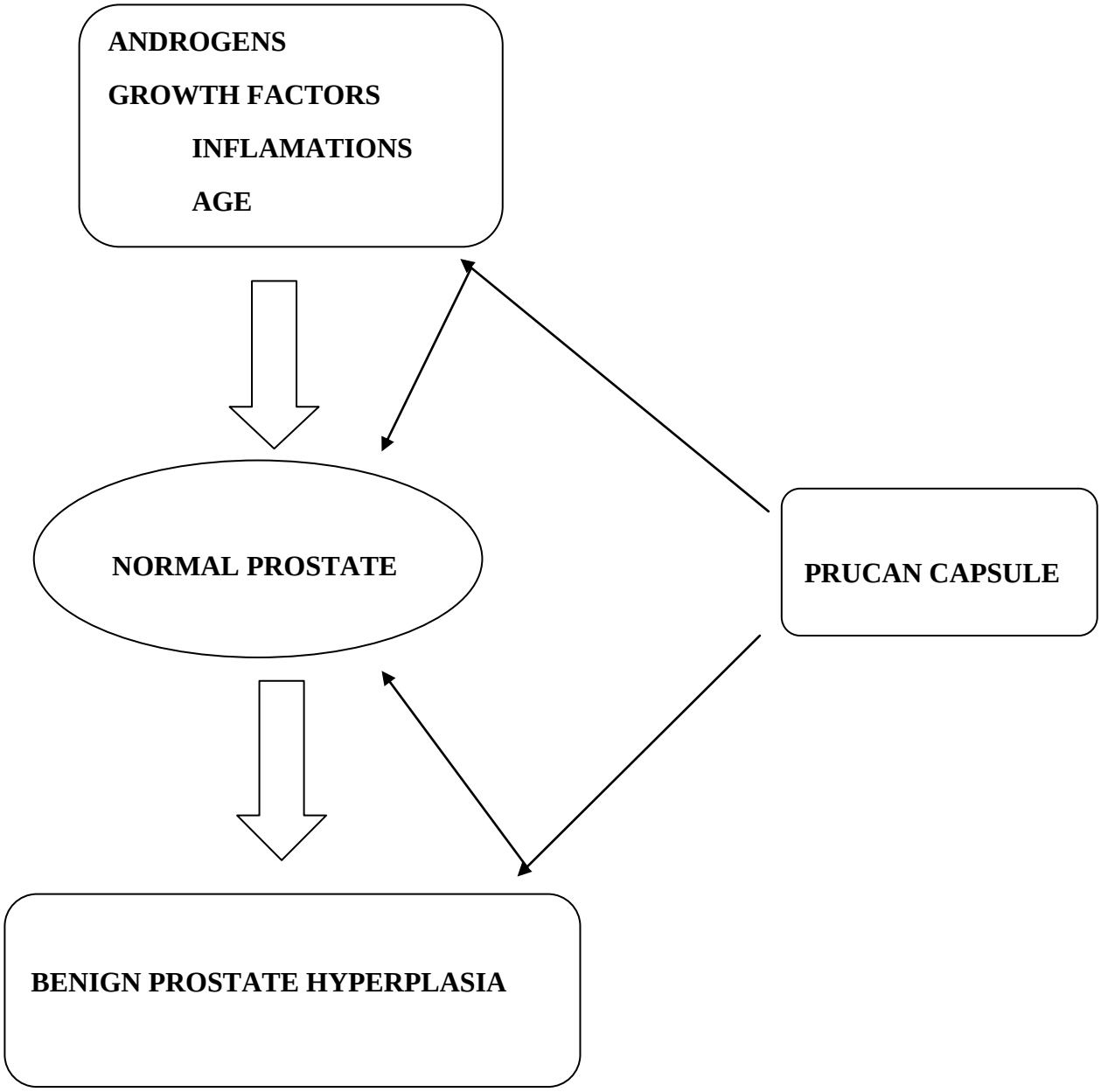
Prucan capsule contains phytosterols and lipids which exert an anti-swelling effect of the prostate synergistically, Prostaglandins are inflammatory hormones that tend to accumulate in the prostates causing swelling and increase in size. The phytosterols in *Prunus Africana* interfere with the formation of these prostaglandins.

1.2 Problem statement

The prevalence of both histological and clinical BPH is on the rise among men of all races, it is a significant health care problem due to its high prevalence and the cost associated with its treatment. The management of clinical BPH has been relying on medical therapy and different drugs have been used singly or in combination therapy, some of the commonly used drugs include finasteride dutasteride, tamsulosin, and prazosin. However using these drugs for management of clinical BPH has been associated with many side effects like impotence, loss of libido, ejaculatory dysfunction, gynecomastia, tiredness, dizziness, hip fractures, and occasionally postural hypotension (Wilt, Howe, and MacDonald, 2002; Auffenberg, Helfand and McVary, 2009b; Yoshida *et al.*, 2011).

All these side effects together with the total costs encountered during medical therapy for clinical BPH have decreased the compliance and adherence to medications in patients who undergo such management. Henceforth the quality of life has been progressive severely affected and the number of complications due to poor management of clinical BPH has also been increasing. Alternatively, several herbal medicines that appear to have limited adverse effects have gained increasing popularity in the use for treating BPH (Zhong *et al.*, 2015), *P .Africana* has been used in Europe for more than 35 years totreat mild to moderate LUTS (Dedhia and McVary, 2008). Prucan capsule contains an extract of *P. Africana* available at the institution of Tradition Medicine of Muhimbili University of health and allied sciences and it is indicated for the management of BPH, despite its significance in improving LUTS to patients with BPH there are limited studies which show its histological changes to the prostate of a patient with BPH and animal model too, this applies to many of *P .Africana* extract products already in the market. This study aims to find out the histological changes in normal rat prostates and pathological ones treated with *P .Africana* extract to further inform its clinical beneficial and non-beneficial use in the clinical management of Benign prostatic hyperplasia.

1.3 Conceptual Framework



1.4 Rationale

The finding of this study will provide scientific evidence of two categories either positive or negative effects of *P .Africanum* extract to the prostate. Positive histological findings established by this study will supplement the body of knowledge known so far from previous studies about clinical ethnobotanical use of *P. Africana* extract for the management of symptomatic BPH. It will also complement information reported by patients about the

usefulness of this therapy in improving the bothersome clinical symptoms of BPH, and by doing so it can be promoted locally and be used as an alternative therapy for helping patients especially the victims of medical therapy's side effects and those who are not able to afford, comply and adhere to the recommended medical therapy. Positive ethnopharmacological effects of Prucan capsule to the prostate at the histological level will provide additional information which may be used by the institute of traditional medicine of Muhimbili University of Health and Allied Sciences in the process of approving and registration of the drug by the Tanzania Medicines and Medical Devices Authority (TMDA) for management of symptomatic BPH.

Negative effects of Prucan capsule will also contribute to the body of knowledge and will open room for more extensive and analytical studies in multiple disciplines of the ethnopharmacological effects of this drug to the prostate of patients with BPH. Many works of literature have reported the therapeutic effects of *P. Africana* extract in the treatment of BPH thorough evaluation of many efficacy parameters such as symptoms score, urinary flow rate, and Quality of Life, however, none of them showed the histological changes of the prostate after such treatment either in rat model or human subject. Not only that but also, the reviewed literature put much emphasis on other products *of P .Africana* extracts either available in the market or prepared locally but no study that assessed Prucan capsule as a brand name and one of the *P. Africana* extract available at the institute of tradition medicine of MUHAS.

The evidence generated by this study will also add knowledge to the limited literature about the histological perspectives of *P. Africanum* extract at large to the prostate of rats as model to normal and benign hyperplastic human prostate. This study is also conducted as a partial fulfillment of the requirement for the degree of Master of Science in Human Anatomy (MSc. Anatomy) of the Muhimbili University of Health and Allied Sciences.

1.5 Research question

Are there any histological changesin rats prostate treated with *P. Africana* extract?

1.6 Hypothesis testing

1.6.1 Null hypothesis; there are no histological changes in rats prostate treated with *P.Africana* extract

1.6.2 Alternative hypothesis; there are histological changes in rats prostate treated with *P .Africana* extract

1.7 Research objectives

1.7.1 Broad objective

1. To evaluate the histological changes in rat prostates treated with *P.Africana* extract

1.7.2 Specific objectives

1. To assess the microstructural changes in the prostate of normal rats treated with *P. Africana* extract

2. To determine the reversal effects of *P. Africana* extract treatment in rats with testosterone-induced BPH.

3. To assess the preventive effects of *P. Africana* extract treatment in high-risk rats, by simultaneous administration of exogenous testosterone and *P. Africana* extract

1.8 Literature review

1.8.1 Phytotherapeutics

Phytotherapeutic products are food supplements derived from plants (typically extracts from roots, seeds, bark, or fruits) and patients with BPH and LUTS commonly use them as a substitute for proven medications (8). In a 1999 survey in the United States of patients undergoing nonsurgical treatment for LUTS secondary to BPH 14% were using phototherapy alone and 20% were using prescribed drugs and phytotherapy for symptom treatment (15). Herbal medicines represent nearly half the medications dispensed for treatment of BPH in Italy, compared with 5% for α-adrenergic antagonists and 5% for 5-α-reductase inhibitors. In other European countries (Germany and Austria), phytotherapy is the first-line treatment for mild-to-moderate LUTS and represents about 90% of all drugs prescribed for the treatment of BPH. In the USA, phytotherapies for BPH are above all available as dietary supplements (14). While there are more than 30 botanical compounds for BPH treatment, four phytotherapy that has undergone the greatest degree of evaluation are Serenoarepens (saw palmetto), *Pygeum Africanum* (African plum), Secalecereale (rye pollen) and Hypoxisrooperi (South African star grass (15). Concerning the present research, we will focus on *Pygeum Africanum* (*P .Africanum*) as one of the phytotherapy used for BPH treatment.

P .Africana (synonym *Pygeum Africana*) belongs to the Rosaceae family, it is a widespread evergreen tree, growing at an altitude of 1500–2000 m, usually 10–25m high with alternate leaves, a straight cylindrical trunk, a dense rounded crown, and small white or cream fragrant flowers (16). It has blackish-brown bark, shining foliage, and greenish or white flowers.*P. Africana*is found in mountains and underlying islands in 22 countries mostly on the eastern side of Africa,it is also found in central Africa (Katanga, Congo), in West Africa, Comoros, and Madagascar. Bark extracts of *P. Africana* are used to treat benign prostate hyperplasia (17). Pygeum is the powdered bark of *Prunus Africana*, both the powder and a lipophilic extract are sold commercially under the same name (18).

Prostafx, Tadenan, Pygenil, and Prucan capsules are some of the herbal preparations of *P. Africana* in the market. Extracts from the stem and root barks contain phytochemicals with anticancer, anti-inflammatory, and antiviral effects (17, 19).

The pharmacological efficacy of the wild tree bark extracts is thought to be due to the synergistic effect of various compounds some of which are known and others unknown.

1.8.2The contents of *P.Africana* extract

The major parts of *P .Africanum* which are used as medicines include, leaves roots and stem bark, the stem bark can be powdered and boiled in water to make a decoction or the powdered bark can be used to make capsule and orally administered to treat and manage BPH (Komakech and Kang, 2019). The available studies have indicated that *P.Africana* stem bark contains many bioactive phytochemicals such astarpenoids, including ursolic acid, oleanolic acid, and β –amyirins. Flavonoids, including ferulic acid, phytosterol including β –sitosterol, fatty acid including lauric acid and myristic acid,tannins including atraric acid and N-butylbenzene-sulphonamide. The synergistic interactions of these phytochemicals have made *P.africanum* significantly a potent traditional medicine for many diseases and conditions such as benign prostate hyperplasia (Komakech and Kang, 2019; Thompson, Katz and Sheehan, 2019).

1.8.3 Proposed pharmacological effects of*P. Africana*phytochemicals

Pygeum Africana extract has been shown in vitro to affect stromal fibroblast cell proliferation of the prostate induced by EGF, bFGF, and IGF-I, it also acts in part by protecting neuronal and sub-cellular membranes from ischemia-induced damage, and by this means it protect the

contractile function of the bladder. Thus *P.Africana* inhibits the proliferation of fibroblasts from human hyperplastic prostate and bladder which leads to improvement of lower urinary tract symptoms (Renganathan, Cartwright and Schaefer, 2010). Ursolic and Oleanolic acids inhibit glucosyl-transferase activity and have an anti-edematous activity to the prostate; β-sitosterol and β-sitostenone have an anti-inflammatory effect by suppressing the production of prostaglandins and thus prevent swelling of the prostate.

Ferulic acid esters (n-tetracosanol and n-docosanol) and their derivatives have antitumor and hypocholesterolemic activity on the prostateleading to decreased levels of testosterone and dihydrotestosterone (Nyamai *et al.*, 2015; Thompson, Katz and Sheehan, 2019). Beta-sitosterol is among the common phytosterol found in *P. Africana* extract, the compound inhibits 5-alpha-reductase and therefore can reduce the amount of dihydrotestosterone in the prostate and thereby prostate growth. The compound also exhibits general anti-inflammatory and muscle-relaxing activity and may act as an alpha-blocker (Thompson, Katz and Sheehan, 2019).

Myristic acid exhibits antioxidant activity by alterations in membrane structure that limit the susceptibility of the membrane to lipid peroxidation, therefore repressing the progress of lipid peroxidation is likely to contribute to the benefits associated with *P .Africanum* therapy (Hass *et al.*, 1999). Atraricacid, N-butylbenzenesulfonamide (NBBSA) compounds have been identified as an androgen receptor antagonist and they are active against BPH (Thompson, Katz, and Sheehan, 2019). In terms of anti-inflammation *P.Africanum* has been shown to cause the inhibition ofleukotriene synthesis in human polymorphonuclear cell cultures (Dedhia and McVary, 2008). Lauric acid can inhibit 5-alpha –reductase enzymes hence resulting in the blockage of testosterone conversion to dihydrotestosterone crucially preventing testosterone cancer (Komakech and Kang, 2019).

1.8.4 Efficacy and Ethno pharmacological Use of *P. Africana*in Treatment of BPH

According to Komakech and Y. Kang, in traditional medicine the bark decoction of *P .Africa num* has been used for the treatment of BPH since time immemorial. Several ethnobotanical surveys have confirmed the use of *P. Africana* in the treatment of BPH (Komakech and Kang, 2019). Kipkore *et al* showed that ethnobotanical medicine in the Marakwet community of Kenya has been integrated into the health management system of different diseases. This herbal preparation is used either single or commonly in combination and they are prescribed

by herbalist, and the decoction of *P. Africana*stem bark is used for the treatment of enlarged prostate in that community residing in the rift valley region of Kenya (Kigen, Wanjohi, and Rono, 2014).

Noumin his study demonstrated that the stem bark of *P. Africana* in powdered form or decoction was mostly cited by the informants regarding its usefulness in treating prostate adenoma and there is widespread use of *P.Africana* stem bark among the tribes of Foumban Cameroon (Noumi, 2010). Tadenam is a *P.Africana* extract available in many countries, including those in central and Eastern Europe, for the treatment of mild to moderate BPH. Breza*et al* demonstrated the efficacy and acceptability of Tadenam in improving clinical symptoms of BPH and quality of life.Some of the investigated efficacy parameters are IPSS and QoL, and their change in score after two month period of treatment with Tadenam was statistically significant with a mean improvement of 40% and 31% respectively, also the nocturnal frequency was reduced and was statistically significant. Hence *Pygeum Africana* extract induces significant improvement in IPSS and uroflowmetry parameters with the overall resu1t of a substantial improvement in QoL (Breza *et al.*, 1998).

African have been using tea made of African prune Pygeum for a long time to treat urinary symptoms, Pygeum not only has a positive effect on the prostate but also on the bladder. Pygeum provide a moderately large improvement in urologic symptoms and flow measures such as nocturia and peak urinary flow(Ishani *et al.*, 2000; Pagano *et al.*, 2014). Bodeker*et al* 2014 showed that the trades in *P. Africana* bark extract for the treatment of Benign prostatic hyperplasia (BPH) led to a very lucrative international market worth approximately US$220 million in the late 1990s, and this led to the concerns of long term sustainability of harvesting and conservation of the plant species because more than 3300 tons of bark were collected annually to satisfy the demand (Bodeker, Van'T Klooster and Weisbord, 2014).

1.8.4 Prostate size reduction effects of *P. Africana* extract treatment

Most of the reviewed literature reported that there is no change in prostate volume(size) after several duration of treatment with *P. Africana* to the patient with BPH as it has been reported by J Breza et al, E. Pagano et al and M. Papaioannou (Breza *et al.*, 1998; Schleich *et al.*, 2006; Pagano *et al.*, 2014). This is an area of interest and there is a missing gap because if patients reported improvement in LUTS we expect the size of the prostate to be reduced following treatment, but the literature has reported the contrary, so this study is going to find

out gross and histological changes that are associated with the treatment of *P .Africana* in rat animal model.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Study area

The study was conducted at Muhimbili University of Health and Allied Sciences (MUHAS) School of Medicine, in the Department of Anatomy.

2.2 Sample size calculations

This was an experimental study involving animals placed in different groups, thus the conventional sample size calculation formulas were not applicable since various prerequisites for calculations are not available. Therefore the sample size was calculated by the Resource equation method whereby value E is calculated based on the decided sample size (Charan and Biswas, 2013).

E = Total number of animal – Total number of groups

For optimal sample size E value should lie between 10-20, if < 10 more animals are needed and if >20 sample size should be decreased.

In this study, there were 6 groups and the decided sample size is 20 animals, this gave us the optimal sample size based on the equation above,however, more extra animals were added to 42 rats, out of the sample size to counteract the loss due to death of animals.

Therefore forty-two male Wistar rats (200±50g) were purchased from the animal breeding unit at Sokoine University of Agriculture. The weight was chosen because of the previously published study (Ammar et al., 2015), rats were housed in a standard air-conditioned atmosphere, at a temperature of 22°C ± 2°C with alternate 12-hr light and dark cycles. Animals in different groups were housed in cages 7 animals per cage and were fed a conventional rodent laboratory foodwith an unlimited supply of drinking water. The animals were acclimatized to laboratory conditions for one week before the beginning of the experiment.

2.3 Study design

This was an experimental study design, the stem bark of *P. Africana* extract was collected, cleaned, dried, and ground to reduce the thickness then soaked in 98% of ethanol for three days, later the solution was filtered with cotton wool in a separating funnel. Finally, the solution was concentrated in a round bottom flask to remove ethanol.The crude obtained was placed at room temperature for further removal of ethanol remained, this gave us a dry crude which was ground by mortal and paste into powder for easy measurement and dose calculations depending on rats weight

- **Animal dosage of *Prunus Africana* extract**

The usual dispended dose of Prucan capsule to patients with mild to moderate BPH at MUHAS-ITM is either 100mg twice a day,or 200mg twice a day which is a human beneficial dose per day depending on the severity of LUTS (Breza *et al.*, 1998).In rats, the dose of 100mg/kg body weight has been used repeatedly in different kinds of literature, from previous studies an oral dose of 10 and 20 mg/kg/day of the *Pygeum African* was administered for 21 days for evaluating anti-BPH activity. As 20 mg/kg/day dose showed better activity, so this dose was used subsequently to evaluate the efficacy. Similarly, for anti-inflammatory activity, initially, a single dose of 20, 100, 200, and 400 mg/kg of the tested drugs were selected (Jena *et al.*, 2016). Toxicity studies of *Prunus Africana* extract in a single dose of lipophilic extract administered intragastrically to mice and rats up to 8g/kg body weight was well tolerated. Repeated dose toxicity at short term (I month) and long term (6month) by intragastric administration of the extract to rats of 750mg/kg/day cause no adverse effects on hematological biochemicalor anatomical/pathological parameters.No adverse reactions were observed after daily intragastric administration of the extract to mice at 60mg/kg or rats at 600mg/kg bodyweight for 11 months. Oral administration of the extract to rats at up to 1g/kg body weight daily for 8 weeks did not cause clinical or pathological signs of toxicity but moderate rises were observed in serum alanine aminotransferase (ALAT) and blood urea nitrogen levels. These findings confirmed the safety of the extract at therapeutic dosages since signs of toxicity were observed only at very high dose levels (Medicines Agency, 2016).

Therefore by considering *Prunus Africana* extract safety at the therapeutic dosage and the common dose of Prucan capsule dispensed at MUHAS-ITM being 100mg or 200mg twice a day, the decided dosages to use in this study was 400mg/kg body weight in all groups that were given *Prunus Africana* extract intragastrically.

2.4 Data collection methods

A completely randomized block design (CRBD) was used in the experiment, animals were divided into six groups, each group containing 7 rats. The first group was a Negative Control group,this grouphelped us during data analysis to compare rat of this normal group and the rest of the groups to elicit the changes of the prostate with respects to each specific group, this group was given a vehicle (olive oil) by subcutaneous injection, the dose weight per volume was calculated where 1kg has to receive a total of 10mls per day. The second group was a BPH groupto establish a rat model of human BPH to assess the effects of interventions given and was also used as a reference for comparison with treatment groups, BPH was induced by a subcutaneous administration of Testosterone propionate (B.M pharmaceuticals) 3mg/kg body weight/day for four weeks (Shin *et al.*, 2012; Ammar *et al.*, 2015; Swaroop *et al.*, 2015). The third group was a treatment group wherethe reversal effects of *Prunus Africana* extracton the diseased prostate was assessed grossly and histologically,rats with testosterone-induced BPH were given crude of *Prunus Africana* extract dissolved in normal saline by oral gavage for four weeks (Medicines Agency, 2016).

The fourth group was a preventive group,the inhibition effect of Prucan capsule towards the development of BPH in rats with the risk of BPH (testosterone) was assessed grossly and histologically, where rats were administered with testosterone propionate simultaneously with Prunus extract for 4 weeks. The fifth group was a Prucan group, rats were only administered with prunus extract 4 weeks. This group assessed histological changes of this medication to the normal rat's prostate. The sixth group was a Positive control group where BPH induced rats were given a standard regime Finasteride (Aurobindo Pharma Ltd) 5mg/kg body weight intragastrically (Chung *et al.*, 2016).

2.4 Sample Collections and Histopathological Analysis

At the end of the experiment period, all animals were fasted overnight and euthanized using pentobarbital at 100 mg/kg body weight injected intraperitoneally (Shin *et al.*, 2012). Ventral Prostate tissues were excised, rinsed, and weighed immediately, and then the ratio of the

prostate weight to body weight was calculated (Prostatic Index). The prostatic index (PI) is calculated as PW/BW x100% (Zhong *et al.*, 2015). Sections of the ventral prostate lobe were fixed in 10% neutral buffered formalin,fixed tissue was dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in liquid paraffin wax. The tissues were sectioned at 5µm using the Heitz 150 rotary microtome (Cambridge model). The sections were then subjected to Erlich's Haematoxylin and Eosin (H&E) staining technique using Baker and Silverton method for histological examination. Histological slides were scanned using the Digital slide scanning system Motic Easy Scanner (Motic Incorporation Ltd Hong Kong).

2.5 Histologic morphometric or quantitative tissue analysis

With the use of morphometry, the prostate can be classified into distinct and measurable tissue components (i.e., epithelium, fibromuscular stroma, and glandular lumina). Morphometry might help identify histological parameters that predict an outcome to alternative pharmacotherapy for BPH (Marks *et al.*, 1994). Histomorphometric parameters of epithelia, fibromuscular stroma, and glandular luminal in this study were measured.By using four non-overlapping fields, the histomorphometric analysis was performed to measure the epithelial height, acinar luminal area, and stromal area, the images were obtained under an x10 magnification (four fields per prostate). For the epithelial height analysis, the images were obtained under x40 magnification (four fields per prostate), assessment of the histological images were done by two separate individuals who were not aware of the study.

The prostatic epithelial height was measured by manually drawing a line through the acinar epithelia (30 measures per field). The acini luminal area was measured by drawing a line around the luminal perimeter and calculating the acini area. The stroma was measured by subtracting the total field area by the total acinar area, all histomorphometric data were collected and analyzed using Image J software (Gonzales *et al.*, 2008; Nasr El-Din and Abdel Fattah, 2019).

2.7 Data processing and analysis

All quantitative data were packed and analyzed by SPSS statistical software (version 20). All values of parameters were expressed in mean ± SD (standard Deviation).

Results between groups were compared by using a one-way analysis of variance (ANOVA), followed by the Turkey Post Hoc testto determine their level of significance. Differences at

$p<0.05$ was considered statistically significant, other histological findings were described accordingly.

2.8 Ethical Issues

The ethical clearance to conduct this study was obtained from MUHAS ethical committee, and that animal keeping and their use in the laboratory were according to recognized standards and guide for animal care and use in scientific purposes. All the procedures adhered to the ethical guidelines and animal welfare for investigations in laboratory animals (Close et al., 1997; Sarkar, 2011; Mohr et al., 2016). At the beginning of the experiment, animals underwent a daily injection of testosterone and all its associated pain and discomfort were well tolerated and adapted naturally. At the end of the experiment, before extraction of organs animals were euthanized by a recommended triple strength anesthetic agents which is sodium pentobarbitone (dosage of 200 mg/kg for euthanasia) intraperitoneal (Close *et al.*, 1997) which gave full anesthesia before the neuromuscular blocking agents took the effect to prevent distress to the animal, animal carcasses were incinerated after the experiment.

2.9 Study limitation and mitigation

2.9.1 Study Limitation

Lack of electronic Microscope, Expensive immune-histochemical kit, the loss to die animals

2.9.2 Mitigation

Due to the absence of more sophisticated tools and advanced histological techniques to be used in this study, we tried to use the available resources and practiced techniques like a normal conventional bright-field light microscope and Hematoxylin and Eosin Stain to obtain maximal and valuable results.The loss to die animals will be counteracted by the addition of more samples than the optimal sample size calculated

CHAPTER THREE

3.0 RESULTS

3.1 Effect of *Prunus Africana* extract on prostate weight

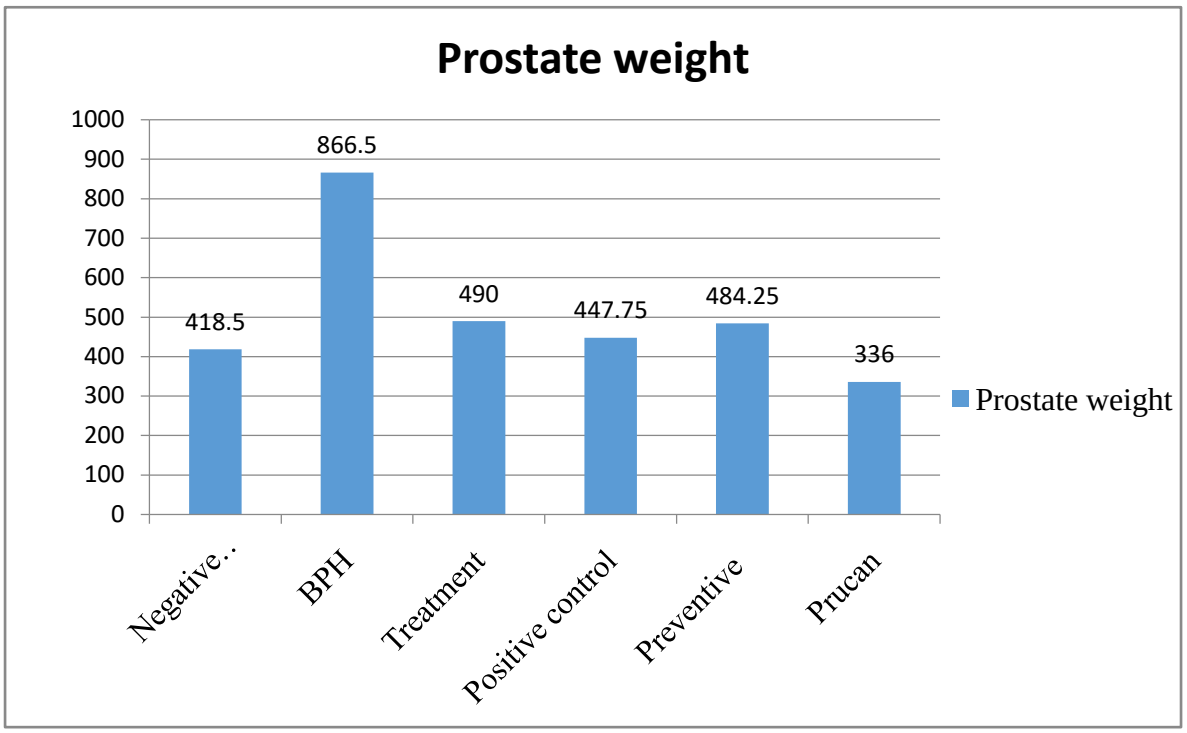
Rats in the BPH group showed absolute and relative prostate weight that were significantly greater than those of rats in any other group, whereas prostate weights in the finasteride-treated group were decreased markedly compared with the BPH group. Prunus treated group also showed a statistically significant decrease in absolute and relative prostate weight compared with the negative control group. Relative prostate weight ratio in BPH recorded 3.88 times heavier than the control group, 2.19 heavier in treatment,2.01heavier in the positive control, 2.16 heavier in the preventive and 1.51 lighter in Prunus treated group compared with the control group.

Table1. Effects of *Prunus Africana* extract on prostate weight and prostate index, Values are mean ± SD

Group ratio(mg/g)	Prostate weight(mg)	p-value	Prostate Index	p-value
Normal control	418.5 ± 9.04		1.86 ± 0.06	
BPH	866.5 ± 63.98	0.000*	3.88 ± 0.19	
Positive control	447.75 ± 51.27	0.817*	2.01 ± 0.33	0.000**
Treatment	490 ± 00.00	0.071*	2.19 ± 0.11	0.000**
Preventive	484.25± 3.77	0.112*	2.16 ± 0.13	0.000**
Prucan	336 ± 4.32	0.029*	1.51 ± 0.10	

* compared to negative control ** compared to BPH

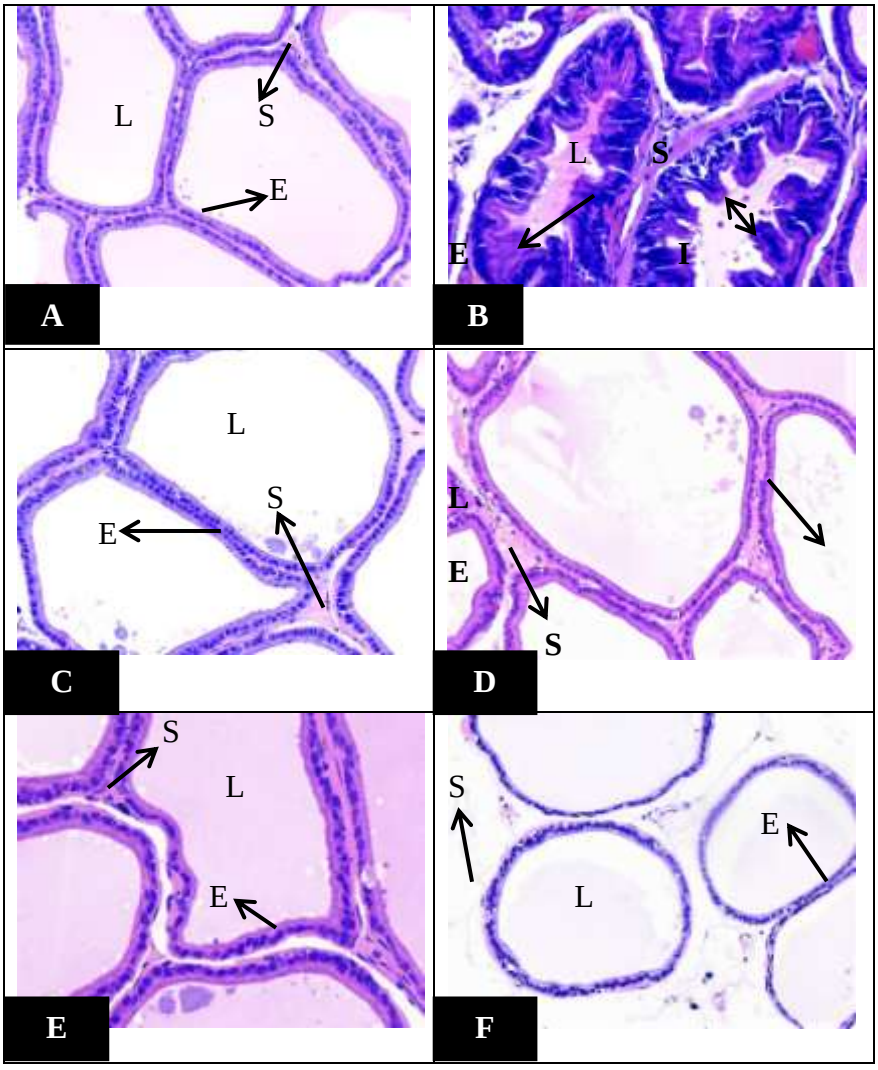
Figure 1- Prostate weight, it is higher in BPH compared to other groups while the Prucan group is lighter than the negative control group, values are Mean ± SD



3.2 Histopathological results

For the control group, the cells under the microscope demonstrated normal architecture, and the prostate gland is surrounded by a layer of smooth muscle with a distinct nucleus. The lumens are filled with prostatic secretion and show a single layer of epithelial lining at x40. The BPH group revealed remarkable histological alterations. There were large stromal spaces of prostate and glandular hyperplasia with epithelial proliferation, the glandular epithelium became thicker and formed numerous papillae (involutions) projecting into eosinophilic prostatic secretions. The connective tissue blood vessels have dilated compared to the control indicating increased activity or demand by hyperplastic cells. The treatment with finasteride and *Prunus Africana* extract in different groups exhibited some features of prostate glandular hyperplasia, but with very much reduced epithelial proliferation, acinar epithelium height with absent involutions and reduced stromalspaces of the prostate as compared to the BPH induced group. The administration of finasteride and Prunus protected the overall morphology and restored the histological features of the prostate to nearly normal.

Figure 2 – Photomicrographs of HE-stained (×40) sections of the prostate: (A) Negative control group showing normal prostatic tissue, with acini lined by a single layer of low columnar epithelial cells {E}, scanty stroma {S} and narrowed acini lumen {L}. (B) BPH group showing the narrowed acinar lumen {L} by thickened epithelial {E} projecting into large involutions {I}, with abundant stroma in between the acini {S}. (C) and (D) are Positive control and Treatment group both showing flat cuboidal epithelial {E} with the absence of involutions{I}, widened acini lumen {L}and moderate stroma{S}. (E) Preventive group showing flat cuboidal epithelial {E} and moderate stroma{S}. (F) Prucan group showing decreased acini lumen with flat cuboidal and thin epithelial {E} and increased stroma area. {S}



3.3 Histomorphometric results

The decrease of the epithelial height in the Prucan group was statistically significant when compared to the control one. On the contrary, there was a significant increase of epithelial height in the BPH group, when compared with control (positive and negative) and Prunus treated groups. On the other hand, administration of *P .Africana* extract to the treatment and preventive groups showed a significant decrease in epithelial height, when compared with the BPH group, however, there was an increase of epithelial height in treatment and preventive groups compared to the negative control group but the increase was not statistically significant.The epithelial height of the positive control group was higher compared to that ofthe treatment group but was not significant. When compared with the negative control groupa statistically significant decrease of the luminal area in both the Prucan group and BPH groups was observed. There was an increase in the luminal area in the positive control, preventive, and treatment group however this increase was not significant in each of these groups when compared with the negative control group. The luminal area of the positive control group was higher than that of the treatment group but was not significant, in treatment, positive control, and the preventive group there was a significant increase of luminal area when compared to the BPH group. There was an increase in the stromal area in the Prucan and BPH groups and when compared to that of the control group and it was statistically significant. With the application of *P.Africana* in the preventive and treatment group and finasteride in that of the positive control group the stromal area was statistically decreased and significant when compared to the BPH group. The stroma area of preventive, treatment and positive control groups was lowered statistically close to the normal of the control group but were not significant, the stroma area in the treatment group was higher than that of the positive control group but not significant.

Table 2 Epithelial height, acinar luminal area, stromal area, results expressed in Mean ± SD

Group	Epithelial height [μm]	p-value of height	luminal area [μm^2] × 10^3	p-value of lumen	Stroma area [μm^2] × 10^3
Negative control	16.14 ± 0.37		348.5 ± 1.29		246.12 ± 1.09
BPH	27.15 ± 0.96	0.000*	299.5 ± 1.29	0.001**	341.12 ± 1.26
Treatment	17.1 ± 0.32	0.126*	350.22 ± 1.1	0.365**	248.28 ± 0.49
Positive control	16.55 ± 0.48	0.856*	349.9 ± 1.67	0.576**	247.21 ± 0.32
Preventive	17.18 ± 0.33	0.086*	350.80 ± 0.83	0.120**	249.30 ± 0.49
Prucan	14.97 ± 0.12	0.000*	345.59 ± 0.75	0.030**	623.52 ± 6.82

* compared to negative control ** compared to the negative control

CHAPTER FOUR

4.0 DISCUSSION

The present study aimed to evaluate the histological changes in rat prostates treated with *P. Africana* and measuring of prostatic epithelial cell proliferation, stroma expansion, and glandular lumen size. We have found that there is a restoration of the histological architecture and histomorphometric features of the induced BPH prostate nearly to normal.

Our study has found that the BPH group had a significant increase in absolute and relative prostate weight as compared with the negative control group (P = 0.000), this was what we expected, the same finding was also reported by Nasr El-Din and Abdel Fattah, 2019 the possible reason of having similar finding is because they also used the same induction technique with different dosage but above the known established dose for BPH induction (Nasr El-Din and Abdel Fattah, 2019). Animals that were induced with BPH and then treated with finasteride (P=0.000) and the *P. Africana* (P=0.000) showed a remarkable reduction in the prostate weight as compared with the BPH group, this is telling us that there is a possible reversal effect in ameliorating the testosterone-induced BPH specifically with *Prunus Africana* extract because finasteride is already known to have ant BPH activity in vivo. These results agree with the study done by Jena *et al* 2016 who also treated animals with finasteride and Prunus species (Jena *et al.*, 2016). However prostate weight in the positive control group was lower than that of the treatment group (P=0.817), this is not a surprising finding because finasteride is among the first-line therapy for BPH that its efficacy has been proved beyond doubt in the literature (Auffenberg, Helfand and McVary, 2009a). Animals treated with P. Africana extract alone exhibited a significant decrease in prostate weight compared with the negative control (P=0.029).

Regarding the Prostate weight index in this study, the BPH group had a statistically significant increase in weight when compared with that of the normal control (p=0.000), with the administration of the finasteride and *Prunus Africana* the prostate was heavier (2.69) in treatment than (2.60) in the positive control group. These findings are similar to the results obtained by Wael *et al*, 2019 although they assessed other herbal formulations we have similar findings because their products also have anti-BPH activity and they also used the same induction techniques (Shin et al., 2012; Nasr El-Din and Abdel Fattah, 2019).

Histological findings of this study have demonstrated that in the treatment and preventive group there were cuboidal or flat epithelium with the absence of involutions, widened acini lumen, and moderate fibromuscular stroma, these features are further supported by histomorphometric findings that we analyzed which showed a significant decrease of epithelium height (P=0.00) and stroma area (P=0.00) and increased luminalarea (P=0.00) of the treatment and preventive group compared with that of the BPH group. To the best of our knowledge, this is among the few studies to describe the histological and histomorphometric changes associated with *P .Africana*. These findings are suggesting the possible anti-BPH activity of *Prunus Africana* histologically and this conforms to what was reported by Komakech *et al*, 2019 that the bark decoction of *P. Africana* has been used for the treatment of BPH since time immemorial (Komakech and Kang, 2019). However, the histomorphometric parameters of the positive control group in this study were superior to that of the treatment groupand this might be attributed to the fact that finasteride is a known gold standard treatment for BPH with known efficacy and mechanism of action.

The histological and histomorphometric results we obtained in this study with the ant-BPH effect are in favor of what has been reported in the literature and at our own local context by patients that *Prunus Africana* extract do improve LUTS of BPH (Breza *et al.*, 1998; Ishani *et al.*, 2000; Pagano *et al.*, 2014). This is a very useful finding since it supplements the already known information about the efficacy of this extract (Noumi, 2010; Komakech and Kang, 2019)and this can be used locally to promote the use of *Prunus Africana* extract as an alternative therapy to medical ant-BPH treatment because medical therapies are associated with many side effects and some are intolerable (Yoshida et al., 2011). Medical therapy does also have cost implications, all these factors tend to reduce the adherence and compliance to medications and thus increases the chances of complications and enhance the poor quality of life (pinto et al 2015). In European countries, phytotherapy has been used as the first-line treatment for mild-to-moderate LUTS and represents about 90% of all drugs that are prescribed for the treatment of BPH (McKELVIE *et al.*, 1993), therefore it's not surprising at all if we introduce the use of *Prunus Africana* extract in our local setting as the alternative or one of the treatment option for mild to moderate BPH.

Even though we used routine methods of staining technique we managed to obtain good histological images and findings but more intensive study with more advanced histochemical

techniques are needed to appreciate a fine histological feature like prostate receptors that can show hormonal and extract levels in the prostate.

In the present study, we have found that the histological and histomorphometric features of the Prucan group vary markedly with that of the negative control group, and the difference we observed was significant when we compared the epithelial height (P=0.00), luminal area (P=0.03) and stroma area (P=0.00) with that of the negative control group. We think that these features are consistent with the protective effect of the extract to the prostate of rats and this thought is also supported by Jena et al 2016 that Prunus has been shown to maintain the health of the prostate (Jena *et al.*, 2016), *Prunus Africana* is believed to work in the glandular part of the prostate and this is manifested by flattening of the epithelium and decreased luminal area, these findings are in line with Gathumbi 1995, who found atrophy of the glandular epithelium with the decrease of acinar secretion in toxicity study of *P .Africana* (Gathumbi, 1995). The huge expansion of the stroma component observed in this group might be a compensatory mechanism of the fibromuscular stroma towards the reduced glandular area in the prostate by the extract. This is an area where further studies are needed to explore how the overall can weight of the prostate decrease with the increase in the stroma area.

CHAPTER FIVE

5.1 CONCLUSION

This study has demonstrated that there is a restoration of histopathological findings caused by exogenous administration of testosterone to rats following treatment with *P .Africana* extract and that *Prunus Africana* seems to have a potentially inhibitory histological effect when it was administered simultaneously with testosterone through improving the histopathology influenced by that hormone in the prostate. *Prunus Africana* showed protective effects when administered to normal rats by decreasing histological and histomorphometric findings compared to negative control. All these histological findings have been supported by gross observations of ventral prostrates and their size in this study however the microstructure of finasteride-treated rats was superior to that of *P. Africana* treated rats.

5.2 Recommendation

Basing on the strength and weaknesses observed in this study we recommend the following, another intensive histological study to be done with advanced histochemical technique to elicit more histological changes and possible mechanisms of anti-androgen activity of *P. Africana* extract. A clinically based study should be done involving human subjects to assess *P .Africana* extract histological changes in human prostates, this will foster the use of *P .Africana* extract (Prucan capsule) for management of BPH in our setting.

CHAPTER SIX

6.0 REFERENCES

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